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SIX LECTURES ON THE PREVENTION OF ENCEPHALITIS EPIDEMICS IN SIBERIA

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Annotation. This review summarizes the work of large teams of researchers to prevent two separate encephalitis epidemics in Siberia. The first three lectures sum up an extensive effort to study and control the *Tick-borne encephalitis* (TBE) epidemic in the Kemerovo region of Western Siberia. The study has helped to create a mathematical model that details the TBE epidemic process and offers a quantitative approach to the development of strategies for preventing TBE epidemics. Ten-year effort to combat TBE in the Kemerovo region led to a significant and sustained reduction in TBE morbidity and mortality. Fifty years after completion of this work, the proposed strategy has not been tested in other endemic regions, although the incidence of TBE worldwide has almost doubled, taking hundreds of lives and causing disability in thousands.

The second disease described here is *Viliuisk encephalomyelitis* (VEM), first discovered 150 years ago in a small rural population of Eastern Siberia. The disease later spread to densely populated areas of the Republic of Sakha (Yakutia), reaching epidemic proportions. The three lectures on VEM provide an overview of multi-year studies on clinical presentation, neuropathology, pathogenesis, etiology, and epidemiology of VEM. We report here for the first time how a prolonged hospitalization of VEM patients during the acute and subacute phases of the disease prevented transmission to susceptible individuals in their families and communities, which has helped to put an end to the further spread of this deadly disease. VEM is a new example of a local disease that has spread to a large territory and could potentially invade other countries if left unchecked.

This review is based on a series of lectures delivered to different audiences at different times. The purpose of combining discrete topics in a single review is to emphasize approaches to solving problems, to illustrate the main results of the fight against Siberian epidemics and, when possible, reflect on the individual contribution of each researcher.

Key words: Tick-borne encephalitis; Kemerovo region; Kemerovo virus; TBE vaccine; acaricides; Viliuisk encephalomyelitis; Republic of Sakha (Yakutia); Viliuisk virus; Autosomal dominant spinocerebellar ataxia type 1 (SCA1), Hereditary spastic paraplegia.

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INTRODUCTION

Siberia is enormous; it extends east from the Ural Mountains to the Pacific coast, occupying about 10% of the Earth's surface and spanning eight time zones. Siberia is a combination of extremes. It is known for long frigidly cold winters and short hot summers, high mountains and ultra-deep valleys, the long and wide rivers Ob, Yenisei and Lena, millions of lakes, including the world's largest Lake Baikal. Geographically, the region consists of the West Siberian Plain and the East Siberian Plateau. Much of Western Siberia is covered with dark coniferous forests called *taiga.* A significant part of Eastern Siberia is covered with treeless tundra. The Altay, Sayan, and Tuva mountains rise on the southern edge of Siberia. Siberia is rich in coal, oil, and gas, and is mined for gold and diamonds, silver, manganese, lead, zinc, nickel, cobalt and molybdenum.

The development of Siberia's natural resources intensified in the 20th century, with large cities and industrial towns appearing in the region. The world's longest Trans-Siberian Railway (5,000 miles) and the longest oil and gas pipelines were built to provide energy to Europe. In 2008, the Siberian Economic Region contributed 25% of the Russian Federation's GDP. Up to 70% of the population (about 30 million people) live in cities. The largest cities are Novosibirsk (1.6 million residents), Omsk, Krasnoyarsk, Barnaul. However, Siberia remains one of the most sparsely populated regions on Earth. The rural population of Western Siberia is 4.1 million people, and of Eastern Siberia - 2.6 million.

The population of Siberia is ethnically diverse. It includes the descendants of early Slavic settlers (Russians and Ukrainians) who moved to Siberia from the European part of Russia, and more than 120 indigenous peoples, some of whom still practice their traditional nomadic way of life. The largest are Sakha (Yakuts), Evenki, Buryats, Tuvans, Altai, Tatars, Koryaks, Kalmyks, Chukchi, Khanty and Mansi. Siberia has traditionally been used as a place for prisons, labor camps, and exile.

Siberia is also known for major epidemics of infectious diseases that have claimed the lives of many thousands of people. Descriptions of smallpox epidemics are terrifying. An outbreak of 1610 wiped out the entire city of *Narym.* The *Irkutsk* epidemic of 1752 claimed most of the city's population. The smallpox epidemic in *Zashiversk* in 1832 killed all its residents [1]. *Thousands* of Siberians died during the cholera epidemic in 1852-1860. The civil war of 1918-1922 was at some point stopped by mutual consent of the warring armies, so that they could bury 60 thousand victims of epidemic typhus. Reports of lepers living as outcasts in Siberian forests are examples of extreme human suffering [2]. Anthrax was endemic in many parts of Siberia. Tuberculosis has killed and is still claiming thousands of lives.

In this review, two separate types of encephalitis in Siberia are described. The first, *Tick-borne encephalitis* (TBE), was discovered during an expedition to the Far East region of the USSR in 1937 and later found to be endemic in Western Siberia, the Urals, Central and Eastern Europe. The first three lectures summarize the results of an extensive collaborative effort to study and fight the TBE epidemic in the Kemerovo region of Western Siberia. I worked closely with recognized leaders in this field, Professors M.P. Chumakov, E.S. Sarmanova, D.K. Lvov, K.G. Umansky, A.N. Shapoval, doctors G.N. Naydich, N.F. Chumak, I.A. Selyutina, and others. Ten-year effort to combat TBE in the Kemerovo region has led to a significant and sustained reduction in TBE morbidity and mortality. The results of this work were published in disparate and often inaccessible dissertations and meeting proceedings but never summarized as a full narrative.

The second disease described here is *Viliuisk encephalomyelitis* (VEM), first discovered 150 years ago in a small Tungus-Sakha population of Eastern Siberia. The disease later spread to densely populated areas of the Republic of Sakha (Yakutia), reaching epidemic proportions. Lectures on this topic provide an overview of studies conducted in collaboration with brilliant researchers and scientists, including Professors P.A. Petrov, M.P. Chumakov, D.K. Gajdusek, R.S. Tazlova, C.L. Masters, doctors A.I. Vladimirtsev, G.V. Lyskova, V.A. Vladimirtsev, F.A Platonov, V.L. Osakovskiy, T.M. Sivtseva and others. In addition to analyzing and interpreting the accumulated data on the clinical manifestation, etiology, pathogenesis and epidemiology of VEM, we report for the first time that prolonged hospitalization of patients in the acute and subacute phases of the disease prevented its transmission to susceptible members of their families and communities, which led to the elimination of this deadly disease. After 2012, no new cases of VEM were reported.

TICK-BORNE ENCEPHALITIS

TBE was discovered in the Far Eastern region of the USSR in the 1930s. The locals were dying of encephalitis, and even more alarmingly for the Soviet authorities, some soldiers guarding the border with China and Japan also contracted the disease.

Lev Aleksandrovich Zilber

A Moscow expedition led by Professor *Lev Aleksandrovich Zilber* was dispatched in 1937 to study the Far Eastern outbreak of encephalitis. Professor Zilber organized two teams of virologists and epidemiologists working in parallel in the Khabarovsk and Vladivostok border districts. The Khabarovsk group included virologists *Elizaveta Nikolayevna Levkovich, Mikhail Petrovich Chumakov,* and several laboratory assistants. Based on his analysis of epidemiological data, Professor Zilber realized that an unknown pathogen is likely to be transmitted to humans by *Ixodid ticks* [3]. Indeed, very soon Dr. M.P. Chumakov succeeded at isolating the causative virus from *Ixodes persulcatus* ticks [4]; many other virus strains were obtained from the blood and cerebrospinal fluid of patients with TBE. This was in every respect a highly successful expedition.

Professor Zilber summed up the results of the first Far Eastern expedition of 1937 as follows: "*Within three months, we established the existence of a new, previously unknown form of encephalitis, identified 29 strains of the pathogen, detailed the epidemiology of the disease and its vector, described the clinical features and neuropathology of the disease.*" The subsequent expeditions of 1938-1941 fully confirmed these results.

The work on TBE carried great dangers for the team. During the 1937 and subsequent expeditions, five team members were infected and developed TBE. Dr. M.P. Chumakov accidentally cut his finger while performing an autopsy and contracted a severe encephalitic form of TBE.

He survived, but from the age of 27, he was left with a permanently paralyzed right arm and severe hearing loss. *Dr. Nadezhda Veniaminovna Kagan* and laboratory assistant *Natalya Yakovlevna Utkina* died from TBE while working to create the first TBE vaccine. Parasitologist *Boris Ivanovich Pomerantsev* died of TBE after being exposed to numerous tick bites. *Valentin Dmitrievich Solovyov* and *Vasiliy Sergeyevich Mironov* recovered from a less severe form of TBE.

Historical photograph of the first expedition to study Tick-borne encephalitis in the Far-Eastern region. In the foreground, Elizaveta Nikolayevna Levkovich, laboratory assistant Galina Nikolayevna Zorina-Nikolayeva, standing - Mikhail Petrovich Chumakov. Oboz, Khabarovsk district, 1937.

Another devastating tragedy struck the participants of the first Far Eastern TBE expedition when on their return to Moscow Professor L.A. Zilber and two team members, virologist *Aleksandra Danilovna Sheboldayeva* and epidemiologist *Tamara Mikhailovna Safonova,* were arrested and without due process wrongly convicted of subversive activities – *"spreading the Japanese encephalitis virus with criminal intent to harm military activities in the Far Eastern region and attempting to spread the disease in Moscow under the guise of conducting scientific research on a new virus*" [5]. The first report on the etiology and epidemiology of TBE was published in 1938 with the names of arrested participants missing from the list of authors. Professor L.A. Zilber was released from prison two years later, arrested again and released in 1944.

Despite his disability, M.P. Chumakov continued his work on TBE. He organized several medical expeditions and, in 1939, discovered that TBE was also endemic in the Urals and Western Siberia [6, 7]. Dr. M.P. Chumakov later determined that TBE existed in the European part of the USSR, where it was transmitted to humans by *Ixodes ricinus,* a different species of ticks. In the 1950s, Professor M.P. Chumakov founded the *Institute of Poliomyelitis and Virus Encephalitis* in Moscow, which became the center for vaccine development. The other participants of the 1939 expeditions, notably E.N. Levkovich, A.K. Shubladze, and V.D. Solovyov, have become distinguished leaders in the field of virology.

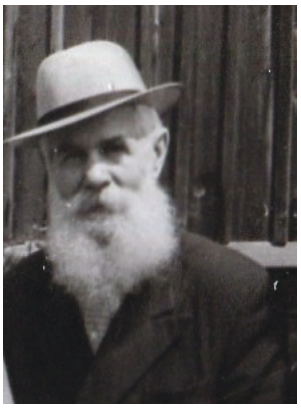
The next, less well-known but no less heroic wave of TBE researchers, epidemiologists, virologists, and parasitologists, responded to the call to contain the TBE epidemics, which occurred in many parts of the USSR in the 1950s and 1960s [8]. They devoted their work to developing and applying measures for protecting the local population and people moving to TBE endemic areas for post-war economic development.

At the risk of possible omissions, I will name the researchers whom I regularly met at meetings and worked with in expeditions: *L.G. Tatarinova, I.N. Polenova* and *G.N. Leonova* in Vladivostok; *L.A. Vereta* in Khabarovsk; *I.V. Uspenskiy,* a research-scientist from the Institute of Parasitology who worked in the Amur region; *A.A. Vasenin* and *O.Z. Gorin* in Irkutsk and Transbaikalia; *L.V. Babenko, M.A. Rubina, E.I. Fastovskaya, L.A. Prisyagina,* and *R.L. Naumov,* researchers from the Institute of Parasitology who worked in the Krasnoyarsk Territory; S.P. *Karpov, B.G. Trukhmanov* and *A.R. Yavya* in Tomsk; *N.M. Vlasenko, P.I. Chudinov* and *V.I. Prigorodov* in Novosibirsk; *N.S. Gorbunov* and *V.V. Kuklin* in Altai; *G.I. Netsky, F.F. Busygin* and *O.V. Ravdonikas* in Omsk; *A.V. Dubov* in Tyumen; *S.S. Magazanik* and *L.S. Subbotina* in Sverdlovsk; *E.I. Korenberg,* a researcher from the Gamaleya Research Institute who worked in Udmurtia; *A.L. Dumina* and *S.A. Shilova* in Perm, *G.Kh. Gilmanova* and *V.A. Boyko* in Kazan; *V.I. Votyakov* and *I.I. Protas* in Minsk.

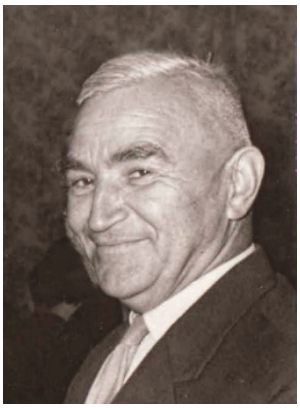
The undisputed leaders of this second wave of TBE studies have become *E.N. Levkovich, M.P. Chumakov, V.N. Beklemishev, V.V. Kucheruk, E.S. Sarmanova, A.N. Shapoval, V.V. Pogodina, D.K. Lvov, N.N. Gorchakovskaya, S.P. Karpov, E.I. Korenberg.* At numerous conferences, methodological approaches and new projects were discussed. *Lidiya Mikhaylovna Ivanova* coordinated all activities on TBE prevention in the Russian Federation. She managed a network of institutions that included the *Sanitary and Epidemiological Stations* and research *Institutes of Epidemiology and Microbiology* all over the country. The work carried out in the Kemerovo region was part of the general strategy to combat TBE.

LECTURE 1. Tick-borne encephalitis in the Kemerovo region

The rapid industrial development of Western Siberia during and after World War II led to an increase in the number of people exposed to the dormant inherent foci of TBE. Massive outbreaks, which attracted the attention of the government and local medical services, occurred in the Kemerovo region [9, 10]. Leading TBE researchers, E.N. Levkovich and N.N. Gorchakovskaya, came from Moscow to take part in the search for the causes of an unprecedented 1952 outbreak in the town of *Barzas* near Kemerovo. The local press readily commented on the arrival of famous Moscow scientists with unforgettable photos of Professor E.N. Levkovich, heading to *Barzas* on a high cart, drawn by a pair of horses.



Nikolay Fedorovich Chumak



Aleksey Nikitovich Shapoval

Systematic large-scale measures to combat TBE began in 1953-1954. There was no Institute of Epidemiology and Microbiology in Kemerovo, therefore, all the functions of dealing with epidemics were carried out by the Kemerovo Regional Sanitary and Epidemiological Station. *Nikolay Fedorovich Chumak,* an experienced and respected doctor who defeated malaria in the Kemerovo region, led the Division of Parasitology.

Now he has focused his Division's efforts on fighting the TBE epidemic. The virology laboratory, which was launched in connection with the TBE epidemic, focused on serological testing of patients with suspected TBE. Nikolay Fedorovich and members of his group circulated endlessly in endemic areas — *Taiga, Angero-Sudzhensk,*

Yaya, Mariinsk and *Tisul* in the north, *Novo-Kuznetsk, Osinniki, Kuzedeevo, Myski* and *Mezhdurechensk* in the south and a dozen places in between. We regularly visited local, district, and city hospitals that admitted TBE patients to coordinate uniform clinical investigations, collect and deliver specimens for serological testing, and ensuring accurate case reporting.

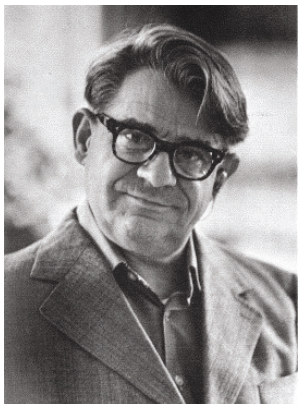
Professor *Aleksey Nikitovich Shapoval,* the recently elected Chair of the *Department of Neurology at the Kemerovo Medical School,* participated in many trips. Professor A.N. Shapoval was a good teacher. He had extensive experience with TBE, having participated in the first Far-Eastern expedition of 1937, and was in the process of completing his text on the clinical characteristics of TBE [11]. I learned a lot from him.

In the winter of 1960, a conference on TBE was held in Kemerovo. Leading researchers from Siberian scientific institutions were presenting their results. N.F. Chumak's group prepared a report for this conference. Although the presentation was considered *"of local significance"*, that is, primitive compared to other scientific reports, it may have contributed to the decision of the *Institute of Poliomyelitis and Virus Encephalitis* to launch a comprehensive TBE research program in the Kemerovo region.

Institute of Poliomyelitis and Virus Encephalitis

One day in March 1961, we were hosting three representatives from the Institute. The Institute director, Professor Mikhail Petrovich Chumakov, came with two other scientists to determine whether the conditions in Kemerovo were satisfactory for intended research. Mikhail Petrovich was only 52 years old but by any standards, he was an outstanding, world-famous, very successful scientist. In the 1940s, his expeditions discovered and characterized new viral diseases - hemorrhagic fever with renal syndrome, Omsk and Crimean hemorrhagic fevers. In the 1950s, Professor M.P. Chumakov founded the *Institute of Poliomyelitis and Viral Encephalitis* in Moscow, which became the center for the development of vaccines against dangerous viral diseases. Under his leadership, the Institute carried out the development, production and clinical trials of the live polio vaccine. Mass vaccination in the USSR, and later in 60 other countries, led to the almost complete elimination of poliomyelitis.

Visiting scientists received a warm welcome and decided that the Kemerovo region was the optimal place to carry out their program. In preparation for the collaborative research, half of the newly built two-story



Mikhail Petrovich Chumakov

Elena Semenovna Sarmanova

laboratory building was given to the virology laboratory. Well-trained staff was assigned to the project, and modern equipment was purchased.

The work was expected to focus mainly on the serological diagnostic testing of patients. Studies with live viruses were not planned, but later, when the need arose, the necessary equipment was added. The enthusiastic participation of the local medical services led by the Head Physician, *Dr. Grigoriy Naumovich Naydich,* the director of virology *Isabella Andreyevna Selyutina,* and Dr. N.F. Chumak was of critical importance.

Elena Semenovna Sarmanova from the Institute of Poliomyelitis oversaw the entire expedition and took the lead in creating a functional virology service in Kemerovo, which has mastered modern methods of serological and virologic diagnostics.

During the expedition, numerous new strains of TBE virus from ticks and patients were discovered. According to the latest taxonomy, the TBE virus *(TBEV)* is a member of the genus *Flavivirus* in the *Flaviviridae* family. The mature virion is composed of 3 structural proteins - capsid (C), membrane (M), and envelope (E). Protein E induces the production of antibodies that neutralize *TBEV.* Recently published nucleotide and amino acid sequences confirmed the existence of three *TBEV* subtypes named: 1) European, 2) Siberian, and 3) Far Eastern. A strain of *TBEV Kemerovo 67-08,* isolated in the Kemerovo region, has *Thr* at the polymorphic position 175 and *His* at position 234 and has been identified as a prototype of the South-Siberian *TBEV* phylogenetic lineage [12].

Field studies were carried out by two groups: epidemiological, led by *Dmitriy Konstantinovich Lvov,* and clinical, led by *Konstantin Grigoryevich Umanskiy.* The main objective of both groups was to evaluate the effectiveness of the new cell-culture vaccine against TBE,

which required further improvement of clinical and laboratory diagnostics, accurate patient registration, a quantitative assessment of the level of endemicity, and a complete clarity of which population groups needed protection from TBE. It was a large and complex program with many moving parts.

Clinical manifestations of Tick-borne encephalitis

TBEV infects humans through tick bites. A human victim does not immediately notice the tick bite because the tick's saliva contains an anesthetic, as well as other pharmacologically active molecules that allow the virus to penetrate the body's defense mechanisms. Locals know that on return from the forest, they need to check for ticks on their clothes and body, but many tourists do not do that.

The patient or parent usually remembers the effort of removing the tick, which is an essential diagnostic indication. The incubation period between the tick bite and the onset of the disease lasts on average from 7 to 14 days. The disease has a sudden onset with progression within a few days. Clinical presentation, in most cases, is meningitis *(meningeal form)*. Initial symptoms are fever, headache, chills, confusion, nausea, repeating vomiting, and sensitivity to light. Irritation of the meninges is manifested by stiff neck and leg muscles *(Kernig's sign)*. Often, there is an increase in intracranial pressure, moderate pleocytosis and an increase of total protein in the cerebrospinal fluid. In patients with an uncomplicated meningeal form, symptoms of acute disease subside and disappear within about 20 days.

The *encephalitic form* is manifested by severe headache, nausea, vomiting, and disturbed consciousness of varying degrees from drowsiness to stupor to coma. Patients may have delusions, hallucinations, psychomotor agitation with loss of orientation in place and time, and in some cases, frequent epileptic seizures. *TBEV* kills motor neurons in the upper segments of the spinal cord, affecting the muscles of the neck, shoulder girdle, upper limbs, and sometimes intercostal muscles and the diaphragm. This can lead to permanent lifelong muscle weakness and atrophy (Figure 1). There is an increase in intracranial pressure, lymphocytic pleocytosis and an increase in the total protein in the cerebrospinal fluid. Patients with respiratory muscle paralysis need urgent ventilatory support.

