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TICK-BORNE ENCEPHALITIS AND VIRAL HEMORRHAGIC FEVERS

Materials of a Conference,
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CERTAIN ASPECTS OF THE PROBLEM OF VIRAL ENCEPHALITIS AND HEMORRHAGIC FEVERS IN SIBERIA AND IN THE FAR EAST


Siberia and the Far East serve a special role in the accumulation of knowledge on viral encephalitis and hemorrhagic fevers, when we take account of the discovery in the Far East (1936-1938) of tick (KE Kleshchevoi entsefalit; tick-borne encephalitis) on Japanese (Ya. E. Yaponskoy entsefalit; Japanese encephalitis) (mosquito-borne encephalitis and hemorrhagic fever with renal syndrome (Far East hemorrhagic nephrosonephritis, GNN hemorragicheskiy nefrozonefrit). The discovery in Siberia of the Omsk hemorrhagic fever (OGL Omskaya hemorragicheskaya likhoradka), similar to but not identical with KH (1946-1948) was also highly significant in coping with the problem of arboviral infections.

These infections are etiologically and epidemiologically dissimilar, being representatives of three groups of viral infections which have natural foci; viruses of the tick-borne encephalitis group, where the OGL virus is included transmitted by Ixodes ticks; Japanese encephalitis virus, among the arboviruses transmitted by mosquitoes, which determines important characteristics of epizootology and epidemiology; the category of the GNN virus as a possibility of its transmissive contagion has not been established.

This report is intended to present a brief characterization of the determining main directions in further research on regional epidemiology and prevention of these infections in Siberia and in the Far East.
Geography, Typology, and Spatial Relationships of Landscape Types of Foci

The existence of natural foci of KE has been established throughout the entire forest zone from the Primor'ye to the Ural chain, that is, in the zone in which the tick Ixodes persulcatus flourishes. In addition, natural KE foci have been established beyond the limits of the forest zone, where Ixodes ticks of the Dermacentor and Gemafisalis genera dominate in the Ixodes fauna, where in several locations spontaneous infection with KE virus has been established for all species of Ixodes ticks found there, both the dominating as well as the second-importance ticks in the local Ixodes fauna.

The presently established areal of the OGL virus occupies only a small part of the areal of the KE virus, limited to the lake portion of the West Siberian forest-steppe, that is, in the zone in which ticks of the Dermacentor genus abound.

The question of studying interrelationships of these two closely related, but not identical, viruses, and the organism of different species of ticks of the Ixodes and Dermacentor genera has become pressing.

The largest number of studies of the role of vertebrate animals in circulation of the KE virus have been conducted in the USSR for the territory of Siberia and the Far East.

Studies of the susceptibility of different species of Ixodes ticks to viruses must be supplemented by study of the comparative susceptibility and sensitivity to the viruses of different, above all background, species of vertebrate animals. These investigations must be the first necessary step in advancing from study of carriers and their geographic distribution to study of conditions under which viruses exist in different landscape types of foci in order to substantiate typology of foci, landscape-epidemiological regionalization, and epidemiological prognosis of territory.

The main parameters of the living conditions of the virus in a given foci include the extent of contamination by the virus of the Ixodes tick population and the amplitude of its possible changes.

The goal of further investigation consists in establishing the effect on dissemination of the virus in an Ixodes population of periodic changes in tick and host population, changes in structure of foci, and their involvement with neighboring foci.
The most important condition for success of these studies is improvement in the methods of determining whether viral infection of populations of the Ixodes ticks and their hosts has taken place, providing for the possibility of investigating mass material in a short time.

In studying natural foci, the investigator encounters the widespread phenomenon of combination of foci of different infections over common territories (common reservoirs and carriers of causative agent).

The problem consists in discovering the types of combinations of arboviral and other infections characteristic of a given landscape, study of the conditions under which the infections exist and their reflection in human pathology.

Thus, if in West Siberia, study of the possibility of the KE and OGL foci being combined is a task of practical urgency, when we consider the overlap of their distribution zones, at least in part, in the Primor'ye the question of the spatial relationships of KE and YaEf foci remains unstudied, this question is all the more urgent because the actual distribution zone of the latter YaEF has not been delimited in the Primor'ye.