A particularly unfavorable course is observed if the damage extends to the brain stem, medulla, and the pons, vital areas of the brain that regulate breathing and blood circulation. Symptoms during the acute phase include coma, seizures, muscle paralysis, and respiratory failure. The brain damage is sometimes so severe that the patient dies within a few days after the disease onset. The mortality rate in the Kemerovo region reached 1 to 2%. Survivors of this most severe clinical form developed permanent disability with dysarthria, difficulty swallowing, breathing problems, and vascular instability.

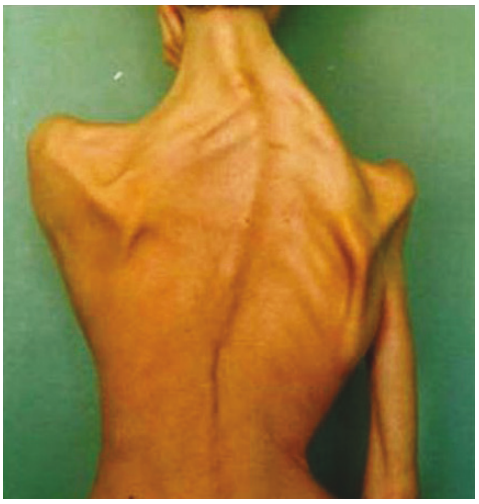


Fig. 1. Consequences of an encephalomyelitis form of Tick-borne encephalitis in a 12-year-old boy. He supports his head with a less affected left hand; otherwise, the head will fall on his chest. Illustration from a textbook.

The good news was that just under 50% of patients with TBE had a mild, uncomplicated course with no significant neurological damage. Fever usually lasted 3-5 days. Patients complained of headache, malaise, fatigue, myalgia, but recovered within 10-15 days. Inflammatory changes in the cerebrospinal fluid were not observed. We called this form *"only fever"* or *"febrile form"*. The febrile form is clinically indistinguishable from many other febrile non-TBE conditions.

Diagnosis. A reliable diagnosis of TBE, especially in the febrile form, requires evidence that the patient developed antibodies against *TBEV* during illness. Since people living in endemic areas may have had antibodies from previous asymptomatic (subclinical) infections, patients are tested twice — in the first few days of the disease and during recovery - to detect an increase in the antibody concentration. The increase is interpreted as a response to a new infection. For this purpose, a sufficiently sensitive and specific *hemagglutination inhibition (HI)* test was used in the 1960s [13]. Antibodies appear or grow in the titer, as evidence that the body is fighting the virus.

Formal diagnostic criteria for TBE included acute febrile illness with severe headache requiring hospitalization, visits to an endemic area of TBE, a history of tick bites, and a four-fold or larger increase in the titer of TBE-specific antibodies. Newer diagnostic methods have recently been introduced - reverse transcriptase polymerase chain reaction *(RTPCR),* which specifically detects *TBEV* RNA in the blood during the viremic phase, and tests for early TBE-specific IgM *(immunoglobulin M)* antibodies [14].

Post-mortem studies revealed turbid, diffusely infiltrated meninges, and severely inflamed brain and spinal cord. Microscopically, there was evidence of neuronal damage in the cervical part of the spinal cord, medulla, brainstem, and the cerebellum. Marked perivascular lymphocytic infiltration is present in these areas. The accumulation of phagocytic microglia cells and lymphocytes around neurons indicates possible neuronophagy.

At the end of each epidemic season, a designated committee of neurologists, epidemiologists, and virologists gathered to study each patient's record and determine the final diagnosis. Vaccination records were then added to the files. Formal TBE diagnostic criteria were introduced partly in 1960, fully in 1961, and did not change significantly over the next decade, making annual estimates of TBE incidence comparable during the years 1961-1970. Strict adherence to diagnostic criteria, which included mandatory serological and, in many cases, virologic testing of patients with suspected TBE [13], allowed to avoid diagnostic errors that were made in other regions at the time [15].

Differential diagnosis. Kemerovo virus fever. Many fully studied patients clinically identical to TBE have failed to show the presence of anti-*TBEV* antibodies in serological tests. It has been suggested that ticks may transmit pathogens other than *TBEV.* False-negative and false-positive results of serological tests could potentially be a major obstacle to the objective assessment of the effectiveness of the new TBE vaccine. In 1962, Professor M.P. Chumakov set up a study aimed at identifying microorganisms other than the *TBEV* that can be transmitted by ticks. He invited a team led by a renowned virologist *Helena Libikova* from the *Institute of Virology in Bratislava,* Czechoslovakia, and *Dr. Jacob Brody* from the *U.S. National Institutes of Health.* The participation of foreign researchers became possible during the political thaw of the early 1960s.

One of the notable results of the 1962 expedition was the isolation from ticks *Ixodes persulcatus* of the novel *"Kemerovo virus"* [16, 17]. The virus was thoroughly investigated, included in official classifications and subsequently identified in many other countries. To determine whether *Kemerovo virus* is pathogenic for humans and, if so, what disease the new virus may cause, *Dr. Mart Martson* from *Tallinn, Estonia,* an expert in pediatric infectious diseases, came to Kemerovo the following year to investigate.

In the area where the *Kemerovo virus* was isolated, every patient with a fever was examined. Of course, there were too many such patients; they were grouped into several categories that differed from each other according to a set of symptoms. *Inna Stepanovna Mikhailova* tested these patients for antibodies to the *Kemerovo virus* and eventually determined which of our clinical group turned out to be caused by the *Kemerovo virus.* Most patients had a febrile disease, and two showed meningeal signs [18].

Many years later, in the early 1990s, another tick-borne microorganism was discovered, the spirochete *Borrelia burgdorferi.* This spirochete causes *Lyme disease.* Signs of Lyme disease were present in dozens of our patients, they were classified as *"erythema migrans".* It never occurred to us to look for spirochetes. If we did, we could have discovered the disease 20 years before *Willy Burgdorfer* [19].

Tick-borne encephalitis outcomes. Recovery from TBE is often incomplete even after the febrile and meningeal forms. Patients complain of constant or intermittent headache, apathy, irritability, memory impairment, difficulty concentrating. Those who had the encephalitic form were left with flaccid muscle paresis or paralysis, which can be restored by treatment, but in some cases, they persist for 1–2 years or for life. The most dramatic was the chronically progressive form of TBE. Although relatively rare (around 1% per epidemic season), they accumulated in endemic areas. The *amyotrophic* type of chronically progressive TBE manifests with muscle weakness and atrophy extending to previously unaffected muscle groups. Another type, *Epilepsia partialis continua,* or *Kozhevnikov's epilepsy,* manifests as persistent myoclonus, mainly in the muscles of the arms and face, and recurrent generalized epileptic seizures. *Kozhevnikov's epilepsy* develops immediately or within 1-2 years after the acute disease. TBE-related origin of these types of progressive neurological disease has been confirmed by virus isolation in several cases [20].

According to the final count, about 1% of TBE patients died during the 1960 epidemic season (before mass vaccination). The encephalitic form was diagnosed in 10% of patients: 4% recovered and 6% remained paralyzed. The meningeal form was diagnosed in 45%, and the febrile form in 44%. The proportion of children under the age of eighteen was 30% [21].

In the Khabarovsk and Vladivostok regions of the Far East, according to data collected by our 1978 expedition, TBE was fatal in 22%; the encephalitic form developed in 34%, of which 17% recovered and 17% remained paralyzed. The meningeal form was diagnosed in 29%, and the febrile form in 15% [22].

The causes of the unequal pathogenicity of *TBEV* in different geographical areas are being studied. The separation of *TBEV* strains into three genetic subtypes does not correlate with pathogenicity [14]. Another approach to the issue of pathogenicity is based on the theory that environmental conditions can influence viral load in individual ticks. More on that in the next lecture.

LECTURE 2. Tick-borne encephalitis: assessing the level of endemicity

The topography of the Kemerovo region presents an intricate pattern created by the forests of the plain *West-Siberian southern taiga ecoregion* and three mountain systems, Salair, Kuznetsk Alatau, and Altai, surrounding the Kuznetsk Valley. This complex topography produces soil and vegetation diversity. Mountain foothills are covered with coniferous-small-leaved forests *(foothill taiga),* while the Kuznetsk Valley and the North-eastern part of the region present a combination of forests and grassy plains *(forest-steppe ecoregion).* Thus, in the relatively small Kemerovo region there are several different ecological zones, each of which is populated and economically developed to varying degrees.

Level of endemicity

In preliminary studies, the *"risk of infection",* a measure of endemicity, was assessed according to several indicators: the frequency of forest visits, the exposure to tick bites, the frequency of seroconversions, the prevalence and level of *TBEV*-specific antibodies acquired through repeated infections, and the TBE incidence. The frequency of forest visits and the exposure to tick bites were estimated using short questionnaires; hundreds of thousands of residents of rural and urban areas took part in regularly conducted surveys. The data was analyzed with the help of a contractor who had access to powerful analytical tools. The presence and level of antibodies against *TBEV* was determined by testing serum samples from thousands of people.

It was clear that the risk of infection indicators were interdependent, but we did not know how to combine information into a single index characterizing the level of endemicity. Dr. D.K. Lvov discussed the problem with

Login Nikolayevich Bolshev

someone at the *V.A. Steklov Institute of Mathematics,* a leading institution in various fields of mathematics, and convinced Professor *Login Nikolayevich Bolshev* in the Department of Mathematical Statistics to take up this project.

First, Login Nikolayevich asked to describe in detail everything that is known about the mechanisms of the TBE epidemic process. This logical question turned out to be a complex one. Many experts believed that humans accidentally get into a natural habitat where *TBEV* is circulating. Therefore, according to them, there was no epidemic process, only an accident. It can be an accident if you think about a single person getting into trouble. However, epidemiologists need to think about a human population that is exposed to the tick-borne infection. In the not quite consistent story, Login Nikolayevich skillfully highlighted critical parameters: how often people are exposed to tick bites, how many people become infected, how many people become immune, what level of immunity prevents the disease, etc.

We agreed that the TBE epidemic process could be formalized as a transition of the human population through several successive states; epidemiological parameters determine in which state the population is at a certain point in time, and parameters of transition between the states determine the speed of the process. The main point was that we were dealing with a step-by-step process, rather than a person who is accidentally infected.

Modeling. Professor Bolshev was an outstanding expert in mathematical statistics. During World War II, he flew a small military single-seat fighter. His plane was shot down. Login Nikolayevich suffered a traumatic brain injury and spent almost a year in intensive care at various hospitals. After the war, he managed to graduate from the *Department of Mechanics and Mathematics at Moscow University,* then the graduate school in the same Department. Since 1955, he was a research fellow at the

Institute of Mathematics and professor at the Department of Mathematical Statistics and Cybernetics at Moscow University. He was elected a Corresponding Member of the Academy of Sciences.

Login Nikolayevich loved all sorts of mathematical applications. For example, he offered a solution to the puzzle of which Soviet figure skater won the decisive competition determining who should be selected for the Olympics. He identified two of the nine judges who appeared to be biased. It became clear from the distribution of the ratings they gave. If the ratings of these two judges were excluded, the first-place athlete would be second. Login Nikolayevich proudly joked: *"I judge judges"*. He also served as an expert in the *World Health Organization,* researching the dangers of tobacco smoking and alcohol consumption. His comment was: *"Smoking is bad, but alcohol is harmful only in large doses"*.

Based on preliminary discussions, we constructed a flowchart of the TBE epidemic process (Figure 2).

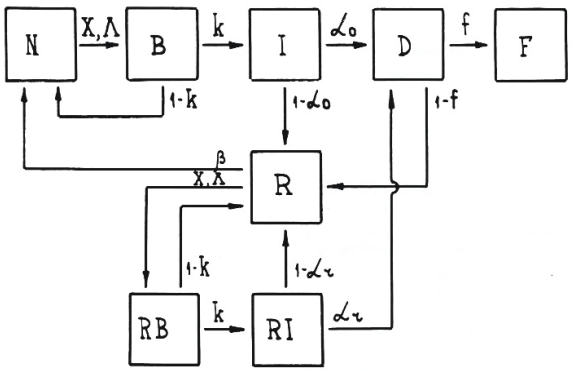


Fig. 2. Flowchart of the Tick-borne encephalitis epidemic process.

The diagram shows the overall structure, successive states and transitions in the TBE epidemic process. An uninfected susceptible person bitten by a tick passes from the initial state N to state B (N → B). The tick-bite rates X and Λ (transition parameters) can be determined from a questionnaire. If the person was not infected, then after the tick was detected and removed, the person returns from state B to state N (B → N). If the person is infected, he/she moves from state B to state I (B → I). The proportion of infected ticks *(k)* can be determined through laboratory testing. An infected person can develop the disease and transfer to state D (I → D) or die (D → F). However, if the person remains alive and healthy, he/she acquires immunity, transitioning to state R (I → R).

Immunity after the initial infection lasts for several years. Loss of immunity causes a return to state N (R → N). An immune person (R) may be re-exposed to infection, transitioning to states designated as RB and RI (R → RB → RI); he/she may develop the disease (RI → D), but most likely will return to stage R (RI → R) with enhanced immunity [23]. As a rule, re-infection strengthens immunity, further reducing the likelihood of disease. In high-intensity endemic regions, the process R → RB → RI → R can be repeated multiple times, leading to stronger and more stable immunity. Those recovering from TBE also become immune, transitioning to R (D→R).

At any given moment, each person is in one of the indicated epidemiological states. The epidemic process consists of transitioning from one state to another, as indicated by arrows. The speed of transition is determined by a set of transition rates shown above or beside the arrows. The *infection rate* determines the speed of the entire process.

Professor Bolshev concluded that such a process can be adequately described using methods proposed by the *queuing theory* (for a comprehensive text on queuing theory see [24]). This well-developed theory is used to analyze the sequence of phone calls on a single line, queues in department stores, street traffic, and other similar processes.

The local human population of an endemic region can be divided into subgroups according to age. *G(t)* is a subgroup at age *t* years at the onset of the epidemic season. The ratio of persons bitten by ticks at age *t* can be expressed by equation:

$$X(t) = \frac{m_t}{n_t} \quad (1),$$

where n_t is the number of respondents from group *G(t)*, and m_t is the number of those who have been exposed to tick bites.

The average number of tick bites per person is *Λ(t)*:

$$\Lambda(t) = \frac{1}{n_t}\sum_{z=1}^{z_{max}} z \cdot m_z \quad (2),$$

where z is the number of tick bites per person, and m_z is the number of persons with z tick bites.

The probability of the occurrence of exactly z tick bites per person during the epidemic season is distributed according to Poisson distribution:

$$P_z(t) = \frac{1}{z!}[\Lambda\,(t)]^z \cdot e^{-\Lambda(t)} \quad (3).$$

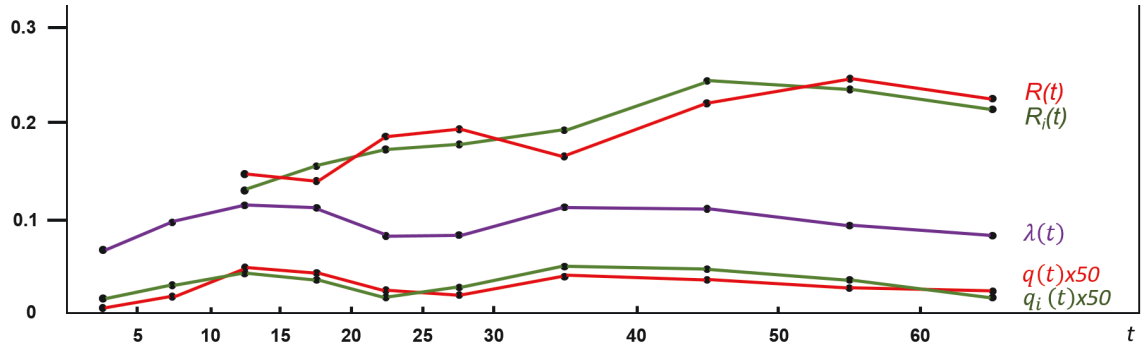


Fig. 3. Analysis of the Tick-borne encephalitis epidemic process using the simple model. Shown on the graph: the age distribution of infection rate, λ(t) (purple curve) having an average slightly higher than 0.01; the age distribution of the observed TBE incidence rate, q(t)and the observed prevalence of immune people R(t) (red curves); calculated $R_i(t)$ u $q_i(t)$ (green curves). The incidence rate $q_i(t)$ is calculated from the model using a single parameter $\alpha_o = 0.0122$. Estimate for $k = 0.23$. The accuracy of conformity between the calculated $q_i(t)$ and the observed q(t) [df = 6; $\chi^2 = 3.11$]; between the calculated R(t) and the observed $R_i(t)$: [df = 17; $\chi^2 = 13.1$].

The probability of no tick bites (z = 0) is:

$$P_o(t) = e^{-\Lambda(t)} \quad (4)$$

and the probability of having one or more tick bites at age *t*:

$$X(t) = 1 - P_o(t) = 1 - e^{-\Lambda(t)} \quad (5).$$

Formula (5) can be used to calculate the average number of tick bites per person during the epidemic season, $\Lambda(t)$ from the $X(t)$ data obtained from the questionnaire. However, if the process does not occur in accordance with the Poisson distribution (this is easy to establish), $\Lambda(t)$ needs to be estimated using formula (2), which will require more detailed data from the questionnaire.

The central characteristic of the epidemic process - the infection rate, $\lambda(t)$ sometimes called *"the force of infection"*, is the average number of infections per person during the epidemic season. It is proportional to the tick-bite rate, $\Lambda(t)$, and the fraction of infected ticks, *k*:

$$\lambda(t) = k \cdot \Lambda(t) \quad (6).$$

The infection rate, in turn, determines the TBE incidence rate, $q(t)$:

$$q(t) = \alpha_o \cdot \lambda(t) \quad (7),$$

where α_o is the probability that a non-immune person of any age develops TBE, if infected.

Thus, all parameters of the N → B → I → D process can be calculated by formulas (2), (6), and (7). α_o can be assessed by the chi-square-min method. The above *simple model* describes a low-intensity epidemic process in large cities or rural populations in ecological zones where the number of people in state R is small. The simple model allows to ignore *recurrent infections,* i.e., R → RB → RI → R transitions; a satisfactory agreement between the calculated $q(t)$ and the observed TBE incidence can be reached by using a single parameter α_o (Figure 3).

The hyperendemic model takes into account the effects of re-infection in R → RB → RI → R and RI → D transitions. *G. Macdonald* [25] investigated a hyperendemic situation in his classic work on malaria epidemiology. If an agreement between the calculated $q(t)$ and the observed TBE incidence using a single value of parameter α_o is unattainable, a hyperendemic model should be used. Our calculations show that the estimate of average λ equal to 0.02 can serve as the threshold for separating between simple and hyperendemic TBE models (here and below we take the value of λ for the local population between the ages of 30 and 49 as the average infection rate for a settlement or an ecological zone).

Assuming that immunity increases in increments with every new infection and decreases exponentially with parameter β, the probability of being at an immune level r for any person, $U_r(t)$, is:

$$U_r(t) = \frac{1}{r!}[g(t)]^r \cdot e^{-g(t)} \quad (8),$$

where $g(t)$ is the average level of immunity and $e^{-g(t)}$ the probability of remaining non-immune by age t:

$$g(t) = \int_0^t \lambda(\tau) \cdot e^{-\beta(t-\tau)} d\tau \quad (9).$$

Based on this, the prevalence of immune persons with any level of immunity at age t, $R(t)$ will be:

$$R(t) = 1 - e^{-g(t)} = 1 - \exp\left[-\int_0^t \lambda(\tau) \cdot e^{-\beta(t-\tau)} d\tau\right] \quad (10).$$

The new parameter α_r is the risk of contracting TBE by an immune person of any age if infected. In the case of $\alpha_r = 0$ the immune person is fully resistant; other values of α_r correspond to a risk of developing TBE above zero. The TBE incidence rate $q(t)$ in a hyperendemic region can be determined by summarizing subpopulations grouped by the strength of their immunity and the corresponding risk of developing the disease, including the non-immune group:

$$q(t) = \lambda(t)[\alpha_o S_o(t) + \alpha_1 S_1(t) + \cdots + \alpha_r S_r(t)] \quad (11),$$

where $S_o(t)$, $S_1(t)$, … $S_r(t)$ are the sizes of the subpopulations with increasing immune levels at age t. The sizes of these subpopulations can be determined by serological testing; $\lambda(t)$ can be calculated as before using formula (6). Parameter α_r can be estimated from (11), assuming that r-distribution is exponential:

$$\alpha_r = \alpha_o \cdot e^{-vr} \quad (12),$$

where v is the rate of reduction in the TBE incidence with increasing immunity.

The results of tentative calculations of the incidence rate $q_i(t)$ using a single parameter a_o, as if in a simple model, does not allow a satisfactory agreement with the observed $q(t)$ (Figure 4). This indicates that a hyperendemic model must be used.

The incidence rate $q_{ii}(t)$ calculated with a series of parameters α_o, α_1, α_2 ... α_r for subpopulations with increasing levels of immunity, as required by the hyperendemic model, is consistent with the observed $q(t)$. The calculated $R(t)$ also closely corresponds to the proportion of immune individuals determined by the *HI* sero-

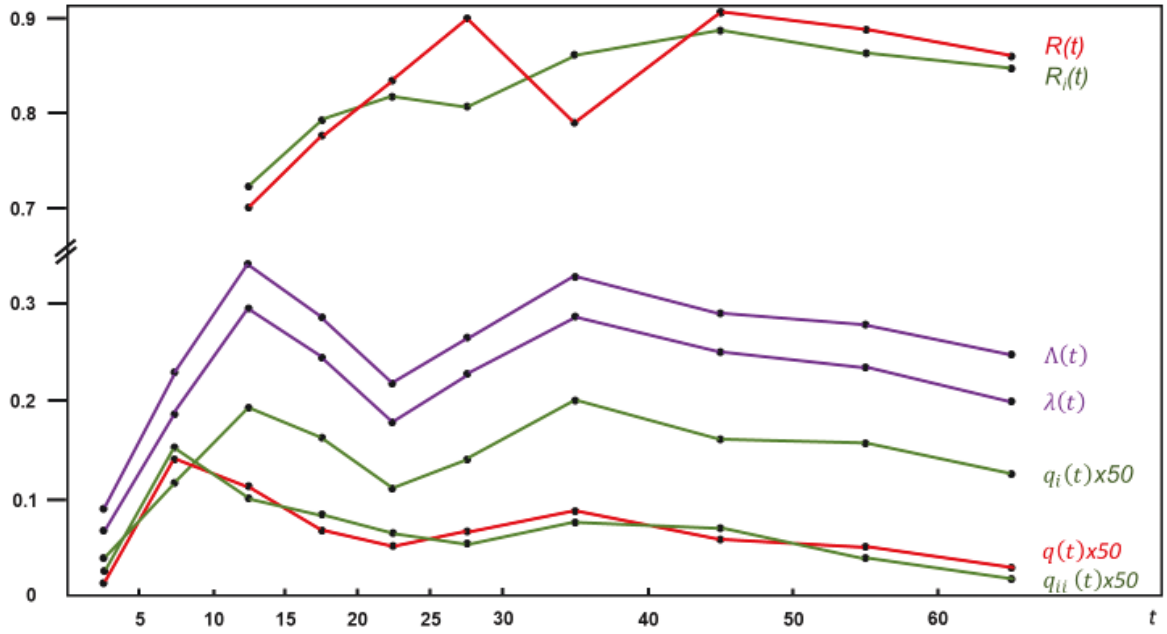


Fig. 4. Analysis of the Tick-borne encephalitis epidemic process in a high-incidence region using a hyperendemic model. Curves show: the age-related tick-bite rate, Λ(t) and the infection rate, λ(t) (purple curves); the observed incidence rate, q(t), and the proportion of immune persons at age t, R(t) (red curves). The incidence rate, $q_i(t)$ was first calculated using a single parameter α_o as if in simple model. The calculation was repeated with a series of parameters α_o, α_1, α_2 ... α_r as required by the hyperendemic model to determine the incidence rate, $q_{ii}(t)$ (green curves). Estimates for the parameters: $k = 0.28$; $\alpha_o = 0.0325$; $\alpha_1 = 0.0241$; $\alpha_2 = 0.0148$; $\alpha_3 = 0.0091$. The accuracy of conformity of $q_i(t)$ to $q(t)$ [$df = 9$; $\chi^2 = 191$]; of $q_{ii}(t)$ to $q(t)$ [$df = 9$; $\chi^2 = 5.7$]; of calculated $R(t)$ to the observed prevalence of HI antibodies in the local population by age t, Ri(t): [$df = 7$; $\chi^2 = 7.91$].

logical test, thus confirming the observation that the *HI* titer corresponds to the immunity level.

Compliance with the real epidemiological data; insights from the model. The simple model satisfactorily described the epidemic process in the villages of *Ussuri lowlands* (average infection rate $\lambda = 0.0021$) and *Sikhote-Alin* foothills ($\lambda = 0.0014$) of the Vladivostok region, the *Middle-Amur lowlands* of the Khabarovsk region ($\lambda = 0.0013$), and the *mid-ecoregion of the Western Siberian taiga* in the Tumen region ($\lambda = 0.0172$). Hyperendemic model was needed to describe the epidemic process in the *southern taiga ecoregion* ($\lambda = 0.22$), *forest-steppe ecoregion* ($\lambda = 0.058$), *foothill taiga* ($\lambda = 0.069$) within the Kemerovo region and the *Prionezhskaya lowlands* in the Belozerskiy and Kirillovskiy districts of the Vologda region ($\lambda = 0.057$). The use of infection rate as a measure of TBE endemicity allows comparing different geographical areas, villages and cities, population groups, etc., based on a single indicator. It has also become possible to estimate all other parameters and variables included in the flow chart: the risk of contracting TBE for a non-immune and immune person, the ratio of asymptomatic to clinically overt infections, and the longevity of immunity. Thus, the mathematical model (a series of formulas describing the epidemic process) links all basic elements of TBE epidemiology and allows to estimate quantitative epidemiological characteristics in various ecological zones and types of settlements.

One of the assumptions included in the model was that the risk of developing TBE after the first-ever infection (a_o) should be significantly higher than the risk of developing TBE for an immune individual after reinfection. Analysis of the hyperendemic focus (Figure 4) confirms the validity of this assumption. There is a category of immune people whose risk of disease is very close to zero; in the southern taiga ecoregion ($\lambda = 0.22$), the size of this category is about 20% of residents, which corresponds to the 1:160 - 1:320 titer of *HI* antibodies. In the *forest-steppe ecoregion* ($\lambda = 0{,}058$), the size of this category is about 7%.

Further analysis of the model confirmed that the duration of immunity after the first-ever infection T_1 is shorter than after reinfection T_2 with a level of significance P = 0.0004. The estimates of $T_1 = 5.19$ years and $T_2 = 10.58$ years were confirmed in all epidemiological situations.

The model predicted that the proportion of infected ticks *(k)* was 23 and 28% in the above examples (Figures 3 and 4). Estimates in other regions were even higher: 70% in the *taiga ecoregion* and 60% in the *forest-steppe ecoregion.* The laboratory methods used to determine

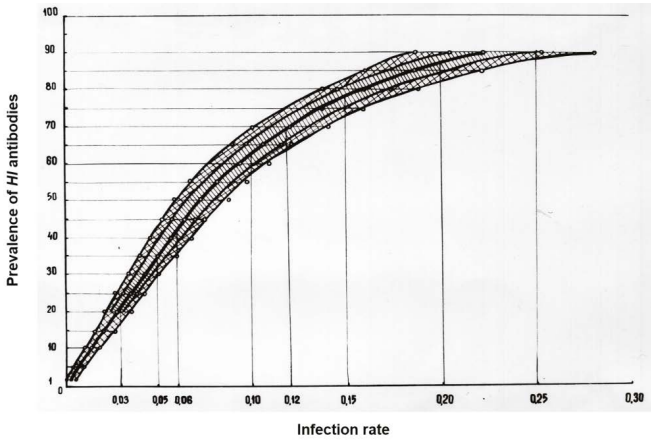


Fig. 5. Nomogram for assessing the average infection rate based on the prevalence of HI antibodies against TBEV among the local nonvaccinated population between the ages of 30 to 49 years (percent). The curve in the middle represents the average infection rate; the intermediate curve corresponds to a standard deviation; and the outer curve to double standard deviation.

tick infectivity at that time were flawed: ten or more ticks were grounded together for a test. Laboratory practice has now been revised by introducing testing of individual ticks. The results have shown that 36% of the tested *Ixodes persulcatus* from Western Siberia were infected [26]. The viral load in individual ticks varied from 2.1×10^2 to 8.5×10^3 PFU/ml [27]. The number of infected Far Eastern Ixodes persulcatus averaged 14%, but the viral load in individually tested ticks was from 10^3 to 10^7 PFU/ml with the largest 8.7×10^6 and 2.5×10^7 PFU/ml [28]. These results were later confirmed in more extensive studies [29, 30]. Improved methods support the conclusion, first suggested by the model, that many more ticks carry infectivity than previously thought.

The elevated viral load in the Far Eastern ticks can be explained by particularly favorable conditions for the development of *Ixodes persulcatus* in light-coniferous and mixed coniferous-broadleaved forests [31]. It is possible *(an unproven hypothesis!)* that the increased frequency of paralytic forms among the Far Eastern TBE patients is associated with a massive infection from ticks with high viral load in a location characterized by a relatively low average infection rate ($\lambda = 0.0014$) and, therefore, a low chance of being immune (i.e. protected against TBE).

If only an approximate estimate of infection rate is required, in order to avoid complex calculations, a nomogram (Figure 5) can be used. The nomogram is based on a close correlation between the infection rate and the prevalence of *HI* antibodies in the local nonvaccinated population aged from 30 to 49 years.

A preliminary report on the TBE epidemiological model was published in the *Journal of Medical parasitology* [32] and a detailed description in the *Proceedings of the Institute of Poliomyelitis, 1970,* vol 18, edited by M.P. Chumakov. The series was published under the title *"Epidemic process in Tick-borne encephalitis"* and consisted of three parts: 1) *Parameters* [33], 2) *Mathematical modeling* [34], and 3) *Predictions* [35]. The simple model was separately described in [36] and the hyperendemic model in [37]. A similar methodology was applied to the analysis of epidemics of *Crimean hemorrhagic fever* [38] and *rubella* [39]. Reprints of the published papers or a copy of the unpublished manuscript can be provided to interested parties.

After completing the mathematical part of the study, our next task was to adjust the implementation strategy of expensive and labor-intensive TBE preventive programs and evaluate their effectiveness in various epidemiological conditions.

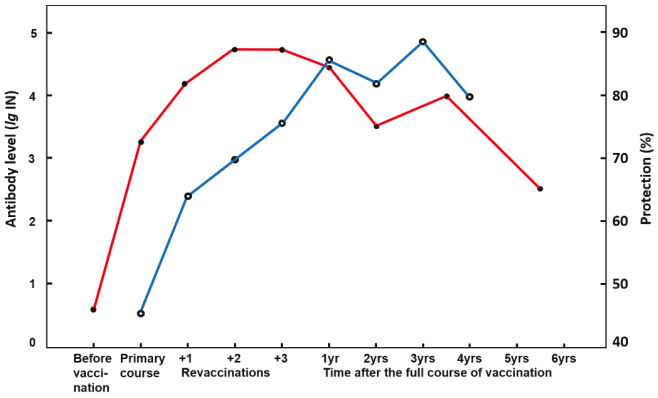


Fig. 6. Results of vaccination with a Tick-borne encephalitis vaccine produced in the Institute of Poliomyelitis and Virus Encephalitis. The levels of neutralizing antibodies in volunteers (red curve, lg of the neutralization index) and levels of protection against TBE in field trials (blue curve, the proportion of TBE incidence rate in vaccinated in relation to the nonvaccinated population) after the primary four-fold vaccination and subsequent three annual boosters (+1, +2, +3), after which the observations extended for further 5.5 years.

LECTURE 3. Tick-borne encephalitis: prevention and control

It was hard to believe that 20 years after the discovery of this disease, there was still no specific therapy, except for the immunoglobulin produced at the *Institute of Epidemiology and Microbiology* in Tomsk by immunizing horses with *TBEV*. This therapy was ineffective. Besides, some patients developed allergic reactions from the introduction of an alien-species protein. The TBE vaccine, also produced in Tomsk from the brain tissue of infected mice and inactivated by formaldehyde, was unsafe for large-scale use [40].

The development of the mining industry attracted a large number of new arrivals from non-endemic areas at a time when the TBE incidence rates rose to 300 per 100 thousand in rural areas and 100 per 100 thousand in some major cities of the Kemerovo region. There was intense pressure from the Kemerovo administration and the local Communist party leaders, who were responsible for the rapid industrialization of the region. They encouraged and paid for the implementation of the most efficient prevention programs. Kemerovo region had become a testing ground for the development of programs for prevention and control of TBE.

Development and testing of a new Tick-borne encephalitis vaccine

In 1959-1960, the Institute of Poliomyelitis and Virus Encephalitis developed mass production of a new variant of the TBE vaccine by culturing the virus *(Sofjin strain)* in a carefully controlled primary cell culture of chicken embryo fibroblasts [41], rather than in the mouse brain. The virus was inactivated with a minimum amount of *formaldehyde* (200μg/ml), further purified by separation, clarification, and sterile filtration, followed by absorption of the antigen with *aluminum hydroxide.* Human albumin (1mg/ml) was used as a preservative. The new vaccine was cleaner and much better tolerated than the mouse brain vaccine. There were no adverse events, local reactions were noted in 19.5% of the vaccinees and expressed as redness of the skin and soreness at the injection site. The most effective vaccination schedule was defined as three injections in the fall (1.0 ml for adult recipients and 0.5 for children 4 to 7 years of age, at intervals of 7-10 and 14 days between injections) plus one in the spring [42]. This primary 4-inoculation course was followed with annual revaccinations for 3 consecutive years.

Immunogenicity. The safety and immunogenicity of the TBE vaccine were tested in 42 volunteers who had no previous exposure to TBE infection. Vaccine ability to induce humoral immunity was estimated by two serological tests used in parallel: the standard neutralization test *(NT)* in mice [43] and the hemagglutination inhibition *(HI)* test. It was believed that neutralizing antibodies are best correlated with post-vaccination immunity [44].

The primary four-fold vaccination induced neutralizing antibodies in 77% of participants at an average of *3.2 lg* of neutralization index (NI), which remained unchanged for one year (Figure 6).

One additional booster (5th injection) produced seroconversion in 100% of the participants and increased the average level of neutralizing antibodies to 4.1 *lg* NI, which remained at this level for one year [44]. The response to the second booster administered a year later (6th injection) averaged at 4.7 *lg* NI with positive values in all subjects; the antibody level decreased slightly to 4.2 *lg* NI after one year. The third annual booster (7th injection) returned the average antibody level to 4.7 *lg* NI with individual levels not lower than 3.0 *lg* NI. One year after a full course of 4+3 injections, with no additional boosters, the average antibody level held at 4.2 *lg* NI; two years later it was at the level of 3.5 *lg* NI; after three and a half years it was 4.0 *lg* NI, and after five and a half years - 2.5 *lg* NI. Thus, we observed a persistent presence of neutralizing antibodies for five and a half years after a 4+3 vaccination schedule in volunteers [45].

Protection efficacy in field trials. Vaccine efficacy is measured by the reduction of disease incidence in the vaccinated group compared to the nonvaccinated (control) group under ideal conditions. The conditions for testing the TBE vaccine in the Kemerovo region were not ideal. It was a vaccination campaign, not a double-blind placebo-controlled clinical trial. According to field observations, the primary four-fold schedule provided 45% protection (Figure 6). An additional booster administered next spring (5th injection) protected 64%. The second booster dose the following spring (6th injection) increased the level of protection to 70%, and the third booster dose (7th injection) to 76%. One year after the full 4+3 course of vaccination, 86% were protected; two years later, with no additional boosters, 82% were protected; after three years - 89%, and after four years - 80% [46]. Most likely, the protection was supported and enhanced by periodic exposure to infection in high-risk areas [47].

At various stages of this study, up to 16% of the participants were asked about forest visits and exposure to tick bites. We did not introduce numerical adjustments for the risk of infection when calculating the vaccine efficacy; however, the estimated frequency of tick bites among vaccine recipients was 23.4% vs. 15.5% in the control group in the first and second years of the trial, and 28% in vaccinated vs. 15% in controls in the third and fourth years.

Vaccination status was blinded to clinicians and epidemiologists working in the field during the epidemic season. Vaccination records were provided for the designated diagnostic committee, which met at the end of each epidemic season to examine each patient's records and determine the final diagnosis (see above). A significant number of vaccinated people, especially those who have received only the primary vaccination, developed laboratory-confirmed TBE. The disease was milder and the disabling paralytic forms were less frequent than in the nonvaccinated group, although detailed data on this topic is unavailable.

Effectiveness of tick control by acaricides

The first experiments on tick control were carried out in the Kemerovo region by *Natalya Nikolayevna Gorchakovskaya* [48, 49]. The use of 16% *Hexachloran* (HCH) has shown its limited potential. 10% *Dichlorodiphenyltrichloromethylmethane* (DDT) proved to be more promising for suppressing the tick population. Large-scale use of DDT began in connection with a sharp increase of TBE incidence in many parts of the country. In 1957, extensive use of acaricide for the extermination of ticks had become the main element of the TBE control programs in the Kemerovo region. Small aircraft were used to spray 10% DDT into forests in early spring after the snow has melted, but the leaves on the trees were still small. DDT was used in the amount of 0.4-0.5 grams per $1m^2$ of the forest surface. The acaricidal impact, as measured by the standard method of flagging for one hour at the peak of tick activity, reached 65% in the first year and 96% in the second year after treatment with DDT, compared to untreated areas. Effectiveness remained at 88-96% for seven years. The tick population was gradually recovering after the 8^{th} year [50]. Factors influencing the effectiveness of acaricidal action are discussed by *Uspensky and Ioffe-Uspensky* [51].

It was later recognized that DDT, the most effective known pesticide, not only eliminates ticks and mosquitoes that spread infectious diseases, but also kills beneficial fauna and disrupts biological cycles in nature. *Rachel Carson* in her book *Silent Spring* (1962) alerted the public to the fact that DDT accumulates to toxic levels. In 1971, the World Health Organization called on all DDT users to develop strategies that limit the use of DDT to a minimum. It is expected that synthetic acaricides less harmful to the environment will be developed in the near future. But in the 1950s and the 1960s, large-scale acaricide intervention was irreplaceable in some epidemiological situations (see below).

Strategies for Tick-borne encephalitis prevention and control in various epidemiological settings

The most effective methods to combat TBE, mass vaccination and acaricidal treatment of frequently visited forests have become the basis for strategic planning of TBE prevention and control. These methods were suitable for large-scale use, but were costly and required outstanding organizational capacity. The key to developing a rational program for each settlement in the Kemerovo region was a skillful choice of prevention and control methods appropriate to the level of endemicity and their use in sufficient quantity. I will describe some typical situations and decisions made in the following sections.

Rural population in ecological zones with high or moderate Tick-borne encephalitis infection rates. Programs were designed individually for specific sets of villages. A rural community in Russia *(sel'sovet)* corresponds to a small county in the American hierarchy of settlements. According to the program, all residents aged 4 years and older in counties located in ecological zones with an estimated average infection rate (λ value) between 0.12 and 0.36 should be protected by mass vaccination. A total of 22 counties with 148,5 thousand residents belonged to this category [52]. It took several years to complete the program (Figure 7).

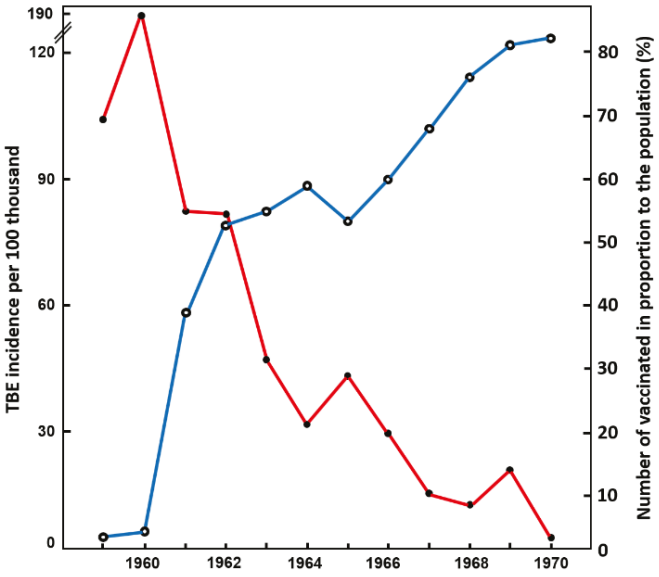


Fig. 7. Vaccination coverage (blue curve) and the annual Tick-borne encephalitis incidence per 100 thousand (red curve) in the rural population of the high-risk ecological zone of the Kemerovo region. The difference between the incidence rates in 1961 and 1967-1970 (all cases laboratory-confirmed): Fisher's exact test, P < 0.001.

38% of the residents were vaccinated in 1961, 51% in 1962, 55% in 1963, 59% in 1964, 53% in 1965 (loss in the number of vaccinated rural population in 1965 was caused by an increased migration to cities), 60% in 1966, 68% in 1967, 76% in 1968, 81% in 1969, and 82% in 1970. Close to 100% of schoolchildren and working adults were vaccinated. Since 1965, an increasing share of vaccinated people received the full 4+3 vaccination schedule.

Prior to the vaccination campaign, TBE incidence in these areas reached 81.9 per 100 thousand (in 1961). It fell to 41.5 per 100 thousand in 1965 and to 6.5 per 100 thousand in 1967-1970 [52, 53]. High vaccination coverage was made possible due to awareness of this dangerous disease and the commitment by local health workers and administrators.

In another group of counties located in ecoregions with a moderate infection rate (λ values from 0.06 to 0.12), only target groups that were subjected to increased tick-bite rates based on survey data were vaccinated. The total population of these 24 counties amounted to 174,9 thousand people. In 1961, 10% of the residents were vaccinated, 17% in 1962, 25% in 1963, 33% in 1964, 37% in 1965, 30% in 1966, 28% in 1967, 31% in 1968, 48% in 1969 and 1970. Vaccination was not considered necessary for 4 to 7 years old children. The TBE incidence rate in these areas before the vaccination campaign was 77.1 per 100 thousand (in 1961). In 1965 it was 16.8 per 100 thousand and fell to 10.2 in 1970.