Epidemiological survey of natural foci of GNN and their combinations with KE foci have been carried out throughout the entire forest zone between the Urals and the Far East, that is, the regions where GNN has been found to exist.

In recent years, a new aspect of the study of arboviral infection foci in Siberia and in the Far East has become pressing -- study of the role migratory birds play in the historical formation of foci.

This problem is of first-priority importance in the region of Siberia-India, since there are arboviruses similar to KE present in India, first of all the virus of Kyasanur forest disease, similar to OGL.

Regional Epidemiology of KE, Epidemiological Basis for Choice of Antencephalitis Measures and the Problem of Sanitation of the Territory

A most important problem of combating KE is formulation of the principles under which antiencephalitis measures are to be chosen for protecting contingents of different types based on the classification of epidemiological types of the disease. As practice has shown, in order to correctly chose measures of prevention, account must be taken of landscape and viral infectivity of the tick-carrier population, in addition to epidemiological type of the disease, and also the seasons and "perimeter" of contact between the population and ticks and the extent of the population's latent immunization.

The following epidemiological types of KE can be distinguished in Siberia and the Far East:
Rural type of KE incidence.

Two extreme variants:

a) annually revived epidemic (epidemic form of incidence);
b) episodic individual cases of the disease in individual settlements.

Urban type of KE incidence.

a) annually revived epidemic due to infection of the focal territory adjoining the city;
b) diseases due to infection beyond the limits of the urban zone during excursions.

Incidence among newly arrived large nonimmune contingents (new construction sites, new tree farms, sanitation establishments, etc.).

Incidence among mobile groups. (geological survey and other circulating groups).

Experience has shown that tactics of combating KE must be varied in relation to, above all, these indicated epidemiological types of incidence.

Accordingly, in recent years, two main directions have been laid out in the study of regional epidemiology of KE:

a) study of the immunological structure of the population and its determining factors in different kinds of settlements and landscapes;
b) finding out the actual incidence and its relationship to the immunological structure of the population.

The concomitant study of epidemiological types of incidence, the actual incidence of the disease, and the immunological structure of the population opens up prospects of developing in the very near future more refined criteria by which contingents are to be selected for specific preventive measures.

However, the problem of combating KE can be solved only by sanitation of the nidi. Study of the problems of clearing out territories of epidemiologically dangerous KE foci has only begun.

The broad use of DDT to exterminate tick-carriers in KE foci of Siberia opened up the possibility of using aviation spraying to render nidi. However, this prospect led to the problems which are now the object of study:

first, the problem of the effect DDT has on vegetation and useful entomofauna, demarcation of cattle grazing land protected from DDT fallout,

second, the question of whether the virus is retained in treated areas due to circulation by "round-about" ways (Arthropoda -- parasites living in the fur and burrows of small mammals),

third, the question of the modes and times of restoration of Ixodes tick populations.

- 4 -
There are wide ranging possibilities in Siberia and the
Far East to develop tactics of combating KE and test the effectiveness of preventive agents in epidemiological experiments under conditions of different landscapes and adjusted to different contingents.
CERTAIN PROPERTIES OF RIBONUCLEIC ACID ISOLATED FROM
VIRUSES OF THE TICK-BORNE ENCEPHALITIS GROUP

Following is the translation of an article by T. M. Bragina, G. D. Zasukhina, and A. A. Poniklenko in the
Russian-language book Kleshchevoy entsefalit i virusnyye gemorragedcheskiye likhoradki (Tick-borne
Encephalitis and Viral Hemorrhagic Fevers), Materials of a Conference held 10-13 December 1963, Omsk, 1963,
pages 33-34.