Large cities (population 100,000 or more). Kemerovo region is one of the largest coal basins in the world and the most densely populated part of Siberia. About 86% of people live in cities. In the 1960s, the largest cities were *Novokuznetsk* (410 thousand inhabitants), *Kemerovo* (305 thousand), *Prokopyevsk* (292 thousand), *Leninsk-Kuznetsky* (140 thousand), *Angero-Sudzhensk* (120 thousand), and *Belovo* (118 thousand).

Urbanization changes the epidemiological characteristics of TBE. Some part of the urban population never visits forests. Most city residents visit only nearby parks and forests for a short day's rest. Those who work outside the city (in construction, etc.), as well as those who collect forest garlic and edible fern leaves shortly after snow melts (early sources of vitamins after a long winter), and families spending time in their gardens outside the city, must be vaccinated. However, mass vaccination of the population of large cities is impossible and unnecessary. The most efficient way to protect the urban population from TBE was to exterminate ticks in suburban forests.

The goal was to determine which forest areas were most visited and to delineate their boundaries. We used

a short questionnaire to establish which forest parcels a person visited last summer (using a detailed map) and how many tick bites occurred at each visit. Over the years, 45,103 people were interviewed in 76 cities and towns of the Kemerovo region with a coverage of at least 2% of the city population [54].

As an example, we rated 170 forest parcels in a recreation area around the city of *Angero-Sudzhensk*. Thirteen of them, occupying only 6.6% of the territory, accounted for 33.1% of all registered tick bites. The next group of 19 parcels, occupying 8.1% of the suburban forest-covered recreation area, accounted for 31.9% of registered tick bites. Thus, visits to 32 designated parcels (less than 15% of the entire forest-covered recreation area) contributed 65% of tick bites. Tick control at these selected sites was planned with the prioritization of the most tick-infested areas [55].

It took many years to complete the program. The TBE incidence in the city of *Angero-Sudzhensk* decreased from 132 per 100 thousand in the years preceding the tick-control measures (1955-1956) to 25 per 100 thousand in 1961-1962 after acaricide treatment of about 22% of the designated area, and further to 3 per 100 thousand in 1967-1970 after processing an additional 15% of the forest-covered area (Figure 8). Processing the additional 15% became necessary because by then, the attendance of the more remote forests had nearly doubled. With the construction of new roads, the development of public transport, and the growing number of families owning cars and suburban cottages, the need to re-evaluate the epidemiological situation and make changes to programs has become vitally important. Another demanding circumstance was the need to re-treat areas where the tick population was slowly recovering.

Similar TBE control programs were carried out in six other cities of the Kemerovo region *(Belovo, Kemerovo, Kiselyovsk, Mezhdurechensk, Novokuznetsk, Prokopyevsk)* with similar results. 22-74% of the frequently visited suburban forests were treated, leading to a 10-fold reduction in TBE incidence. The tick-bite rate associated with the treated forest areas declined from 7.74 per 100 visits to 0.76 [56].

Smaller towns (population from 10,000 to 100,000). This category of settlements included district centers in rural areas and independent small industrial towns. We have studied 67 settlements of this type located in high- and moderate-risk ecological zones. As an example, we report results achieved in a coal mining conglomerate *Berezovka-Barzas* with a population of 31 thousand people. The town is located in a high-risk ecological zone. Tick control in the most visited forests began in 1958

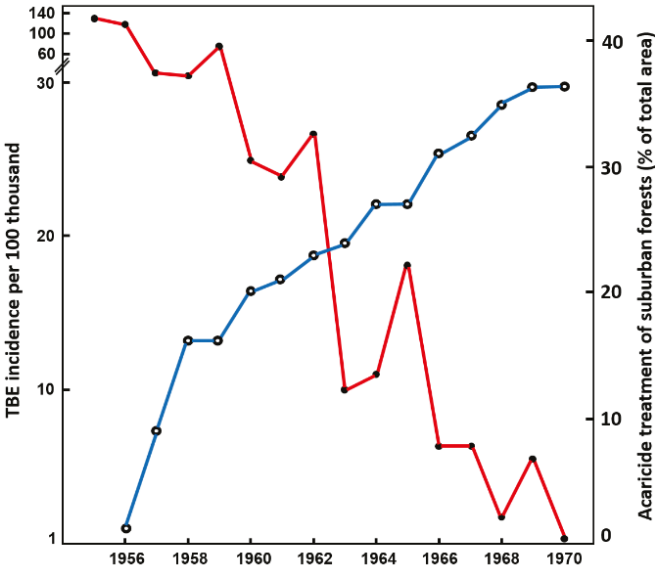


Fig. 8. Results of acaricidal treatment of selected suburban forests in proportion to the total recreational forested territory (blue curve) and the annual Tick-borne encephalitis incidence per 100 thousand (red curve) in the city of Angero-Sudzhensk. The difference between incidence rates in 1961-1962 and 1967-1970 (all cases laboratory confirmed), Fisher's exact test, P < 0.001.

and covered 19% of the recreational area around the town. The effects of tick destruction in treated forests persisted until 1966 and reduced TBE incidence from 145 to 54 per 100 thousand. Meanwhile, mass vaccination, which has been carried out since 1961, covered 71% of the town population, which led to a further decrease of TBE incidence to zero in 1970.

Individual prophylaxis was aimed at preventing tick bites by modeling protective clothing, using repellents, and following the recommended procedure for removing a tick. These measures were very effective for people who strictly followed the rules. In the 1960s, the use of immunoglobulins after a tick bite was practiced on a limited scale, since the effectiveness of this measure was not confirmed in numerous observations.

Overall results of Tick-borne encephalitis prevention efforts in the Kemerovo region

Efforts to combat TBE in the Kemerovo region have resulted in *a significant and sustained reduction* in morbidity and mortality in a densely populated and ecologically diverse region. It was achieved through a quantitative analysis of the TBE epidemic process and choosing the most effective prevention and control methods adopted for large-scale use in relevant epidemiological sit-

uations. By the end of the decade-long campaign, more than 800 thousand people (27% of the total population) were vaccinated, and 980 thousand hectares (3,783 square miles) of tick-infested territory (7.6% of the total forest-covered area of the Kemerovo region) underwent acaricide treatment.

Preventive measures on a smaller scale were also carried out in Tomsk, Novosibirsk, Irkutsk and Krasnoyarsk regions. However, the total volume of control and preventive measures in the Kemerovo region was much larger, it amounted to about 50% of all implemented in the Russian Federation [57]. The share of the Kemerovo region in the Federation's TBE incidence has decreased. Compared with all other regions of Western Siberia taken together, the TBE incidence in the Kemerovo region in 1963–1970 decreased 4-9-fold (Figure 9).

The decrease in TBE incidence in the Kemerovo region in the 1960s occurred due to large-scale use of the most effective prevention and control methods. It was the first demonstration that a targeted intervention strategy can control the TBE incidence in a densely populated and ecologically diverse region.

The work on TBE prevention and control in the Kemerovo region was largely discontinued in the 1970s for several reasons. First, we became victims of our success - a significant reduction in the TBE incidence made further research unnecessary. Secondly, the use of DDT for the extermination of ticks had to be stopped due to DDT toxicity. Thirdly, the most active participants in the project ceased to participate in it for various reasons: Dr. N.F. Chumak, *"the motivator"* of all this TBE work, was now in the mid-70s and slowed considerably; Dr. G.N. Naydich, *"the decider",* left to become a professor at the Kemerovo Medical School; *Ekaterina Dmitriyevna Chigirik,* one of the most active participants, has died. By 1970, the Institute of Poliomyelitis switched to studies of Viliuisk encephalomyelitis. *P.A. Arkatovskiy* and *S.V. Istratkina* tried to continue the work on TBE at some level before both retired in the 1980s.

It is interesting to see what happened in the Kemerovo region after work on TBE moved to a less active stage. The situation was characterized by *A.R. Efimova et al.* [58] in their longitudinal study on TBE incidence in the Kemerovo region (Figure 10).

The annual TBE incidence decreased substantially in 1963-1970 (for statistical analysis, see the publication of *A.R. Efimova et al., 2015*), and it remained low for the next ten years, from 1970 to 1980, while the effects of mass vaccination and acaricidal intervention persisted. The TBE incidence gradually increased over the next two decades to return to indices of the pre-control

Fig. 9. The annual Tick-borne encephalitis incidence (per 100 thousand) in the Kemerovo region (curve 1) compared with the TBE incidence in other West Siberian regions combined (curve 2).

era. The subsequent slowdown that began in 2000 may have been caused by a new wave of mass vaccination, in which up to 250 thousand people at risk were vaccinated. Recognizing the uneven spread of TBE over its wide range and fluctuations over time, a marked decrease in the 1960s was mainly due to large-scale preventive measures [58, 59].

The resurgence of TBE in the 1980 and 1990s required a reassessment of TBE prevention strategies, given the spread of TBE to new areas of European Russia, as well as the Eastern and Western Europe, and changes in epidemiological profiles. Significant shortcomings were identified in the main methods of TBE prevention: the existing TBE vaccine required too many revaccinations and did not provide protection above 80%; the use of DDT, the most effective acaricide, has been largely discontinued due to unacceptable toxicity. A live vaccine derived from the *Langat virus* antigenically related to TBE [60] was rejected due to numerous cases of post-vaccination encephalitis.

Further purification of the inactivated vaccine using adsorption chromatography and gel filtration chromatography [61] has enabled a new generation of commercial TBE vaccines produced by the M.P. Chumakov's Institute. The new vaccines demonstrated a high level of immunogenicity and an excellent safety profile: 100% seroconversion was achieved after two inoculations at an interval of 1 to 7 months [62]. Mass vaccination with sufficient coverage led to a significant decrease in TBE incidence: 4-fold after vaccination of 55% and 8-fold after vaccination of 72% of the population [63]. Epidemiological efficacy at the 99% level has been achieved in

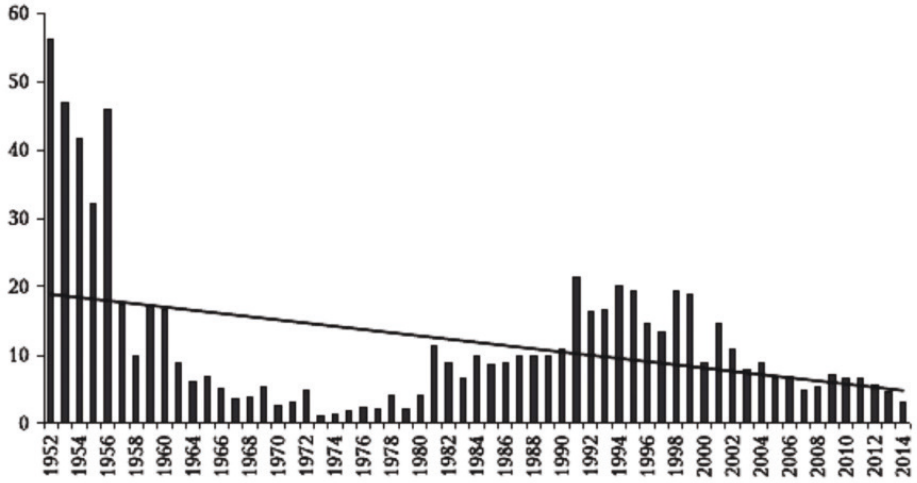


Fig. 10. Annual incidence rates of Tick-borne encephalitis per 100 thousand in the Kemerovo region, 1952-2014 [reprinted from A.R. Efimova et al., 2015]

Austria with the highly purified *FSME-Immun* and *Encepur vaccines* if the recommended vaccination schedule was strictly followed [64].

The extensive economic development of Siberia led to a further sharp increase in human population density. Natural environments have been converted into agricultural or recreational areas. Unexpectedly, anthropogenic impact led to a redistribution of tick populations; ticks adapted to secondary birch-aspen forests, which replaced drier original coniferous forests [65]. This brought the ticks closer to people's homes. A large number of ticks remained in places of their former natural habitat near villages and within cities, even in city parks [66]. The use of toxic acaricides in close proximity to residential buildings is problematic; however, the acaricidal treatment of small gardens, farms, or summer camps for children using hand-held equipment seems insufficient. Large-scale acaricidal treatment of carefully selected forest sites should become part of urban TBE prevention programs when appropriate non-toxic acaricides are developed.

SUMMARY

The main purpose of this review was to restore historical memory of efforts to prevent TBE epidemics in the 1950s and 1960s. At the same time, an account on methodology and overall strategy adopted at that time can still be instructive and perhaps used to plan future programs.

For example, the assessment of the level of endemicity needs to be used for the justification of TBE prevention and control strategies. The latest version of the *Neurovirology Textbook* [67] states: *"TBE vaccine recommendations show major variations partly due to difficulties in defining the degree of endemicity and risk for TBE. To date, there is no clear consensus about when TBE vaccination should be recommended or not, and if recommended, at what age primary immunization should be initiated in childhood… An agreement on how to define high, intermediate, and low endemicity would be helpful in designing vaccine recommendations, but unfortunately, no such consensus exists"*. Epidemiological analysis helps to solve such problems.

Epidemiological surveys in the endemic areas of the Kemerovo region have become the basis for constructing a mathematical model of the TBE epidemic process. The calculated parameters obtained from the model were in satisfactory agreement with the observed epidemiological data, which reinforced the view that the model reflects on the most significant characteristics of TBE epidemiology. Determined in 153 settlements, the infection rate varied 36-fold. The ultimate goal of modeling was to develop a robust quantitative strategy for planning costly preventative programs and assessing their effectiveness in a wide range of environmental conditions.

For the first time, the safety, immunogenicity, and epidemiological efficacy of a TBE vaccine produced by the Institute of Poliomyelitis and Virus Encephalitis were studied. In rural areas with a consistently high infection rate at the level of $\lambda > 0.12$, vaccination of the entire local population was the most efficient prevention method; a sustained 12-fold reduction in the TBE in-

cidence was achieved after 80 to 90% of residents aged 4 years and older were vaccinated. In rural areas with a moderate infection rate (λ values from 0.06 to 0.12), vaccination of the population groups in which the risk of infection was above average led to a 7-fold reduction in the TBE incidence.

The extermination of ticks in the frequently visited and tick-infested recreational suburban forests was adopted as a leading method for controlling TBE in cities. The proposed methodology for quantifying the frequency of visits and the exposure to tick bites in designated forest parcels, obtained through representative surveys of about 2% of the city population, helped to determine the forest sites to be processed. In one city, the acaricide treatment of 37% of the recreational suburban forest area reduced the incidence of TBE 8-fold.

For the first time, a sharp long-term reduction in the TBE incidence was achieved in a densely populated and environmentally diverse region by applying adequately selected prevention and control measures in sufficient quantities. The share of the Kemerovo region in the total number of registered TBE patients in Western Siberia decreased 4 to 9 times in 1963-1970. Hundreds of lives were saved, and thousands of people did not become disabled.

VILIUISK ENCEPHALOMYELITIS

The vast sparsely populated territory of the Sakha (Yakut) Republic is located in *Northeastern Siberia,* the coldest region of the Northern hemisphere with an average January temperature of –50°C and a world record of –72.2°C (minus 58°F and 98°F, respectively). The territory is abundantly rich in raw materials, diamonds, gold, and tin ore. Mining is the main focus of the economy. Sakha population descended from a nomadic Central Asian tribe that migrated to the Siberian plains 600 to 900 years ago under the pressure of Mongol expansion [68]. The arrivals brought the culture of cattle/horse breeding and a dialect of the Turkic language. By the time of Russian colonization in the early 17th century, Sakha people were living in a relatively small part of the Lena-Aldan Valley (currently Central Yakutia).

Prior to the establishment of Sakha settlements, the land was occupied by Tungus (Evenki) and Even tribes, reindeer herders and hunters. Being a more civilized population with a more advanced economy, less dependent on harsh climatic conditions, Sakha assimilated the indigenous populations. From the end of the 17th to the end of the 19th century, the Sakha-speaking population grew from 28,500 to 225,000. The current tally is just shy of 500,000. The rural population of 330,000 people is almost entirely Sakha, while the population of the capital Yakutsk is 56% Sakha and 44% Russian. The Tsarist and Soviet authorities systematically restricted the movement of people. Only after the Second World War, when the development of Siberian resources intensified, local people were allowed to relocate to Central Yakutia, which was more favorable for industrial and agricultural development.

LECTURE 4. Viliuisk encephalomyelitis: Moscow and American expeditions

VEM was discovered in several small settlements near *Lake Mastach* in the Viliuisk ulus. Nomadic Tungus tribes inhabited these areas for centuries. Later, Sakha, ethnically different people, came to the area and established their own settlements. The area around *Lake Mastach* was probably the only place in the entire region where the Tungus were not pushed away, but instead allowed to stay and coexist with Sakha peacefully. In the early Soviet period, small nomadic Tungus tribes were still scattered throughout this vast territory, but in the 1930s, the authorities forcibly resettled them in the permanent Sakha villages, to create larger farms.

Sakha and Tungus populations mixed, and it is currently impossible to determine which one was the source of VEM. Most likely, this disease smoldered in the Tungus tribes and spread to the alien Sakha. In the 1950s, VEM incidence in the Viliuisk ulus reached a level of about 840 per 100 thousand per decade. The disease then spread to other districts along the Viliui Valley and, finally, to the densely populated Central Yakutia. The spread of the disease occurred during a well-documented post-war migration of people from impoverished Viliuisk villages.

Early research. To understand the nature of the epidemic and prevent its further spread, *Prokopiy Andreyevich Petrov,* then a 28-year-old neurologist at the *Viliuisk District Hospital,* began a systematic study of clinical manifestations and epidemiology of this fatal disease. Dr. P.A. Petrov is credited with the discovery and the first detailed clinical and epidemiological descriptions of VEM.

Prokopiy Andreyevich was born in Verkhne-Viliuisk, a small town on the Viliui River upstream of the city of Viliuisk. At the age of 18, he graduated from a nursing school in Viliuisk and worked as a paramedic in the northern village of *Abyy* on the Indigirka River.

Two years later, he entered the *Irkutsk Medical School* and graduated in 1951. On his return to Viliuisk, Dr.

P.A. Petrov witnessed a large and growing number of patients who were dying from a mysterious disease resembling encephalitis and could not come up with a diagnosis. He first considered *Economo encephalitis (Encephalitis lethargica),* an epidemic of which swept through many countries. His mentor, Professor *K-B.G. Khodos,* observed and described hundreds of patients with *Economo encephalitis* during an epidemic in Irkutsk. The classic characteristics of the disease, as described by *Constantin von Economo* [69], were fever and insurmountable drowsiness for 3-4 weeks or more, after which a chronic course with hyperkinetic or parkinsonian manifestations developed. Soon, Prokopiy Andreyevich realized that Viliuisk encephalitis was different from lethargic encephalitis or any other known disease. He described this new disease in a series of articles [70, 71] and summarized his observations in a 1964 monograph [72].

Dr. P.A. Petrov's publications attracted the attention of leading experts in neuroinfectious diseases. The first scientist to accept the challenge of studying VEM academically was *Aleksey Nikitovich Shapoval,* a clinician specializing in Neurovirology. By this time, Professor A.N. Shapoval achieved the status of a hero of the Far Eastern Tick-borne encephalitis expeditions (see lecture 1 on Tick-borne encephalitis). Professor A.N. Shapoval organized a series of expeditions to study VEM from 1954 to1957.

Dr. Elena Semenovna Sarmanova took part in Professor Shapoval's VEM expeditions and managed to isolate an unusual virus named *Viliuisk virus* from patients and wild rodents [73].

Since the VEM epidemic showed no signs of abating and was spreading to many areas outside of Viliuisk ulus, surveillance was expanded to cover the entire Sakha (Yakut) Republic. A 60-bed *Encephalitis Department,* entirely dedicated to servicing patients with VEM, was opened in the capital city of Yakutsk. All newly identified patients with VEM were admitted and carefully examined by a group of highly dedicated doctors led by *Afanasiy Ivanovich Vladimirtsev. The Encephalitis Department* provided long-term hospitalization for each VEM patient. Dr. A.I. Vladimirtsev's and his team's neurological assessments were mathematically accurate [74]. After 20 years at the Department, they left a rich archive of medical histories and valuable diagnostic recommendations.

Moscow expeditions to study Viliuisk encephalomyelitis. News about the expanding VEM epidemic reached *Mikhail Petrovich Chumakov.* In 1965-1969, he organized short trips by researchers from the Institute of

Afanasiy Ivanovich Vladimirtsev and Prokopiy Andreyevich Petrov

Poliomyelitis and Virus Encephalitis for consultations and in 1968 proposed to make VEM one of the main projects. He coordinated the studies with the Academy and ministries, attracted other institutions. An active group was formed in Sarmanova laboratory and immediately got to work. The program included a comprehensive study of VEM: clinical and pathomorphological characterization, new attempts to isolate the pathogen, a detailed study of epidemiology, genetics, biochemistry. Dr. E.S. Sarmanova planned to use advanced virologic methods to search for the pathogen that caused this deadly epidemic spreading throughout Yakutia.

The first urgent task was to clarify the clinical and neuropathological characteristics of VEM and to improve case detection, identification, and reporting, so that neuropathologists, epidemiologists, virologists, and biochemists could work with perfectly-identified patients. The reason for this overhaul was the suspicion that other neurodegenerative disorders could have tainted the VEM registry.

Although the clinical signs and symptoms of VEM were described in several earlier publications, significant difficulties had to be overcome before a clinical diagnosis was put on a reliable basis due to VEM clinical polymorphism at different phases of illness, the lack of reliable and specific laboratory diagnostic tests, and the presence in the region of other neurodegenerative diseases with partially overlapping clinical manifestations. The reassessment was urgently needed in order to determine the direction and mechanisms of the further spread of VEM through Yakutia. One of the main tasks

was early detection and hospitalization of patients with suspected VEM. We have developed a complete understanding with our Yakutsk colleagues. Dr. P.A. Petrov, now the Republic's Minister of Health, helped us move around this huge country - an airplane, a helicopter, a car was available to us at the very moment when we needed it.

The first few expeditions of the Institute of Poliomyelitis were successful. We have collected sufficient clinical, neuropathological, and epidemiological data to begin a discussion of the nature of VEM. Several mutually exclusive hypotheses have been proposed. The *infectious hypothesis* was based on the disease onset with an acute febrile syndrome manifested with signs of encephalomyelitis in more than 50% of patients, the presence of inflammatory changes regularly detected in the cerebrospinal fluid and the brain tissue in post-mortem studies, and the spread of the disease into previously unaffected areas of the Republic.

The traditional neurologists, on the other hand, noting an accumulation of patients with chronic VEM in the affected villages, claimed that VEM is a non-inflammatory neurodegenerative disorder similar to *Strümpell-Lorrain's spastic paraplegia.* Based on the analysis of several families from the high-incidence *Viliuisk ulus,* a hypothesis was put forward that VEM is a hereditary disease with autosomal recessive inheritance. There were numerous discussions of infectious vs. genetic hypotheses. The Institute director, Professor M.P. Chumakov, chaired the meetings. From the discussions, it was clear that further research was needed for an in-depth evaluation of each hypothesis, but by 1975-1976 progress in VEM studies had stalled. It was due to the failure of attempts to isolate the VEM pathogen. The enthusiasm of the virology team has suffered from endless discussions that included the possibility that VEM was not an infectious disease. There was a fundamental split in the working group.

American expeditions to study Viliuisk encephalomyelitis. At the beginning of our work on VEM, we were motivated by the ongoing extremely successful research on kuru in New Guinea. Americans have convincingly proven that kuru, a deadly chronic neurodegenerative disease highly prevalent among the Papuans of New Guinea [75], is caused by an infectious agent. The clinical and pathological characteristics of kuru have nothing in common with VEM, but both are fatal disorders affecting ethnically unique isolated populations, and both show a tendency to develop a chronic course. Thus, some of the same research methods can be used.

D. Carleton Gajdusek

The leading researcher on kuru, *Dr. D. Carleton Gajdusek,* who was awarded the Nobel Prize for his research, regularly lectured at our Institute in Moscow, and we enthusiastically read all publications from the Gajdusek group that were systematically sent to us. Each time in Moscow, Dr. Gajdusek asked about VEM. He wanted to see VEM patients and visit Yakutia, but the Soviet authorities would not allow a foreigner to go that far into Siberia, no matter how much M.P. Chumakov begged them. In May 1976, Dr. Gajdusek was shown three VEM patients who were brought to Moscow for this purpose. This patient demonstration only provoked his interest in seeing more. Having received the Nobel Prize, he became the world expert in the field of the so-called *slow viral infections. "Slow"* is an infection with a long incubation period (years) and mainly a chronic course. Dr. Gajdusek made fundamental discoveries in this area.

In the summer of 1979, as a participant of the *XIV Pacific Scientific Congress* in Khabarovsk, Dr. D.C. Gajdusek quietly signed up and paid for a pre-Congress tour to Yakutsk. Without saying a word to anyone about his real purpose, he arrived in Yakutsk on a three-week visit. At a time when our own work on VEM seemed to be stuck in impassable failures and disagreements, when we did not know what our next step should be, the arrival of this energetic, full of ideas scientist was a gift. By this time, we accumulated complete data, well analyzed and sharpened in numerous discussions.

After seeing dozens of VEM patients in many affected villages, the Encephalitis Departments in Yakutsk and Viliuisk and reading through the accumulated materials, Dr. Gajdusek summed up his impressions as follows: *"The finding of extensive meningeal and leptomeningeal inflammatory response, of extensive perivascular cuffing and inflammatory reaction in the brain parenchyma, of necrotic foci with lymphocytic infiltrates, as well as of mi-*

croglial and astroglial activation – and even secondary hydrocephalus from the choroid and meningeal inflammatory responses – is almost certainly a sign of a chronic infection, and this differentiates VEM completely from SSVE's (subacute sclerosing virus encephalitis) *and from ALS/PD* (amyotrophic lateral sclerosis and parkinsonism-dementia complex) *as we know these diseases*". And he continued: "*I cannot imagine any other than infectious etiology in such a disease... If it is a virus infection, it is surely a new one, and it demands a more intensive laboratory study*" [76].

Professor M.P. Chumakov arranged a meeting to listen to Dr. Gajdusek's impressions of his trip to study VEM. During our previous heated discussions at the Institute, Chumakov tried to remain objective without taking sides. Now he wanted to hear an independent opinion. Dr. Gajdusek expressed himself even more distinctly than during the Yakutsk trip: *"the acute phase of VEM is a full-blown encephalitis, and not a "flu-like episode." Irritation of the meninges, generalized seizures, impaired consciousness and muscle spasticity indicate a specific infection, encephalomyelitis, which damages the brain stem functions. Subacute and then chronic phases follow acute encephalomyelitis*". Dr. D.C. Gajdusek was an expert of the highest level (could not be higher!), and he had time to see patients and understand the problem. His conclusions made a strong impression on Chumakov. We needed to set up a close collaboration with Dr. Gajdusek and his group in order to redirect VEM research and achieve decisive results.

Since 1991, full-scale research on VEM was conducted at the *Institute of Health* [77]. Work concentrated on creating a database of VEM patients, performing a retrospective analysis of case histories, examining members of the affected families, and the entire population of affected villages. An enthusiastic group of scientists led by *Vsevolod Afanasyevich Vladimirtsev* and *Fyodor Alekseyevich Platonov* accomplished critical research. The first Institute director, *Vasiliy Prokopyevich Alekseev,* conducted a comprehensive analysis on geography of VEM [78].

With the collapse of the USSR in the early 1990s, the Sakha (Yakut) Republic gained some independence from Russia. Dr. Gajdusek's group used the chance to return to Yakutia for additional VEM studies and made eight summer trips (1992, 1993, 1996, 1997, 1998, 2000, 2004, and 2006) for 3-4 weeks each [76]. *Drs. Ralph Garruto, Martin Zeidler, Neil Renwick, Howard Lipton, Eduardo Dueňas-Barajas, Colin Masters, Alison Green, Richard Knight,* and *Fusahiro Ikuta* participated in the expeditions. Researchers from Yakutia had the opportunity to visit our laboratories in Bethesda.

Clinical studies of Viliuisk encephalomyelitis

Particular attention was paid to the earliest detection and identification of patients with suspected VEM. High fever and severe headache in a resident of an endemic village were sufficient reasons to consult a neurologist. Most district hospitals were staffed by neurologists trained in VEM diagnostics. Patients with developing neurological symptoms were immediately taken to one of the specialized hospitals: *The Neurological Department of the Viliuisk District Hospital* (from 1959 – *Viliuisk Psychoneurological Hospital*) or *the Encephalitis Department in Yakutsk.*

Groups of neurologists and epidemiologists from Moscow and Yakutsk periodically visited each settlement in the endemic zone to identify new, examine known patients, and conduct epidemiological studies.

Disease manifestations. The analysis of the VEM clinical manifestations in the current study is based on a fully verified computerized database of VEM patients. All clinical and morphological studies were carried out in accordance with clinical protocols approved by the *Institute of Health of the Republic of Sakha (Yakutia)* and the *U.S. National Institutes of Health.*

Sudden onset and development of the disease within one to several days was observed in 62% of patients with definite VEM. The first symptoms of the disease were chills, fever, headache, blurred vision, muscle pain, and confusion. Headache, aggravated by movements, bright light, and noise, persisted from several weeks to 3-4 months. Approximately half of patients with acute onset of the disease had impaired consciousness of various degrees: deep coma, stupor with absent or incomplete contact with the patient, psychomotor agitation with disorientation, or an indefinite degree of impaired consciousness, described as confusion or lethargy. In some cases, acute illness with fever and headache occurred after one or more generalized tonic-clonic seizures or an acute psychotic episode. Meningeal symptoms were observed from the first day of the disease - photophobia, stiff neck, *Kernig's* and *Brudzinsky's* symptoms, pleocytosis and increased protein concentration in the cerebrospinal fluid.

Cranial nerve dysfunction, including ptosis, double vision, strabismus, convergence insufficiency, and failure of accommodation, were detected within the first few days of illness. Spastic quadri- or lower paraparesis became apparent after 3-4 weeks of observation. Cerebellar ataxia has been reported in a number of cases but has never been a leading neurological sign. Patients remained in a state of low awareness from a few days to

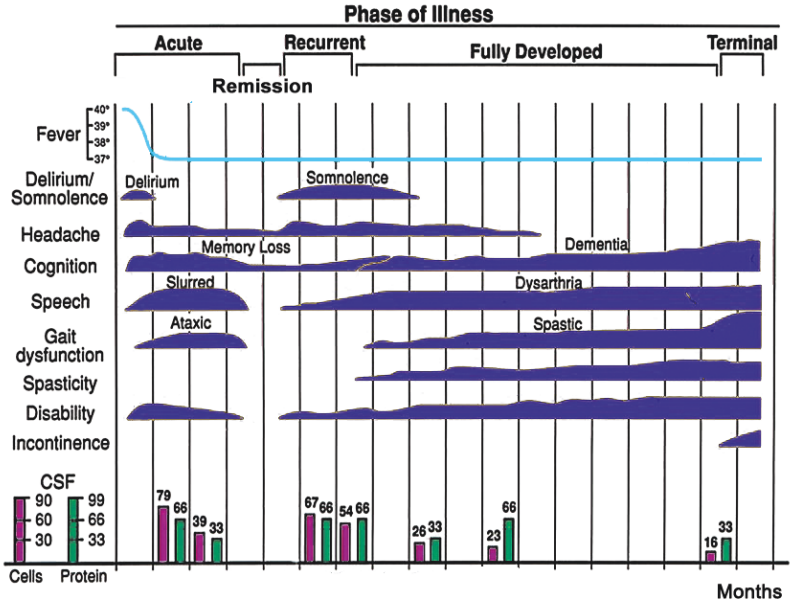


*Fig. 11. Clinical chart of a patient with slowly progressive (subacute) variant of Viliuisk encephalomyelitis. A 38-year-old patient was admitted with delirium, fever, chills, headache, myalgia, nausea, and frequent vomiting. He was mentally and physically slow, shaky, with stiff neck muscles and Kernig's sign. At the end of the tenth week, he recovered enough to return home. After several weeks of relative stability, his speech became slurred, he experienced memory lapses, had hyperactive deep tendon reflexes, bilateral Babinsky sign, decreased muscle strength mainly in the distal lower limb muscles, and a noticeably spastic gait. Within several months, the patient's cognitive abilities further declined, his speech became dysarthric, and he could walk with great difficulty due to spastic paraparesis. Urinary incontinence was another feature. The disease progressed steadily, and he died 17 months after the disease onset. Lymphocyte count in the CSF was consistently abnormal throughout the illness. *CSF – cerebrospinal fluid*

several months. Body temperature remained at a high level for an average of 10 days, after which a sustained low-grade (37.1 to 38°C) fever persisted for additional several weeks and, in some cases, for several months.

Three clinical variants of VEM were identified, depending on the rate of disease progression. The *acute form* of VEM is characterized by prolonged fever (from several days to several months), severe headache, depressed level of consciousness, meningeal symptoms, and persistent neurological impairment, including bradykinesia, muscle stiffness, dysarthria, hyperactive deep tendon reflexes, spastic paresis and in some cases generalized tonic-clonic seizures. Patients often complained of paresthesias, excruciating burning pain in the limbs, a sensation of vibration, and passage of electric current. The disease steadily progressed with no remissions; all patients died within 1–12 months. Inflammatory changes in the cerebrospinal fluid - pleocytosis (up to 660 cells per mm^3, predominantly mononuclears) and increased protein concentration (up to 330 mg per dl) were seen in all but one patient.

Those who survived the acute phase of the disease developed *subacute VEM*. The signs of the acute process gradually subsided, followed by a short remission. The remission lasted from 2 to 12 months (an average of 6.1 months), but there was no full recovery: patients remained depressed, indifferent, and passive. They complained of pain in the limbs, awkwardness when performing complex motor acts, drowsiness, and impaired memory. Patients tried to return to professional activities, or to perform chores at home but were limited in their ability to work. Behavioral abnormalities were observed. Many had difficulty pronouncing polysyllabic words and impaired fluency when asked to read quickly.

After remission, a progressive neurological syndrome characterized by dementia, dysarthric speech, pyramidal quadri- or lower paraparesis and associated bradykinesia, postural instability, and cogwheel rigidity slowly developed. Most patients in this group had a characteristic sequence of phases. A clinical chart reflecting this sequence in a typical patient is presented in Figure 11.

Cognitive decline was evident in 79% of patients, and another 17% complained of memory loss. Bradykinesia and muscle stiffness, postural instability, pyramidal insufficiency with hyperactive deep reflexes, and characteristic spastic-paretic gait were determined in 100% of

patients, a decrease in muscle strength and restriction of active movements in 77%. Progressive dysarthria was observed in 87% of patients. Cerebellar symptoms were rare. Muscle stiffness and spasticity steadily progressed. Some patients showed hypothalamic involvement.

Further down the line, patients became even more deeply disabled. Their movements lost smoothness, confidence, remained incomplete and aimless. Patients looked helpless even in familiar situations and habitual actions, acquiring a characteristic appearance and behavior, defined by the residents of the affected villages as *"bohooror"* - stiffness. Sluggish, obese, with an expressionless face, they remained for a long time in calm complacent mood. The emotional reaction caused by some effort would not subside for a long time. However, patients retained physical strength and could walk a fairly long distance, despite the pronounced spasticity and muscle rigidity. In the developed or late stage of the disease, dysphagia was often noted.

Cerebrospinal fluid studies showed lymphocytic pleocytosis and elevated total protein; the number of cells remained at an average level of about 30 in 1 mm^3 for a year or longer, subsequently decreasing, but not normalizing up to 72 months after the disease onset. Neuroimaging revealed communicating hydrocephalus, the lateral extension of the third ventricle, diffuse cortical atrophy in the frontoparietal-temporal areas. Electroencephalographic recordings showed a diffuse lowering of electric potentials, mainly in the frontal and parietal regions with no pronounced hemisphere asymmetry. The alpha rhythm was replaced by low-amplitude, irregular beta rhythm with superimposed polymorphic slow waves. In some of the acute and subacute cases, ongoing epileptic activity was observed.

The terminal stage is characterized by profound disability, global dementia, immobility, often anarthria. Limb muscle contractures, pressure sores, and renal failure were usually present. Death occurred as a result of the progression of the underlying disease spreading to vital parts of the brain and causing respiratory and circulatory failure about 13 to 72 months after the disease onset.

In about every other patient with a subacute form of VEM, the disease progression stopped at some point, leaving the patient severely disabled over the next 20-30-40 years. *This is the chronic form.* These patients have the same signs and symptoms as patients with the subacute form: intellectual decline, difficulty with pronouncing words, problems with walking, bradykinesia, but in a milder mode. The progression of symptoms is very slow, with long periods of stabilization. Exacerbations can occur periodically, but the terminal stage, characteristic of subacute VEM, is absent. Patients with chronic VEM died from intercurrent infection, trauma, pneumonia, or renal failure.

Finally, in a significant group of patients (38% in the database) the disease began unnoticed. Patients themselves or family members could not name the day, week, and often the month when the disease began. They could not recall an acute episode preceding their chronic condition. Was it due to memory impairment or because the acute phase was too mild to be remembered? The first signs of the disease in these cases were fatigue, muscle weakness, pain in the limbs, shuffling gait. Many had difficulty pronouncing polysyllabic words and impaired speech fluency. Later, the characteristic VEM syndrome with intellectual decline, speech disturbance, bradykinesia, muscle rigidity, and spastic gait became obvious. The clinical criteria for the delate-onset chronic form of VEM are the same as for the acute-onset chronic form of VEM.

The ratio between the acute, subacute, and chronic forms of VEM has changed during the epidemic, a significant but poorly understood phenomenon. Acute and subacute forms of VEM dominated in the 1950s [72, 79]. At the height of the epidemic, in the 1970s, 49% of patients had acute or subacute VEM, 26% developed a chronic form after the acute/subacute phase of the disease, and 25% had a delate-onset chronic form of VEM. In comparison, among patients in the 1980s and 1990s, only 7% had acute/subacute VEM, 43% developed a chronic form after the acute/subacute phase, while 50% had a delate-onset chronic form of VEM, significantly exceeding the frequency of the latter form in the 1970s ($P < 0.01$). The overall duration of illness significantly increased, probably due in part to improved patient care and extensive therapeutic interventions in the acute and subacute phases of illness, which has reduced mortality from complications such as pneumonia and renal failure.

Diagnosis. A multilevel classification of chronic VEM proposed in the early 1960s [74] included diverse clinical forms, such as Parkinson-like, diencephalic, pseudo-neurotic, etc. The widening of definitions contributed to erroneous inclusion in the VEM database of cases lacking the "nuclear" VEM syndrome [80]. The result was clogging of the VEM database with unrelated disorders such as Parkinson's disease, schizophrenia, amyotrophic lateral sclerosis, and vascular dementia [81], while intensive attempts to uncover the etiology of VEM should have been based on reliably diagnosed cases.

Improved clinical criteria for VEM were first proposed in 1965, slightly modified in 1971, and then periodically reviewed [82, 83]. We constantly dreamed of a laboratory test that would help to specifically identify VEM among other similar conditions. The detection of *IgG* produced by individual clones of plasma cells in the cerebrospinal fluid has become a helpful auxiliary test for the diagnosis of VEM. A study of oligoclonal IgG in VEM patients was initiated by *Alison Green* and *T.M. Sivtseva* [84]. The method is based on the electrophoretic separation of proteins at an isoelectric point in a gradient created by ampholines *(isoelectric focusing),* followed by immunoblotting with antibody against human IgG. IgG synthesized by individual clones appears on the electropherograms in the form of reinforced transverse bands on a common polyclonal background.

On the first attempt, cerebrospinal fluid and blood serum from 18 patients with reliably diagnosed subacute or chronic VEM and 15 patients with other neurological diseases, including 10 with non-inflammatory neurological disorders, were studied. Characteristic oligoclonal bands were detected in the cerebrospinal fluid, but not in the blood serum, in 17 of 18 patients with definite VEM. Intrathecal production of oligoclonal IgG was stably present up for 20 years after the disease onset [84]. The test was used for the differential diagnosis of VEM with non-inflammatory degenerative neurological diseases, such as Hereditary Spinocerebellar ataxia and Spastic paraplegia. The diagnostic sensitivity of the test is 93% and the specificity 80% [85].

In a later study [86], all 34 patients with definite chronic VEM showed the presence of oligoclonal bands, while in the group of diagnostically doubtful patients (pleocytosis in the cerebrospinal fluid in 28.6%, dementia in 62.5 %, dysarthria in 45.8%, dysstasia in 8.3%, no dysphagia, extrapyramidal rigidity in 29.2%, muscle atrophy in 4.2%, cortical atrophy on MRI in 63.6% and an average disease duration of 28.6 years) oligoclonal bands were not found.

The database of patients with definite Viliuisk encephalomyelitis. Since 1971, a designated team of neurologists and epidemiologists from Yakutsk and Moscow reviewed each case of suspected VEM using improved clinical criteria and recommendations for differential diagnosis. In recent years, all patients with suspected VEM have been tested for the presence of oligoclonal IgG in the cerebrospinal fluid. For each patient with a confirmed diagnosis of VEM, a standard four-page clinical chart was filled out that documented the patient's symptoms during the acute, subacute and chronic phases of the disease, as well as the results of laboratory, epidemiological, genetic and post-mortem studies. The charts were bound into a directory that was made accessible to every researcher. Anyone who intended to conduct studies on VEM, a pathologist, epidemiologist, virologist, geneticist or a biochemist, had to look up the directory in order to select reliably diagnosed cases. For several decades after the "publication" of the directory, as far as I know, only one diagnostic error was detected. The directory was constantly updated and subsequently computerized. Currently, the electronic database includes 356 cases of definite VEM. The diagnosis was histopathologically confirmed in 66 cases.

Neuropathology

Post-mortem studies conducted in 66 cases have become an essential basis for the identification of VEM as an independent disease entity. Several groups of morphologists simultaneously or sequentially studied the available materials, and all concluded that VEM is a unique disease with specific neuropathological characteristics that differ from all other known types of encephalitis. Doctors *A.P. Savinov, G.L. Zubri, I.A. Robinzon, and A.L. Yurovetskaya* of the Institute of Poliomyelitis characterized the unique features of the disease [87]. Professor A.P. Avtsyn and his team at the *Institute of Human Pathology* in Moscow contributed significantly to the understanding of various pathologic phenomena [88, 89]. Professor *Colin Masters* of the *University of Perth, Australia,* and his team presented convincing evidence that acute, subacute and chronic VEM are phases of the same disease with differences corresponding to the rate of progression and that inflammatory changes or their consequences are clearly expressed at each phase of the disease [90].