Institute of Poliomyelitis and Viral Encephalitis of
the Academy of Medical Sciences USSR

1. Nucleic acids are carriers of the hereditary properties
of viruses. Accordingly, study of the biological properties of
pure nucleic acids of viruses is of great theoretical and practical
interest. Isolation has been achieved from various virus strains
of the tick-borne encephalitis group of nucleic acids whose properties
have been found to be identical to the genetic characters of the
original virus (F. Sokol, H. Libikova, 1959; O. G. Andzhaparidze,
L. G. Stepanova, 1961).

2. In our studies, we carried out experiments on various
methods of extraction of ribonucleic acid (RNA) -- phenolic deprotein-
ization in the cold followed by alcoholic sedimentation; ester treat-
ment, etc. The quantitative indices of RNA yield in growing viruses
in different tissue systems were compared (transplanted cells and
initial explantates), and also methods of RNA extraction from the
brain of infected white mice.

3. The study was conducted with various representatives of
viruses from the tick-borne encephalitis group: the strains Pan,
Khipr [transliterated from the Russian], Sof'in, TP-21, Povassan,
and also strains of Shotland encephalomyelitis, Omsk hemorrhagic
fever, and Klassanur forest disease.

4. Viral RNA induced the formation of plaques in the tissue
culture of chicken fibroblasts according to the methods of Porter-
field and Dulbecco. The capacity toward plaque-formation of viral
RNA in other tissue systems sensitive and insensitive to tick encephalitis virus (fibroblasts of the skin-muscle tissue of the human embryo, skin-muscle and renal tissue of the calf embryo) was studied.

5. The capacity of viral RNA to be multiplied and induce cytopathogenic effect in sensitive and insensitive tissue systems was investigated. Viral RNA induced a clear-cut cytopathogenic effect in tissue cultures of hog embryo kidneys, in a transplanted stock of hog embryo kidney, and induced an irregular cytopathogenic effect in chick embryo tissue cultures.

6. Viral RNA lost its infective, cytopathogenic properties, and also the capacity for plaque-formation when treated with ribonuclease.

7. The sensitivity of viral RNA to desoxycholate, pH of the medium, and certain physical factors (ultraviolet rays, etc.) was determined.

8. Laboratory animals susceptible and nonsusceptible to the tick encephalitis virus were tested for sensitivity to viral RNA.
EFFECT OF GROWTH HORMONE IN TISSUE CULTURES ON REPRODUCTION OF VIRUSES


Institute of Poliomyelitis and Viral Encephalitis of the Academy of Sciences USSR

In the studies of certain authors (Kerlayen transliterated from the Russian, 1962, Royzman, 1962) the effect of cortisone and parathyroid hormone on multiplication of viruses in tissue culture was noted.

Kh. Sh. Li (1962) demonstrated the stimulating effect of growth hormone on protein accumulation and cell multiplication in human liver tissue culture.

Accordingly, we undertook experiments studying the effect of growth hormone in chick embryo tissue culture on reproduction of rabies virus (the PLYURIKHEP strain) and of tick-borne encephalitis virus (the KHABAROVSKII-17 strain).

It has been established that growth hormone in specific dosages has no toxic effect on chick embryo tissue culture and does not suppress multiplication of rabies and tick-borne encephalitis viruses. At the same time, the virus titer was found to be a definite function of growth hormone, which pointed to an essential relationship between growth hormone and virus reproduction. In particular, in the presence of growth hormone titers of rabies virus in culture fluid in biological experiments on newborn white mice were 0.8-2.0 logarithmic units higher than in control experiments. A significant increase in the titer of tick encephalitis virus was achieved in the culture
fluid studied (the hemagglutinin titer was determined according to the hemagglutination reaction). As a rule, the hemagglutinins attained a titer of 1:128, which exceeded by 2-4 times the titer in the control.

Study of the mechanism of the stimulating effect in tissue culture of growth hormone on virus reproduction and on the cellular system is doubtless of interest and calls for further investigation.
ISOLATION OF A CYTOPATHOGENIC VIRUS FROM IXODES TICKS; IN THE KRASNOYARSKIY KRAY WHICH DIFFERS FROM THE CAUSATIVE AGENT OF TICK-BORNE ENCEPHALITIS


Krasnoyarskiy Kray Sanitary-Epidemiological Station
Institute of Medical Parasitology and Tropical Medicine

The isolation in 1962 in Western Siberia (M. P. Chumakov et al) from Ixodes tick-carriers of tick-borne encephalitis -- of a virus differing from the causative agent of tick encephalitis afforded grounds to assume the possibility that this virus circulates in nidi of tick-borne encephalitis of Eastern Siberia.