In cases of VEM, in which the disease progression was rapid, and death occurred within 6-12 months from the disease onset, diffusely edematous cloudy meninges were infiltrated by mononuclear, plasma, and polymorphonuclear cells. Inflammatory changes were especially pronounced in the meninges, overlying the affected cortical areas [87, 90]. Numerous micronecrotic foci consisting of eosinophilic condensed granular material *0.4 mm* in diameter, surrounded by inflammatory infiltrates, were observed throughout the cerebral cortex, basal ganglia, cerebellum, and brain stem (Figure 12A). Identical lesions were found in the anterior and posterior horns of the spinal cord [89]. Massive loss of neurons (by lysis) and dystrophic changes in preserved nerve cells inside and outside the necrotic foci were detected [90] (Figure 12B and C).

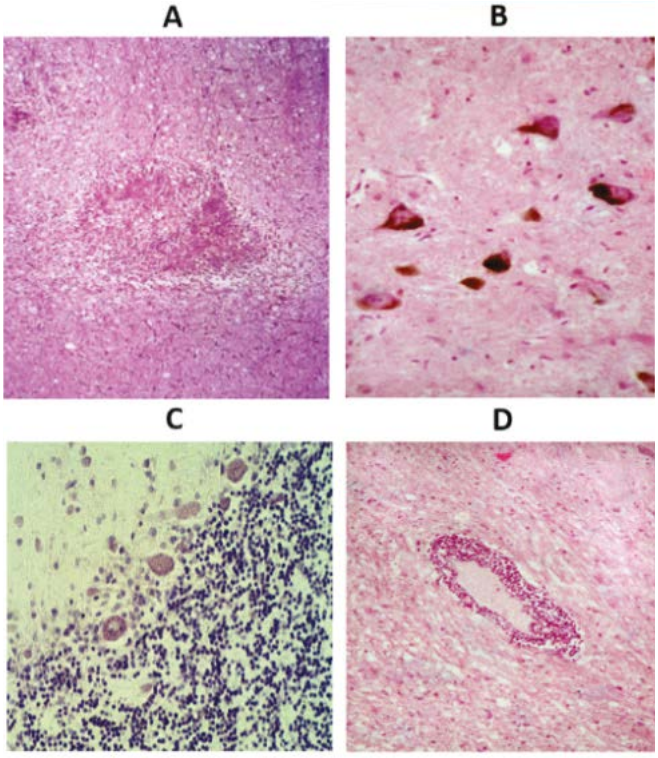


Fig. 12. Histopathological studies of acute and subacute Viliuisk encephalomyelitis. A. A fresh necrotic lesion. Cerebral cortex. B. Dystrophic changes in neurons. Subcortical nucleus. C. Lysis of Purkinje cells. Cerebellum. D. Perivascular lymphocytic cuff, spongiosis. Cerebral white matter.

Perivascular inflammatory cuffs, consisting of activated T-lymphocytes, natural killer cells and natural killer-like cytotoxic T lymphocytes with a small number of macrophages were regularly observed in the affected areas of the cortex and the underlying areas of the white matter [91] (Figure 12D). Such a composition of inflammatory cells indicates that *"a specific cell-mediated immune response that can destroy foreign materials (viruses, protozoa, fungi) by detecting infected cells and annihilating them through apoptosis may be developing in VEM"* [91]. No intranuclear or cytoplasmic inclusions or neuronophagy were detected. Electron microscopy did not disclose viral particles.

In slowly progressing subacute cases, active necrotic foci within the brain parenchyma were less frequent. Organizing lesions, morphologically similar to those in acute VEM, show central lysis of tissue and adjacent reactive gliosis [90]. The ratio of active, organizing, and lytic foci within the brain parenchyma varied from case to case. Extensive loss of neurons was observed in various areas of the cerebral grey matter [87, 89]. In some areas, there was a confluence of lesions resulting in extensive cortical destruction of all cortical laminae, reactive gliosis, and secondary demyelination in underlying white matter. Small vessels within and adjacent to the affected areas had prominent endothelial cell layers and perivascular cuffs of T-lymphocytes. Post-mortem studies confirmed clinical observations indicating that inflammatory process may last 6–7 years [90], or even 11 to 13 years [88]. Since the disease affects various brain structures and proceeds with unequal intensity, variations in morphological manifestations can be striking.

In *chronic* "burnt out" cases, the meninges were fibrous and sticky with minimal residual inflammation. Adhesions impeded the circulation of cerebrospinal fluid, which led to hydrocephalus and severe atrophy of the gray and white matter. In the cerebral cortex, active micronecrotic foci were replaced by single or confluent microcysts framed by gliosis with no active inflammation. There was a severe neuronal loss. In a case with the longest disease duration (22 years), the cerebral cortex contained only a few dystrophic neurons [89]. Scattered lytic foci were also observed in the basis pontis, pontine tegmentum, medulla oblongata, and gray matter of the spinal cord. The walls of the cerebral vessels were thick due to fibrosis and hyalinosis, and the lumens were narrowed. Circulatory failure may lead to a reduction in the microvasculature and further destruction of the brain parenchyma [77, 88, 89, 91].

According to the histopathological features, VEM differs from other diseases - Neurosyphilis, Tick-borne encephalitis, Poliomyelitis, encephalitis in rabies, Multiple sclerosis, Allergic encephalomyelitis, Prion diseases, Multifocal leukoencephalopathy, Subacute sclerosing panencephalitis, Amoebic encephalitis. Systemic lupus erythematosus, and other connective tissue disorders, Vasculitis and Antiphospholipid syndromes [89].

Pathogenesis

Resistance to cerebrospinal fluid circulation and increased intraventricular pressure occur due to blocking at the level of convex surfaces of the hemispheres or in the area of the cisterns at the base of the brain [92, 93]. Hydrocephalus with increased intraventricular pressure is observed in patients with rapidly progressing VEM; surgical treatment of hydrocephalus by the ventriculojugulostomy (ventricular catherization into the jugular vein) procedure helped to alleviate or stop further progression of symptoms in most patients [92]. In patients with chronic VEM, low-pressure hydrocephalus and expansion of the cerebrospinal fluid occur due to atrophy and a decrease in brain volume.

In all cases investigated post-mortem, the most characteristic feature was the widespread and profound destruction of neurons in the cerebral cortex. Accordingly, patients had clinical dementia of varying degrees. The combination of pyramidal, extrapyramidal, and cerebellar symptoms is explained by the presence of lesions in the subcortical gray matter formations – the substantia nigra, pontine nuclei, inferior olives, the reticular formation, the cerebellum, and the spinal cord. The severity of the changes varied from case to case.

Patients with chronic VEM demonstrate persistent inhibition of the production of interferons by leukocytes, in particular, IFN-α [94]. Changes in the physicochemical properties and lipid composition of the plasma membranes of lymphocytes, which maintain their stability, were also identified [95]. The ratio of immunocompetent cells in the peripheral blood of patients with VEM shows a relative scarcity of T-cells.

Intense and, according to some investigators, excessive inflammation in the brain may be influenced by defective genes that support a prolonged inflammatory response. The genotyping methodology has been applied to test for an association between the development of VEM and a set of markers in pro-inflammatory genes. A study led by Dr. *Taras Oleksyk* at the *Cancer Institute of the National Institute of Health* assessed the role of several candidate genes, *CCR2, CCR5, IFNG, IL4, IL6, IL10, SDF* and *CCL5,* known as modulators of inflammatory response [96]. A link was found between VEM and the rare variants *rs2069718* and *rs2069727* in the interferon-gamma *(IFNG)* gene [97]. The association between VEM in patients aged 60–69 years with marker *rs2069718* was significant as compared with unrelated controls of the same age *(P = 0.04)*. All patients at this age had chronic VEM, which suggests that the *IFNG* isoform supports (or does not protect against) a prolonged inflammatory process. Although marker *rs2069718* is located in the *IFNG* intron, it appears that the nucleotide substitution at this site may affect protein function. Similar results were obtained in the study of rheumatoid arthritis: another intron *IFNG* marker showed an association with the rate of disease progression [98]

The protein product of the *IFNG* gene, cytokine interferon-gamma *(IFN-γ),* plays a vital role in the activation and modulation of innate and adaptive immunity and protection against viral and bacterial infections. The concentration of *IFN-γ* in the cerebrospinal fluid of patients with VEM is lower than in patients with other inflammatory and non-inflammatory neurological diseases, and much lower than in patients with multiple sclerosis [99, 100]. Identification of the link with rare variants in the *IFNG* gene and the insufficient concentration of *IFN-γ* in the cerebrospinal fluid, as well as the detection of intrathecal oligoclonal *IgG* production at the time when the inflammatory process is most active, characterize VEM as an *immune-mediated inflammatory disease.*

LECTURE 5. Viliuisk encephalomyelitis: differential diagnosis

To determine the range of diseases with clinical features that overlap with VEM, a census of patients with chronic neurological diseases was conducted in 1971 throughout the Republic with the completion of an individual questionnaire for each patient [81]. Questionnaires for 711 patients completed by local doctors and neurologists were returned. Most of these patients were examined on subsequent visits to the villages by research teams from the Yakutsk Encephalitis Department and the Institute of Poliomyelitis.

Autosomal dominant spinocerebellar ataxia. Among the patients who were ultimately excluded from the VEM database, there was a large group of patients, each of whom had progressive cerebellar ataxia of the limbs and trunk, dysarthria, and variably expressed pyramidal symptoms. The family analysis revealed a classic autosomal dominant type of inheritance. Spinocerebellar ataxia (SCA), as it turned out, was widespread in the Sakha (Yakut) Republic. Dr. *Fyodor Alekseyevich Platonov* conducted a comprehensive study of this disease in the Sakha population. According to his estimates, the SCA prevalence among the Sakha population is reaching the world's highest level [101, 102]. Each year, 4 to 29 patients are newly diagnosed, and 1 to 13 patients die. Three geographic clusters of SCA have been identified [103]. In the Indigirka Valley cluster, corresponding to *Abyyskiy* and *Allaikhovskiy* districts, the prevalence of SCA is currently 585 per 100 thousand; the second cluster, located in the Lena-Amga Valley and overlapping the *Ust-Aldanskiy* and *Tattinskiy* districts has a prevalence rate of 59 per 100 thousand; the third cluster in the upper reaches of Viliui and Lena Rivers, coinciding with the territories of the *Suntarskiy* and *Lenskiy* districts, shows a prevalence rate of 41 per 100 thousand. Most of the patients found elsewhere had family relationships with people from one of these three clusters.

In the early 1990s, genetic technology became available to characterize Siberian ataxia further. The chromosomal location of the gene responsible for ataxia in the Sakha people was studied in Bethesda with the active

participation of Dr. *Astrid Lunkes* from the *University of Dusseldorf, Germany,* who at that time was an intern in our laboratory. Initially, a link was established between the putative Sakha ataxia gene and the *D6S274* genetic locus, which made it possible to localize the mutant gene to human chromosome 6p22.3 [104]. We then collaborated with researchers from the *University of Minnesota* who studied ataxia families from Germany, England, South Africa, and the Netherlands, all linked to genetic loci on chromosome 6p22.3. They identified the *ATXN1* gene and the mutation responsible for ataxia in all studied families [105, 106]. The disease was named *Spinocerebellar ataxia type 1 (SCA1).*

The non-mutant *ATXN1* gene in the Sakha population contains a chain of 25 to 32 three-nucleotide CAG repeats that are interrupted by one or two CAT triplets. In patients with SCA1, the number of CAG repeats increases sharply to 39-72, and the CAT bridge disappears. An uninterrupted chain of CAG repeats lacking CAT complexes becomes unstable: the number of repeats can increase or decrease during meiosis. The *ATXN1* gene mutation was detected in each Sakha patient diagnosed with SCA1.

The age of SCA1 onset ranged from 15 to 57 years and closely correlated with the number of interrupted CAG repeats in the coding region of the mutant *ATXN1* gene (multiple correlation coefficient $R = 0.846$; $P < 0.0001$) [107]. The three youngest patients (two 15-year-olds and a 19-year-old) had the highest number of CAG repeats (58, 60, and 72), while patients with the late-onset disease (45 to 57 years) had the lowest CAG repeat numbers, from 39 to 45 (Figure 13).

In typical cases, SCA1 is a chronic neurological disease manifested by progressive cerebellar ataxia. The gait becomes unstable, with legs positioned wide apart. Early oculomotor symptoms include nystagmus and hypermetric saccades. As the disease progresses, other cerebellar signs become apparent, such as locomotor asynergy with rhythmic intentional tremor, diadochokinesis, and muscle hypotension. Speech becomes slow and explosive. Pyramidal signs - spasticity, hyperreflexia, and extensor plantar responses – were observed in 60% of the studied patients. Bulbar symptoms, notably dysphagia, tongue atrophy and diffuse skeletal muscle atrophy with fasciculations, become pronounced in the relatively late stages of illness. In 15 of 22 patients with the number of CAG repeats equal to or greater than 52, lower motor neuron involvement severely complicated the course of illness, and in two patients led to early respiratory death [108]. Clinical characteristics of SCA1 correlate with the degree of degeneration of the Purkinje cells in the cerebellum and neurons in the cranial nerves' nuclei in the brainstem.

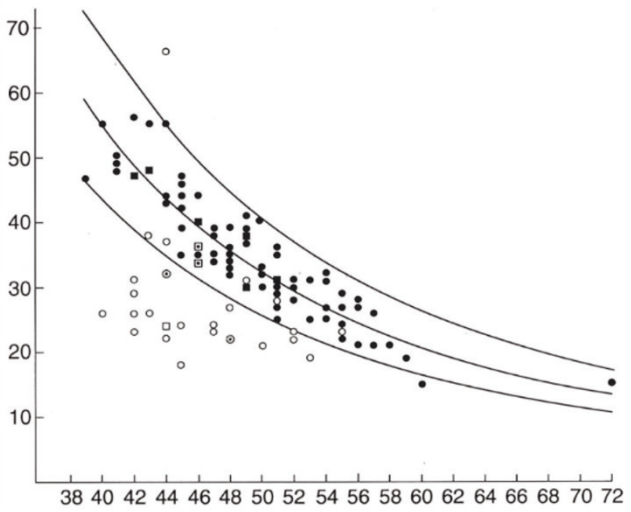


Fig. 13. The age of onset of Spinocerebellar ataxia type 1 and the number of continuous CAG repeats in the mutant ATXN1 gene (filled circles, n = 77). The curves show the average age of SCA1 onset and the 95% confidence intervals. The number of CAG repeats in asymptomatic individuals is shown relative to their current age (unfilled circles, n = 21). The point inside the square or circle marks homozygous individuals.

A negative correlation was established between the number of CAG repeats and the duration of illness ($R = -0.58$, $P = 0.0008$), as well as with the age of death ($R = -0.81$, $P < 0.001$) [101, 102]. In addition to the overall increase in the incidence of SCA1 in the Sakha population, it is alarming that the morbidity has shifted to younger age groups, given that in cases of early-onset the disease progresses faster. The shift of morbidity to younger age groups is due to an increase in the number of CAG repeats during transmission from one generation to another, particularly via paternal transmission.

Since 1994, every new patient suspected of having SCA1 has undergone genetic testing, which was carried out at the *Clinical Neurogenetics Unit, National Institute of Neurological Disorders and Stroke, National Institute of Health in Bethesda, Maryland,* and later at the laboratories of the *Institute of Health, North-Eastern Federal University, Yakutsk,* and the *Research Center, Siberian Branch of the Russian Academy of Medical Sciences in Yakutsk.* A genetic test demonstrating the loss of a CAT bridge and an increase of the CAG repeat number in the *ATXN1* gene provides convincing evidence for the diagnosis. Patients with Viliuisk encephalomyelitis do not have mutations in the *ATXN1* gene.

Genetic counseling for patients with SCA1 and their families has become an essential element of prevention of the further spread of SCA1 in the Sakha community; more than 1800 genetic tests have been performed [109]. Prenatal testing is offered to individuals at increased risk of transmission of the mutant *ATXN1* gene [110]. If prenatal testing is done early enough and the risk of developing SCA1 in adolescence or early adulthood is high, it is recommended that the prospective parents receive all the necessary information and the opportunity to make a decision about medical abortion. Of the 48 women who sought help, 12 showed a fetal *ATXN1* gene mutation. Ten families decided to terminate the pregnancy. The fertility rate in women carrying a CAG repeat number of less than 50 does not differ from the average fertility rate among the rural Sakha population, therefore the *ATXN1* gene mutation has little chance of being eliminated by natural selection [102].

An attempt was made to establish the origin of the *ATXN1* gene mutation in the Sakha population based on the comparison of haplotypes. The results indicate that patients from the three affected regions of the Sakha (Yakutia) Republic have the same haplotype, which confirms the origin of the mutant *ATXN1* gene from a common ancestor. The Sakha haplotype differs from the haplotypes in SCA1 patients from Mongolia, China and the USA. Haplotype-based calculations show that the mutation may have occurred no less than 915 years (37 generations) ago [101, 111]

Autosomal dominant spastic paraplegia. The VEM phenotype at a late stage of the disease acquires features of a neurodegenerative condition. Some of our colleagues, believing that VEM is a genetic disease, have tried to convince us that VEM is a variant of *Strümpell-Lorrain's* spastic paraplegia [112]. Family history in one hotly disputed case, initially diagnosed with chronic VEM, did indeed show an autosomal dominant pattern of inheritance, unlike all other families with typical VEM. Soon, several other families with severe spastic paraplegia, but without dementia, dysarthria, or extrapyramidal symptoms characteristic of VEM, were also identified. Studies conducted by Drs. *A.P. Danilova* and *T.M. Sivtseva* provided a description of six families with 26 fully characterized patients. All had slowly progressive spastic paraplegia.

One of these Sakha spastic paraplegia families with nine patients in four generations was studied using novel genome-wide exome sequencing technology. The disease in each of the nine family members had an insidious onset at the age of 10 to 37 years (mean, 26 years) with spastic gait and muscle stiffness in the lower limbs. Further progression led to severe bilateral weakness in the lower extremity's muscles. Three patients died after an illness lasting from 23 to 32 years. The clinical picture corresponded to upper motor neuron dysfunction; only late in the course of illness did symptoms suggestive of mild sensory changes and distal muscle atrophy became apparent.

Our molecular, functional, and molecular-structural studies have identified a p.R719W mutation in the *Dynamine-2 (DNM2)* gene as the cause of this disease [113]. The mutation results at the protein level in a replacement of arginine with tryptophan, potentially disrupting synthesis of *Dynamine-2.* Modeling in cell cultures showed the destructive effect of this mutation on endocytosis in neurons [113]. This was the first time that the role of *Dynamine-2* was implicated in the pathogenesis of Hereditary spastic paraplegia. None of the other Sakha spastic paraplegia families show a mutation in the same gene [114].

This study demonstrated that another hereditary disorder, Hereditary spastic paraplegia, analogous to what in the past was called *Strümpell-Lorrain's* disease, is prevalent in the Sakha population. In one of the identified families, the disease was caused by a mutation in the *DNM2* gene. *DNM2* gene mutations have not been identified in patients with definite VEM.

Viral encephalitis. The northern boundary of *Ixodes persulcatus* range within Yakutia, according to E.I. Korenberg et al. [115], is between 59 and 60° N. During our 1998 expedition to study patients with VEM in the village of Asyma in Gorny ulus (62°25' N), we were approached by a woman who was attacked by a tick. The tick was identified as a female *Ixodes persulcatus* [116]. As mentioned above, the pathological characteristics of acute Tick-borne encephalitis are significantly different from VEM, but chronic TBE can have various presentations. Mass seroepidemiological screening of 61 patients with VEM [117] for 46 known viruses, protozoa, and rickettsia excluded the role of *TBEV.*

As a result of these efforts, diagnostic and differential-diagnostic criteria for VEM were developed, various neurodegenerative diseases not associated with VEM were identified and excluded; the current electronic database includes 356 cases of reliably diagnosed VEM.

Search for the pathogen

E.S. Sarmanova and *G.G. Chumachenko* [73] isolated a viral agent by inoculating experimental white mice with tissues from VEM patients. One of the virus strains obtained from the cerebrospinal fluid of a patient

with chronic VEM, designated as *V-1 (Viliuisk virus-1),* strongly cross-reacted with *Theiler's Murine encephalomyelitis viruses (TMEV)* and weakly with murine *Encephalomyocarditis virus (EMCV),* both of which belong to the genus *Cardiovirus* in the *Picornaviridae* family [118], natural saprophytes of mice. In subsequent studies, a dilemma arose whether the *V-1 virus* was indeed the causative agent of VEM, or if the isolated strain was contaminated with murine viruses during the isolation process [119].

Howard Lipton's group, known researchers of *TMEV* viruses, tried to solve this problem. To determine whether the *Viliuisk virus* is a human pathogen or a mouse contaminant, they sequenced the viral genome. The nucleotide sequences of the *Viliuisk virus* in some critical regions, especially in the capsid, were only 66% homologous to the classical representatives of *TMEV,* while the similarity of the known *TMEV* strains to each other was at least 90% [120]. These differences indicated that the "*Viliuisk virus*", renamed "*Viliuisk human encephalomyelitis virus*" *(VHEV),* is a separate *Theilovirus* clade, the ancestor of a new group of "*human TMEV"* viruses within the *Theiler's* group [121].

Interest in the *Viliuisk virus* has recently increased in connection with the discovery of a new human *Theilovirus, the Saffold virus,* isolated from children with diarrhea and respiratory diseases in Canada, Germany, Brazil, China, and Japan [122], as well as from children with polio-like flaccid paralysis in Pakistan and Afghanistan [123]. Another variant of the *Saffold virus (SAFV 2)* was isolated from the cerebrospinal fluid of two children with an apparent acute central nervous system infection, one of which ended in death [124]. Numerous new variants *(SAFV 3-11)* were isolated from stool samples of children with gastroenteritis and respiratory diseases; epidemiological studies have shown that the *Saffold virus* circulate in human populations, and children become infected from a very young age [125, 126].

The discovery of the *Saffold viruses* that belong to the same group of *Theiloviruses* as the Viliuisk virus, and the established pathogenicity of the Saffold viruses for humans increased the likelihood that the *Viliuisk virus* was indeed isolated from patients with VEM and was not a product of contamination, as previously thought; it may have recombined when exposed to murine virus population [127]. However, since patients with VEM do not produce neutralizing antibodies to the *Viliuisk virus,* it's etiological role in VEM remains uncertain [128, 129].

Another attempt to detect the VEM causative agent was made in the 1970s using materials from VEM patients, including blood, cerebrospinal fluid, and brain tissue taken during autopsy and biopsy. A new candidate agent, designated *KPN virus,* was first detected by indirect immunofluorescence using VEM patients' sera [130]. The agent was sensitive to lipid solvents (ether, chloroform), sodium deoxycholate, and formaldehyde. *KPN* caused low-grade encephalitis after a long incubation period in Rhesus monkeys. Low titers in tissue culture and in the brains of experimentally infected animals made its further characterization difficult.

K.M. Chumakov and *A.S. Karavanov* [131] demonstrated that the *KPN agent* had been mistakenly thought to be a virus. The purified *KPN* contained DNA, RNA and ribosomes, suggesting that it was more complex than a virus. The 5S-RNA sequences were identical to those of *Acanthamoeba castellanii,* a free-living amoeba. This type of amoeba lives in fresh lake waters, soil, and wastewater. It causes primary *amoebic meningoencephalitis* in immunocompromised individuals, a slow protracted disease defined histologically as multifocal subacute or chronic granulomatous encephalitis. Attempts to re-isolate *KPN* or other pathogens from 13 brain biopsies taken from patients with chronic VEM during surgery for hydrocephalus were unsuccessful. Whether this free-living amoeba has been a chance contaminant or has come from a VEM patient's brain remains unclear.

Quest for VEM etiology remained intense and persistent. In his laboratory in Frederick, Dr. Carleton Gajdusek allocated staff, laboratory facilities, and dozens of experimental animals, including primates [132]. He himself inoculated animals with materials from VEM patients. The serological screening was conducted for 46 known infectious agents, including protozoa, viruses, and rickettsia, in order to find indirect evidence of pathogen exposure [117]. No convincing results were obtained. Virologists at the *Institute of Virology and Biotechnology* in Novosibirsk *("Vector")* under the direction of *Alexander Alekseyevich Chepurnov* conducted pathogen identification experiments using numerous highly sensitive cell cultures. The results were negative.

Thus, a series of attempts to isolate the VEM pathogen failed. Several unique candidates of biological interest have been identified and studied, but it is evident that work on some of these isolates has not been completed. The recent discovery of the *Saffold virus,* which is pathogenic for humans and belongs to the same group as the *Viliuisk virus,* increases the interest to this candidate. Generally, the choice of experimental animals or types of cell cultures or materials for inoculation might not have been appropriate, and transportation of samples over long distances to Moscow or Washington without

understanding the conditions in which the pathogen can survive for at least a few days might have been other reasons for failure.

Genetic studies

Early researchers repeatedly entertained the assumption that VEM was a hereditary disease, based on the exceptional distribution of the disease in the Sakha population, the tendency to the development of a chronic course, and the occurrence of two or more confirmed VEM cases in some affected families. However, disagreements arose regarding the role of genetic factors in this disease. Professor *K.G. Umanskiy* [133] described several families with autosomal dominant inheritance. However, not all patients in these families were diagnosed as definite VEM.

Subsequent genetic studies focused on patients with definite VEM from 5 pedigrees in a set of villages of the Viliuisk ulus, in an area with a high prevalence of VEM. Patients were involved in genetic and non-genetic relationships. The estimated VEM inheritance coefficient calculated using the *Hogben* and *Haldane* models, adjusted for age-dependent penetrance, was 0.1968 (95% CI: from 0.0970 to 0.2966). The range included a theoretical level of 0.25 and, therefore, the result did not formally contradict the hypothesis of autosomal recessive inheritance [112]. The authors believe that VEM may be a variant of *Strümpell-Lorrain's* autosomal recessive spastic paraplegia. In a critical review of these results, Professor P.A. Petrov [134], one of the Principal investigators on the project, disputed the conclusion, indicating that data collected in a hyperendemic region with a VEM prevalence of up to 1340 per 100 thousand people cannot be reliable for segregation analysis, and that the clinical and histopathological features of VEM are inconsistent with the diagnosis of spastic paraplegia.

Indeed, in a similar study based on data from 194 families across the entire VEM distribution region, including regions along the Viliui Valley with a VEM prevalence rate of 42 per 100 thousand and Central Yakutia with a prevalence level of 30 per 100 thousand, and using the same *Hogben* and *Haldane* models, the coefficient of inheritance estimate was 0.025 (95% CI: from 0 to 0.0888), which excludes the 0.25 level and rejects the hypothesis of monogenic inheritance *($P < 10^6$)* [135]. Thus, segregation analysis did not support the conclusion of monogenic inheritance of VEM, although other genetic mechanisms could not be ruled out.

With the help of Dr. *Viktor Mironovich Gindilis,* an experienced geneticist, we evaluated another hypothesis. Viktor developed a methodology for analyzing multifactorial diseases, that is, diseases, in which the genetic predisposition plays an essential background role, while an environmental factor serves as a trigger. The contribution of genetic factors is assessed as *heritability index,* which is interpreted as the *"weight of genetic variation in overall phenotypic variation",* allowing the added effects of environmental factors. We launched a series of calculations to measure the genetic predisposition to VEM based on the threshold liability model proposed by *D.S. Falconer* [136]. The VEM heritability coefficient in the Viliuisk ulus was estimated for the first-degree (parents, siblings, and children) and second-degree relatives as 22.14% and 28.94%, respectively; these results indicate that the aggregation of VEM in affected families is partly under genetic control [135], although the genetic predisposition is small. In comparison, the heritability coefficient calculated for type 2 diabetes in China was 83.42% [137]. Still, we considered it important to evaluate the genetic contribution with newer methods as soon as they become available.

Recently, DNA exome sequencing was performed in two patients with subacute VEM and seven patients with a confirmed diagnosis of chronic VEM. No variants that could reasonably be considered responsible for the development of VEM were uncovered. Although the involvement of *INF-γ* in the pathogenesis of VEM was suspected based on genotypic and clinical laboratory studies, the coding sequences of the *IFNG* gene did not contain variants common to the nine examined patients with VEM [138].

Testing other hypotheses

Several other hypotheses on the origin of VEM have been put forward and discussed at conferences. The *geochemical* hypothesis emphasized that the soil around *Lake Mastach* and some other areas in the Viliui Valley had biochemical anomalies [139, 140]. The *ecological hypothesis* was based on the recognition that the local ecological systems of the Viliuisk ulus support the circulation of the VEM pathogen in wild and domestic animals [141]. The *immunodeficiency hypothesis* suggested that Sakha people living in regions affected by VEM may have congenital immunodeficiency, which can be triggered into a full-blown immunopathological condition by an opportunistic infection, not necessarily a VEM-specific pathogen [142].

There were many lively and productive discussions, and there was no shortage of dramatic situations when

former like-minded researchers turned into ardent opponents. Despite excessive emotions, discussions helped to get rid of unconvincing arguments, to clarify positions, and to determine the direction of further research. Work on some aspects of VEM continues to this day [86, 143]. However, the spread of VEM in genetically diverse populations living in areas with different environmental conditions, and the recent disappearance of VEM preclude rational foundations for each of these theories.

LECTURE 6. Viliuisk encephalomyelitis: epidemiology and prevention

All patients in the VEM cohort were ethnic Sakha, except for six individuals born in Tungus-Tungus and eleven in Sakha-Tungus marriages. All patients were born, and most of them lived all their lives in small villages with a population of 250 to 3000 people. The age of disease onset ranged from 11 to 68 years. There was a shift in the age of disease onset in the course of the outbreak from 30.2 (95% CI: from 27.5 to 33.0) at the beginning to 37.1 (95% CI: from 35.1 to 39.1) years at the height of the epidemic in the 1970s and remained at that level. The ratio between VEM incidence rates among women and men has also undergone a significant change in the course of the epidemic: from approximately 2:1 in the 1950s and the 1960s to 1:1 in subsequent decades. The preponderance of female morbidity coincided with a higher proportion of *acute/subacute* VEM. Patients with a known day/week of disease onset became ill in May (27%), June, July, or August (32%), which coincides with extensive outdoor activities such as hunting, fishing, mowing hay, and grazing due to which people can be exposed to adverse environmental factors that can provoke the disease onset.

Trends in incidence rates

The average annual incidence rate of VEM was 3.5 cases per 100 thousand people in the 1940s (Figure 14).

It increased to 53.7 in the 1950s and remained at a high level of 39.5 and 55.3 per 100 thousand in the 1960s and the 1970s. During the decades, when the epidemic reached a peak, 4 to 16 new cases of VEM were reported annually. The average VEM incidence rate during the decade of the 1980s decreased to 15.8 per 100 thousand people, in the 1990s to 3.6, and in the 2000s - to 0.8 per 100 thousand. No new cases of definite VEM were recorded after 2012. As of January 2018, fourteen patients were alive, all in the late stages of chronic VEM. Prevalence rates followed incidence rates with increasing delay as the duration of illness became longer.

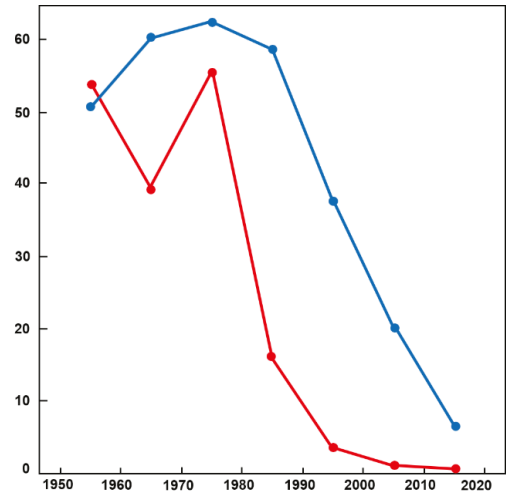


Fig. 14. The decade-by-decade incidence (red curve) and prevalence (blue curve) rates of Viliuisk encephalomyelitis among the Sakha population in 1951-2020.

Trends in territorial spread

VEM was first recorded by a traveler 150 years ago in several small villages around *Lake Mastach* located on the left bank of the Viliui River within the Viliuisk ulus [144]. The first medical description of VEM was made by Dr. *T.A. Kolpakova* [145], who conducted an epidemiological survey of these same villages in the 1920s. The population of the small settlements around *Lake Mastach* was formed as a result of a merger of nomadic Tungus clans, which previously roamed the vast expanses of the East Siberian Plateau and a small group of *"old"* Sakha tribes who have lived in this left-bank area for a long time.

The population of the right-bank villages consisted of *"new"* Sakha tribes, predominantly the powerful *Kangalass tribe* that came from the south a couple of centuries ago and did not mix with the Tungus. In the 1930s, the Soviet government forcibly resettled the population from the *Lake Mastach* area to larger villages on the right bank of the Viliui River to create more productive agricultural communities *("collective farms")*. Early researchers had no doubt that people migrating from the left bank brought this deadly disease with them [72]. The disease spread throughout the Viliuisk ulus, and by the 1950s, the incidence of VEM among the entire rural population of the Viliuisk ulus reached the level of 840 per 100 thousand per decade. Over the next two decades, VEM spread to neighboring regions along the Viliui Valley, where there

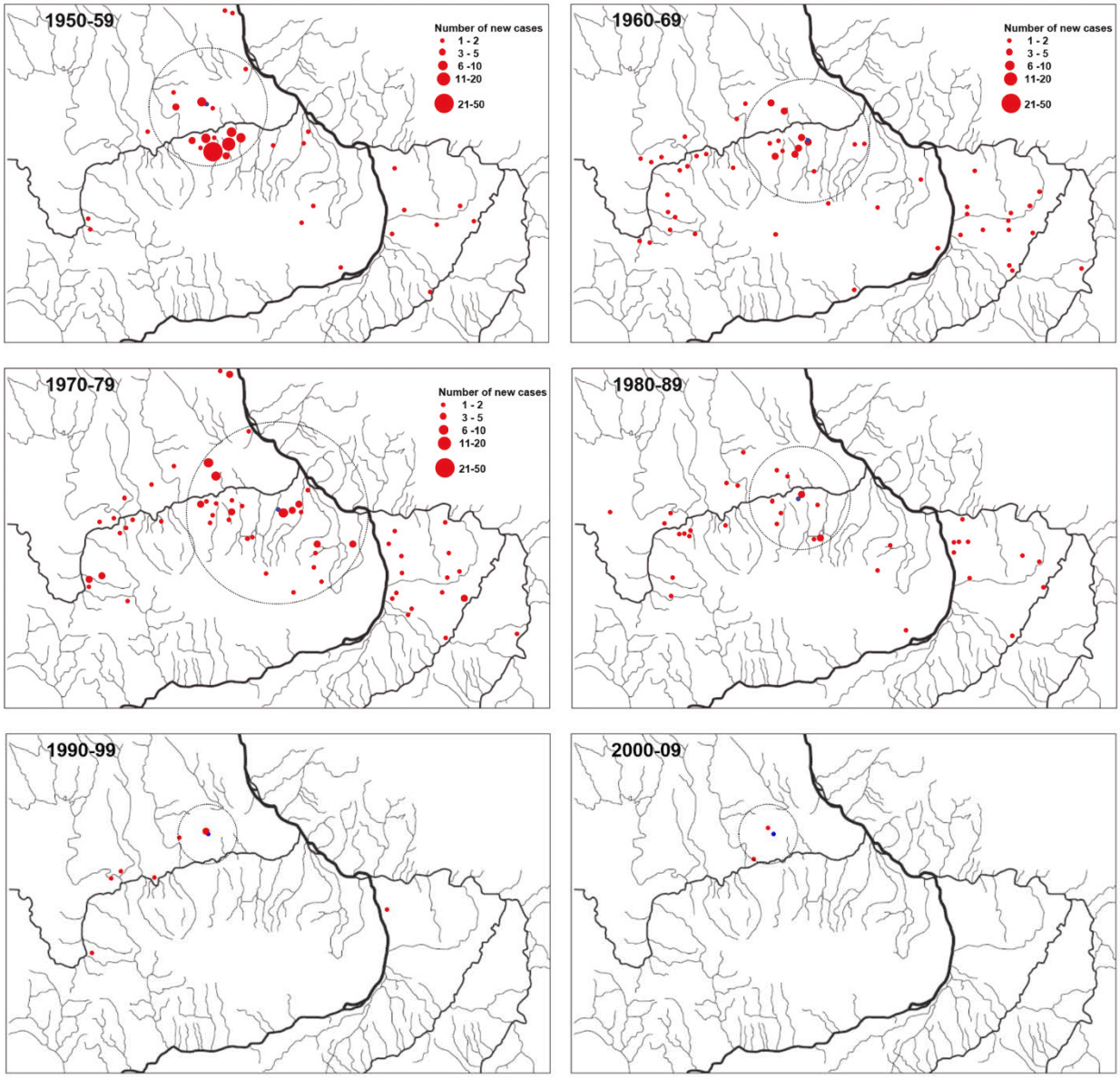


Fig. 15. Location of villages (red dots) in which new patients with definite Viliuisk encephalomyelitis have been registered, per decades. Circles delineate areas of concentration of 90% of new cases; the blue dots indicate the geographical center (Lee et al., 2010 [146], modified).

was only one known case in the 1940s, but additional 7 cases of definite VEM were registered in the 1950s, 22 in the 1960s and 26 in the 1970s, eventually reaching an incidence rate of 42 per 100 thousand (Figure 15).

With the discovery of rich deposits of coal, metals, oil, and gas, people began to move from the Viliui Valley towards the industrial towns of Central Yakutia around the capital city Yakutsk. The Soviet government allowed and even encouraged resettlement in order to develop the mining industry. The spread of VEM in the direction of the densely populated and more developed regions of Central Yakutia was occurring under our watch. With the expansion of the epidemic to the west and south, the distance from *Lake Mastach* to villages, in which later VEM cases occurred, was increasing. The geographic center of the affected area moved 142 km away from *Lake Mastach* in the 1950s, 262 km in the 1960s, and 390 km in the 1970s. The number of affected villages increased from 4 in 1940–1949 to 31 in 1950–1959 to 44 in 1960-1969 and 52 in 1970–1979. Throughout the epidemic, the affected territory increased 15-fold.

We established a chain of VEM cases in the settlements of Central Yakutia, which began with immigrants from the high-incidence Viliui districts

and subsequently involved residents who never left their native villages [146]. Physicians who practiced for many years in the districts of Central Yakutia considered VEM a new disease introduced by aliens from Viliui and called it "*Viliui disease*". There was no folk name for this disease. By the 1970s, almost the entire territory of Central Yakutia was affected.

The spread of the disease occurred during the intensive and well-documented post-war migration of people from the impoverished Viliuisk villages, and this caused a serious epidemic. As the VEM incidence decreased over the 1980s and the 1990s, the geographic center returned to the Viliuisk ulus [146].

The spread of VEM to Central Yakutia, defined as a *"secondary cluster"* [141], is reminiscent of the observations during other epidemics such as HIV infection, avian influenza, coronavirus SARS, and coronavirus COVID-19 from recent examples. The disease spreads from an endemic hot spot to a larger area, taking advantage of favorable conditions for proliferation. When conditions for further spread become unfavorable, the incidence decreases in the secondary clusters, returning to endemic areas.

An essential point of VEM epidemiology is the fact that this disease spread to Central Yakutia during the lifetime of one generation of people (1950–1979) and completely disappeared from these areas within the life of the next generation (1980–2010). This situation characterizes VEM as a transmissible disease and indicates that the environmental agent accompanied the migrating Viliui population to previously unaffected settlements.

Transmission in household settings

P.A. Petrov in his early studies [72] noted that up to 5 family members may be affected in some families. We investigated this phenomenon. Complete family histories were obtained for 194 families. Two family members were affected in 24 and three in 3 families. The types of relationships were: full brothers and sisters in 8 families, brother and sister plus a niece – in one, brother and sister plus a genetically unrelated adoptive sister – in one, half-sibs in one, parent and child in three families, cousins in one, spouses in two families, parent and an adopted child in one, foster brother or sister who did not have genetic ties with the host family - in five families, and unrelated family members (in-laws) living together in four families. Due to harsh environmental conditions, Sakha families adopt orphans and disabled adults; this is a very old tradition, giving the population a chance to survive. Multiple cases of VEM in the same household were observed more often than expected based on random distribution: for genetically related family members *($P < 0.0001$),* as well as adopted family members *($P < 0.001$)*. The average incubation time between the onset of the disease in the initial case and the secondary case was 14.1 years for genetically related and 4.6 years for adopted family members [135].

The most convincing observation, confirming the possible transmission of the disease during a prolonged intrafamily contact, refers to a man who was born in a high-incidence area of the Viliui Valley and at the age of 25 moved to a village in the Central Yakutia, 400 km from the place of his birth [147]. This migrant worker lived in a local family for some time and subsequently married a girl from this family. They had a healthy child. At the age of 46, the husband developed clumsiness in his movements and decreased muscle strength in his lower limbs. A year later, his speech became slurred and dysarthric, and his gait slow and spastic. The couple divorced; the patient moved to a nearby village in the same region where another local woman looked after him during his illness. There have been no VEM cases in this region before. The patient was treated in Yakutsk but was given an erroneous diagnosis of Parkinson's disease. He lost the ability to walk and speak around the 10th year of illness and died 12 years after the onset.

His first wife developed an acute illness when she was 37 years old, seventeen years after the relationship with her husband ended. She sought treatment for high fever, severe headache, dizziness, chills, nausea, and frequent vomiting. Ten weeks later, she developed a substantial mental decline, slowness of movements, limb ataxia, and spastic gait with frequent falls. Six months after the onset of the disease, she gained weight, became severely demented and mute. She died 27 months after the disease onset. Repeated testing of the cerebrospinal fluid showed 65–32–17–45 cells/mm^3 and total protein at the level of 49–66 mg/dL.

The second wife fell ill at the age of 53, five years after her husband's death. The disease started suddenly with high fever, severe headache, chills, nausea, and vomiting. About six weeks after the onset of symptoms, she had another wave of acute illness with high fever, disorientation, and aggressiveness. Ten weeks after the onset, she developed dysarthria, spastic gait, hyperreflexia with foot clonuses, flexor pathological reflexes, and Babinski sign on the right foot. Her condition worsened, and she died 26 weeks after the disease onset. Repeatedly studied cerebrospinal fluid contained 27–11 cells/mm3 and protein at 99 mg/dL. A post-mortem study revealed thick-

ened turbid and fibrous meninges diffusely infiltrated by mononuclear, polymorphonuclear, and plasma cells; in the cerebral cortex - multiple micronecrotic foci surrounded by inflammatory infiltrates with a tendency for these lesions to be replaced with gliofibrotic scars [87].