Ticks, blood, and cerebrospinal fluid of patients with obscured vision /podozreniya/ due to tick-borne encephalitis were studied. The material investigated was used to inoculate at the same time white mice and tissue culture of chicken fibroblasts. Eight-day old chicken embryos were inoculated with the culture fluid during the presence of a cytopathogenic effect in the tissue culture.

The virus strain which had the cytopathogenic effect on tissue culture of chicken fibroblasts was isolated from fasting Ixodes ticks collected in Molye Devich'ye ravine near the construction site of the Krasnoyarskaya Hydroelectric Station, here the death of white mice from infection with the tick suspension was not observed.

The isolated virus strain proved pathogenic for 8-day old chick embryos: when inoculated in the allantois cavity the embryos began to die (after inoculation with a 10% solution of perished embryo body) in two days.
The virus strain had a cytopathogenic effect on tissue
culture of chicken fibroblasts at a dilution of 100 and 10,000-
fold. At the same dilutions, the death of infected tick embryos
commenced.

The death of chick embryos was accompanied by profuse
hemorrhaging throughout the entire embryonic body.

The 10 % suspension prepared from perished chick embryos
proved pathogenic for white mice weighing 7-8 grams when inoculated
intracerebrally.

Thus, of ten inoculated white mice six died by the sixth-tenth
day, and during the first passage in white mouse brain of ten inocu-
lated white mice four perished in the same incubative period.

The isolated virus strain was well preserved in 50 % glycerine
at a temperature of -2° C up to one month (period of observation).

Sera of the blood of patients hospitalized because of a febrile
state after tick bites and who had no antibodies to tick encephalitis
virus in hemagglutination inhibition test were tested by the complement
fixation test with antigen prepared from the virus strain isolated
by the Kemerovskaya Expedition in 1962. The antigen was graciously
presented to us by the Institute of Poliomyelitis and Viral Encepha-
litis of the Academy of Medical Sciences USSR.

Of the 35 sera investigated with the complement fixation test
11 proved positive, and in two of the patients a clear-cut increase in
antibody titer in fresh sera was noted.

Thus, the study made during the epidseason of 1963 confirmed
the circulation in tick encephalitis nidi of the Krasnoyarskiy Kray --
of a virus antigenically identical to the virus isolated by the
Kemerovskaya Expedition in 1962.
STUDY OF A DISTINCTIVE VIRUS ISOLATED FROM A PATIENT WITH PROGRESSIVE COURSE OF TICK-BORNE ENCEPHALITIS, WITH FATAL OUTCOME


Perm' Scientific Research Institute of Virology and Perm' Medical Institute

Chronic viral neuroinfections of man represent a pressing and little studied problem. In our country, among such infections diseases diagnosed as different forms of tick-borne encephalitis marked by a progressive course of the disease are quite well represented. In addition, there is reason to cast doubt on the etiological identity of diseases categorized in this group. This is pointed to, first of all, by the pronounced polymorphism of the clinical forms. Further, tick-borne encephalitis virus in such patients is found so infrequently that its isolation becomes a celebrated rarity. Serologically, the relatively few number of cases also confirms this position. Thus, according to the data of the virological laboratory of the Perm' Scientific Research Institute of Virology only 42% of patients have antibodies to tick-borne encephalitis virus. Finally, the studies of Zil'ber et al (1962) on the etiology of lateral amyotrophic sclerosis also points to the possibility that different etiological stages can exist in the group of chronic neuroinfections which have the same neurological syndrome.