The sequence of events in these families supports the view that VEM can be transmitted through prolonged intra-household contact with a patient manifesting the disease, including transmission to unrelated persons in a non-endemic region. Our analysis also shows that the disease was most severe in secondary cases. However, the exact mechanisms of transmission remain unknown.

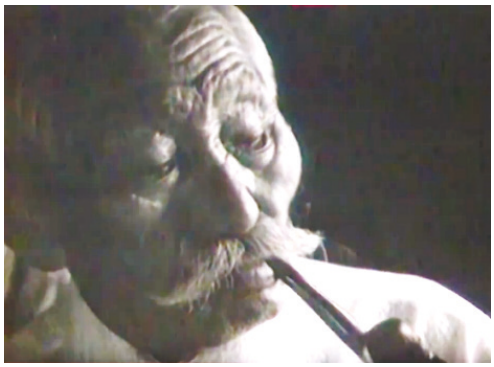
Traditional healers

We noticed that in many households, women and men, young and old, carried and used sharp knives for food processing and other domestic and outdoor activities. Under an impression from the outstanding work of Dr. *Barry Blumberg,* who won the Nobel Prize for discovering hepatitis B transmission mechanisms [148], we suspected that blood-borne infection might be at least one of several transmission mechanisms in VEM.

One after another, VEM patients reported that before admission, they consulted with *"folk healers"* and were treated by bloodletting. According to their stories, bloodletting was done as follows: 1) shaving off hair in the temporal area; 2) inflicting a bleeding wound with a miniature hatchet; 3) attaching the wider end of a cramped cow horn to the wound; 4) sucking out the *"bad blood"* through a small hole on the other side of the horn. The cow horn cannot be sterilized by boiling, and, most likely, the hatchet is never sterilized, either. This suggested that infection could be transmitted from a patient with VEM to anyone who comes next for treatment. While working on this hypothesis, we needed to understand whether our new patient was subjected to bloodletting after a known VEM patient was treated by the same healer the same way on the same day.

A short list of traditional healers who practiced in areas along the Viliui River was at hand. We often came across the name of the highest authority among traditional healers - Nikon. Patients claimed that they were treated by *"Nikon himself"* or by *"Nikon's apprentice"*. In August 1977, we had the opportunity to interview Nikon.

We arrived by car at a summer camp, where Nikon Alekseyevich lived that summer with two other villagers. The villagers cooked on a fire near a tent. After about 20 minutes of waiting, an older man came out of the forest with a log on his shoulder.



Nikon Alekseyevich Vasilyev

He slowly walked past the place where we were waiting, as if he had not noticed us, threw the log behind the tent, then turned around and came up to greet us. He was a sturdy old man, leaning forward slightly, with some asymmetry in his body, but still strong. He sat down with us. Hearing that a doctor came from Moscow to meet him, Nikon pulled himself up. He reported that he was 92 years old and that his father was a shaman. He did not mention that he himself was a shaman and stopped shamanistic rituals only when it was strictly forbidden by the anti-religious policies of the Soviet authorities.

From his youth, he was very interested in medicine. *"I did not study with anyone; I learned everything myself. The first time I realized that I had the ability to heal was at age 16. In a remote village I saw a woman who was "burning." I determined that she had mastitis. I took out my knife* (demonstrates by taking out a knife from a small sheath on his belt), *wrapped the knife in a cloth, leaving a lose 2-3cm tip, cut the skin and tissues over the fluctuating abscess, released pus and applied some leaves to the wound. The woman soon recovered. From this moment, I became convinced that I should be engaged in healing."*

When it got dark, we went to Nikon's house in the village. Nikon described to us his methods: *"I ask the patient the same questions that any doctor asks - where and when he was born, how he grew up, what he ate, what diseases he suffered. I do not record anything; I memorize the answers. During questioning, I watch how the patient behaves, how he expresses himself, the position of his head, arms, and legs. Then I undress the patient and put him in a chair. I walk around and look at his eyes, facial expression, the color of his skin, the pulsation of large vessels, the location of veins, the heart jolt, the breathing movements, the belly. The exam takes up to 20 minutes. No, I am not trying to hypnotize the patient, I truly get the facts and analyze them. Then I lead the patient to urinate to see the urine color and how it goes into the ground. I have a*

stethoscope like yours, but more often than not, there is no need to listen to the heart and lungs, because by that time the diagnosis is already clear to me".

"*I make the same formal diagnoses as you doctors do: tuberculosis, pulmonary emphysema, angina pectoris, arterial hypertension, and cancer. Of course, I know encephalitis* (he said "encephalitis," although the Sakha folk term "bokhooror" was used in the question). *Viliuisk encephalomyelitis runs in families*". To a remark that we have reason to suspect that VEM is contagious, he responded: "*This is also possible. A few decades ago, there were many cases of leprosy. This disease is contagious, but it runs in families*". How do you treat encephalitis, by bloodletting? *"No, I do not use bloodletting. I refuse to treat these patients because encephalitis is incurable.*" How do you treat meningitis? *"I do treat meningitis by bloodletting"*. It is clear that he knows the chronic stage of VEM well, but does not know that meningitis (meningoencephalitis) is part of the acute phase of VEM.

Of several healers who claimed to be "Nikon's apprentices" but treated chronic forms of VEM by bloodletting, Nikon said that many of them lye trying to take advantage of his popularity.

Throughout his story, Nikon Alekseyevich insisted that bloodletting was not the most effective or most used technic. *"Many diseases can be treated with herbs"*. He brings from the other room a *"Directory of Yakut Plants"* published in St. Petersburg in 1913. *"The herbs of Yakutia are much richer in medicinal substances than in other places, because during the very short growing season herbs grow rapidly, accumulating medicinal substances to a higher concentration"*. During the short growing season, Nikon Alekseyevich spends all his time in the forest. He demonstrates how he walks through the forest, holding the open Directory in his left hand and collecting herbs and leaves with his right hand. He tastes them. Taste determines whether the time is right to stockpile this plant. If the time is right, he collects some. *"Back home with a backpack full of herbs and leaves, I feel drunk from their strong juices. That same night, I make extracts from each separate herb and fill bottles.*" He shows several bottles containing brownish liquid. *"Cancer is not treated with herbs. Various other approaches can be used. For example, I cook fossil bones of ancient animals. On hot summer days, the permafrost becomes loose, and if you dig deep enough, you find bones"*.

We left late at night. Nikon was a man of extraordinary physical and spiritual strength. He remained strong for several more years and died at the age of 98. Unfortunately, Nikon Alekseyevich did not live up to the 1990s, the post-Soviet time, when scientists were finally allowed to study the history of shamanism and methods of traditional medicine.

The role of traditional healers in transmitting VEM remains uncertain; our study was suspended due to lack of reliable medical records and complete information about the procedures used.

Prevention of Viliuisk encephalomyelitis further spread

The occurrence of secondary cases of VEM in affected families, including spouses and genetically unrelated adoptive children of the original patient, as well as the emergence of VEM in new geographic areas and populations along the path of mass migration of people from affected areas, indicate that VEM is a disease with a model of spread characteristic of latent and chronic infections. In the absence of specific methods of prevention, the only way to stop further spread of this deadly disease was to reduce contact between patients and susceptible people.

The length of hospitalization. The discoverer of VEM, Dr. P.A. Petrov, realized that patients in the acute phase of the disease could be contagious and, therefore, must be isolated in specialized medical facilities. In the 1950s, the *Neurology Department of the Viliuisk District Hospital* was converted into a 30-bed *Viliuisk Neuropsychiatric Hospital* and transferred to a separate building. In the 1960s, when it became clear that VEM was spreading towards the densely populated Central Yakutia, a building hosting the 60-bed *Encephalitis Department* was equipped to provide long-term hospitalization of VEM patients in Yakutsk. The patients with acute and subacute VEM were kept there for many months, often until their death. Besides, a *Neuropsychiatric Nursing Home* in Sosnovka near Viliuisk was used to house patients with chronic VEM, those who were denied care by their own families. These three facilities were entirely dedicated to long-term hospitalization and care for VEM patients.

Full hospitalization data for 180 patients with a confirmed diagnosis of VEM were available for this analysis. Sixty-six patients had an *acute onset* of symptoms and were admitted to one of the specialized hospitals. The determinants of the acute phase included high (38-40°C) and low-grade (37.1-38°C) temperature, and pleocytosis in the cerebrospinal fluid over 30 in 1 mm3, indicating a sluggish inflammatory process. The duration of the first hospitalization of patients in the acute phase of the disease ranged from 17 to 518 days, on average 114 days, and covered from 80 to 100% of the total duration of the acute phase (Fig. 16, left panel).

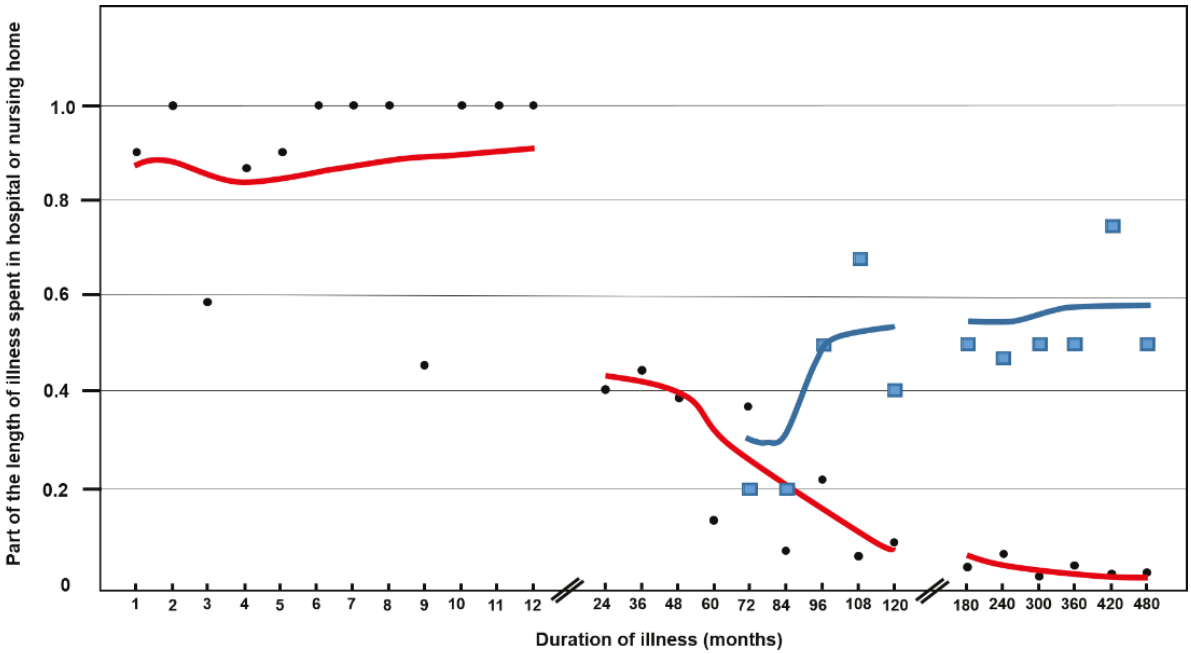


Fig. 16. The duration of hospitalization of patients with Viliuisk encephalomyelitis. Left panel: the red curve represents the duration of hospitalization of VEM patients in the acute phase of illness in relation to the total length of the acute phase (n = 66). Middle panel: the red curve corresponds to the duration of hospitalization of patients with subacute VEM in specialized hospitals (including follow-ups); the blue curve reflects the length of stay in the Neuropsychiatric Nursing Home in Sosnovka, in relation to the total length of their illness (n = 66). Right panel: the red curve shows the time the patients with chronic VEM spent in specialized hospitals (including follow-ups); the blue curve represents the length of stay in the Nursing home in relation to the total duration of their illness (n = 111). Note: some patients who survived the acute phase and transitioned to subacute/chronic VEM status, could have been included twice.

Patients who had survived the acute phase and transitioned into a short remission phase were discharged but returned to the hospital when symptoms worsened. Patients with subacute VEM were repeatedly admitted in the *Encephalitis Department* in Yakutsk for follow-up studies at least once a year to confirm the diagnosis, determine the rate of progression, update the treatment schedule and to certify the disability. The total number of days spent by patients with *subacute VEM* in hospitals ranged from 19 to 2074, an average of 288 days, covering about 20% of the duration of their illness (Figure 16, middle panel). Also, a large group of patients with subacute VEM, about 40%, manifested a more stable course and, depending on family circumstances, were placed in the *Neuropsychiatric Nursing home* in Sosnovka. In total, patients with subacute VEM spent on average 60% of the duration of their illnesses either as patients in specialized hospitals or as nursing home residents.

Of patients with a prolonged *chronic course,* the disabled people who had no other means of subsistence, more than 60% lived in the *Neuropsychiatric Nursing home* in Sosnovka for the rest of their lives (Figure 16, right panel). Long-term hospitalization, treatment, and care helped to control complications such as pneumonia and renal failure, while at the same time, patients were isolated from susceptible individuals in their families and communities. At times, when patients with subacute or chronic VEM remained in their families, they were entitled to the highest level of disability insurance and were not required to work. The relevant law enacted in the mid-1960s made it possible to reduce work-related contacts with fellow villagers during summer field work, when workers lived for extended periods in overcrowded facilities at agricultural camps far from home.

The *Neuropsychiatric Nursing home* in Sosnovka has an interesting history. In 1891, nurse *Kate Marsden* came to Yakutia from the United Kingdom because she heard that there were many patients with leprosy and that Siberians have found a plant that effectively cured leprosy. This brave woman decided to devote her life to alleviating the suffering of these unfortunate people sentenced to disability and death and go to Siberia in search

of this medicine. It took her four months to get from St. Petersburg to Irkutsk on a sleigh, and then to Yakutsk by boat down Lena River.

Kate described in detail the months-long Trans-Siberian journey, including the living conditions in Russia at that time, and the visit to Siberian lepers in the book *On Sledge and Horseback to Outcast Siberian Lepers,* published in 1892 upon her return to the UK [149].

The largest number of leprosy patients concentrated in the vicinity of *Lake Mastach,* exactly where 60 years later, we found the largest number of patients with VEM. Kate was horrified and outraged when she saw the conditions in which lepers lived. In order to isolate them from the rest of the population, patients were driven into the forest wilderness, where they lived in primitive huts and died from cold and hunger. Residents of the nearby villages would leave food for the lepers and run away.

Kate decided to build a colony for these unfortunates in Sosnovka, near Viliuisk. Returning via Irkutsk and other Siberian cities, she collected donations and persuaded five nurses to go to Viliuisk to establish a colony. *Leprosarium Hospital* and a church were built in Sosnovka. The hospital is still there, it now houses the *Neuropsychiatric Nursing home* for VEM patients. At the entrance to the building, there is an inscription: *"The building is named after Kate Marsden"*. In 2014, a beautiful monument dedicated to Kate Marsden was built in Sosnovka.

As for the cure for leprosy, an article was published in a Siberian newspaper about a plant called *Kuchukta,* which the Tungus used to treat leprosy wounds. No research on the effectiveness of this remedy has ever been conducted. As we now know, leprosy is caused by *Mycobacterium leprae* and is successfully treated with the antibiotic *Rifampicin.* In 1969, I saw the last leprosy patient who was on her way from Sosnovka to a larger Leprosarium Hospital in Irkutsk.

Hygiene and social-economic conditions. Poverty, overcrowding, poor nutrition, and dirty drinking water have traditionally been associated with an increased risk of VEM. P.A. Petrov [72] described the unimaginable living conditions in the Viliuisk ulus in the post-war years: *"people lived in dirty, overcrowded and filthy, damp and dark yurts under a common roof with cattle ... The food was of poor quality; the water came from questionable sources."*

We conducted a retrospective social-economic comparative study of families affected and not affected by VEM in villages that used to have a high prevalence of the disease [146, 150]. For this study, 30 patients with definite chronic VEM, 69 members of their families who were in constant contact with the patients, and 39 unrelated residents of the same settlements and approximately the same age (control group) were interviewed.

32% of VEM patients as children lived in a rented room in a house belonging to another family, shared a room with another family, or lived in a dormitory room, compared with 13% of control families *($P < 0.01$).* Generally, in remote villages, where the highest incidence of VEM was observed, people lived in much smaller houses than residents of settlements located closer to the city of Viliuisk *($P = 0.03$).* In the late 1980s and 1990s, life in Yakut villages significantly improved. The villages became more comfortable, wooden houses replaced yurts, so residential yurts became a rarity. Improved living conditions have helped to decrease contacts between VEM patients and susceptible family members.

The source of drinking water in the winter was lake ice, which each family collected individually. In the summer, the water was provided by municipal services. Differences in types of water supply between the groups were not statistically significant. However, the amount of water available for farming and bathing needs in remote villages was not adequate. In the 1950s, there was only one public bathhouse in the city of Viliuisk; in the 1970s, there were 23, but they were open only in the winter, 2-3 days a week. In the 1990s, the number of bathhouses increased considerably. Many families have built their private bathhouse within or outside their homes. 42% of patients' families and 92% of controls used private baths *($P < 0.025$).*

Households produce 72% of their basic staple foods, such as meat, dairy products, and potatoes. The number of livestock and the size of greenhouses belonging to families of VEM patients were smaller than in the control group ($P < 0.05$ in both cases), which was associated with the need for taking care of the patient.

In recent decades, the Republic of Sakha (Yakutia) has achieved significant successes in the healthcare sector. Smallpox was eradicated in 1937, typhoid fever in 1938, malaria and trachoma in the late 1950s, poliomyelitis in the 1960s, and leprosy in 1969. The incidence of diphtheria became insignificant and measles decreased by 7.5 times.

SUMMARY

The results of long-term research establish with full confidence that VEM is a unique disease that, in typical cases, is characterized by acute meningoencephalomyelitis, lasting from several weeks to several months. Some patients die in the acute phase; the survivors develop a slowly progressing neurological syndrome char-

acterized by dementia, dysarthria, spasticity, extrapyramidal muscle stiffness, and long-lasting inflammatory changes in the cerebrospinal fluid. In some patients, the disease stabilizes, and they remain in a state of severe disability for 20-30-40 years.

Post-mortem studies have shown the presence in the cerebral cortex and subcortical gray matter of numerous micronecrotic lesions surrounded by inflammatory infiltrates, massive loss of neurons, and perivascular inflammatory cuffs of activated T-lymphocytes, natural killer cells and natural killer-like cytotoxic T lymphocytes. The neuropathological basis of acute, subacute, and chronic forms of VEM is a single pathological process with differences corresponding to the rate of progression. The inflammatory process is prone to self-elimination in the chronic phase.

Despite the certainty about the underlying clinical characteristics, significant difficulties had to be overcome before the clinical diagnosis was put on a solid basis, due to the clinical polymorphism of VEM, especially in the chronic phase, and its spread in areas where other chronic neurological diseases, to a varying degree mimicking the clinical features of VEM, were prevalent. The detection of intrathecal production of oligoclonal IgG that persists for two decades, has become a helpful auxiliary test. An electronic database has been created. As the epidemic progressed, the ratio between acute, subacute, and chronic forms have changed towards the chronic VEM.

The disease affects predominantly young adults, and in the first phase of the outbreak, the VEM incidence among young women was twice as high as men, similar to the ratio observed in multiple sclerosis [151]. This ratio has changed to 1:1 in the later decades as chronic VEM has become the dominant clinical type of the disease. The incidence of VEM at the height of the epidemic has reached levels equal to or greater than those of common neurological disorders such as amyotrophic lateral sclerosis, hereditary ataxias, spastic paraplegias, or neuropathies.

Analysis of the territorial distribution of VEM identified the population of the *Lake Mastach* area of the Viliuisk ulus as the primary source of the VEM epidemic. The spread of VEM followed extensive well-documented post-war migration of people from impoverished Viliui villages. Within three decades, 1950-1980, the disease spread to neighboring regions and subsequently to remote localities in the general direction of the densely populated regions of Central Yakutia around the capital of Yakutsk, with new cases occurring among the locals. Since the 1980s, there has been a gradual decrease in VEM incidence, and the disease entirely disappeared from the regions of Central Yakutia, and then from the Viliuisk ulus.

Although the etiology and the exact mechanisms of VEM transmission remain unknown, available data characterize VEM as a transmissible disease that has broken through the confines of a geographically isolated indigenous population around *Lake Mastach* to cause an epidemic of a severe neurological disease involving hundreds of victims. The aggregation of cases in households and small villages suggests that VEM is likely to be transmitted during a prolonged intra-household contact, a mechanism that has also been identified in other chronic diseases, such as tuberculosis, trachoma, and leprosy.

Persistent efforts to isolate patients with acute and slowly progressing forms of VEM in specialized medical facilities have helped to contain and control the further spread of this fatal disease. The incidence of VEM decreased in the 1980s and 1990s and came to zero in the 2010s. No new cases of VEM have been reported since 2012. The social and demographic changes that followed the economic reforms of the 1980s and 1990s, primarily the privatization of farms, which reduced work-related contacts of patients with other villagers and improved their living conditions, contributed to the prevention of VEM. These efforts may have prevented yet another emerging disease from spreading globally.

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