Accordingly, a distinctive filterable virus isolated in our laboratory from white mice is of interest. To inoculate the animals we used culture material obtained from a deceased patient of Kotomtsev who had been given the diagnosis: primary-progressive course of tick-borne encephalitis (poliomyelitic form with syndrome of lateral
amyotrophic sclerosis and early merging bulbar effects). After several blind passages in inoculated mice, a disease was developed whose diagnostic picture was highly distinct. On the 12-18th day after inoculation paralysis and pareses of the hind extremities appeared in the animal, and sometimes of the front extremities. The paralyzed mice, as a rule, did not perish. Some of them recovered completely, in others, persistent paralysis remained. One of such mice was placed under observation for ten months. In spite of sluggish paralysis of the hind paws accompanied by atrophy of the muscles and by seizures, the animal remains viable up to the present.

Preliminary data on a study of histological changes in the nervous system of infected mice revealed that the presence of widespread changes in different sections of the brain and spinal column of the "grave disease" and "chronic disease" types, and also precipitation areas in various nuclear groups. Acute inflammatory signs were not found, and small glial nodules were present only here and there in the brain.

From the time of isolation, the virus completed 20 passages through mice, and the minimum incubative period was not less than 10 days in any instance. The virus titer in the cerebral tissue did not exceed $10^{-2}$.

In studying the serum of the Kotomtsev patient, it was not possible to discover in it either virus-neutralizing antibodies or antihemagglutinins to tick-borne encephalitis virus. Nor did the serum neutralize the isolated virus.

Initially, we assumed that this last fact completely denied the possibility of the etiological role of the virus. However, later it became clear that such "serological muteness" was also observed in experiments on mice. Sera of animals undergoing the disease and recovering also did not neutralize the virus causing the disease.

Thus, a certain immunological parallel can be drawn between clinical and experimental data. The results do not of course afford any definite conclusion, however, there is all the reason to assume that this virus merits balanced and careful study. Even if its etiological role in human pathology is subsequently not confirmed, the disease it induces can serve as a model of chronic neuroinfection in [animal] experimentation.
STABILIZATION AND ACCUMULATION OF TICK-BORNE ENCEPHALITIS VIRUS AS AFFECTED BY SEVERAL FACTORS


Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences USSR

Our previous experiments established that in media containing serum and other protein fillers the stability of tick-borne encephalitis virus is enhanced. The extremely low stability of the virus in nonprotein medium used for vaccine preparation compels us to study the conditions which would promote its best stabilization.

In our experiments, we studied the activity of cultivated vaccine virus in a mixture at different concentrations, in which it was established that MgCl₂ at concentrations of 0.1 M; 0.15; 0.25; 0.5 M (37° and 24 hours, 48 hours, and 72 hours, and at 50° for 5-7 minutes) had no stabilizing effect on the virus. Later, lower MgCl₂ concentrations were investigated — 0.01 M; 0.05; 0.06; 0.07; 0.08 M (50% — 5-7 minutes). In these experiments, stabilization was not constant from experiment to experiment. Virus titer at 0.05-0.07 M in the mixture was either at the control level or 0.5-0.7 lg above the virus titer in the control.

In experiments that followed we investigated the effect of increased MgCl₂ and CaCl₂ concentrations in the maintenance mixture on virus accumulation in the culture. The experiments showed that the amount of CaCl₂ brought to the final concentration of 0.003 M and above promoted a 1-1.5 lg increase in virus yield. Similar results were obtained with MgCl₂ at a final concentration in the medium of 0.003 M. The pH value of the sustained medium also had a fairly
weighty effect on virus accumulation in the tissue culture. In parallel experiments, the medium 199 with pH = 7.5 and 7.8-8.0 was compared.

With constant regularity the virus yield averaged 1 lg unit above the medium with pH 7/8-8.0, and the vaccine proved more immunogenic compared to the controls. The virus titer was 0.5 lg unit higher in the cultures and for the case in which the virus seeded as diluted before inoculation in a medium with a higher pH value (7.8) even when later a medium with pH 7.5 was used.

It can be assumed that in such media conditions for the best linkage of virus and cell and more active reproduction of the virus in the culture are provided for.
TRANSLATION NO. 1168

BIRDS AND MOSQUITOES AS CARRIERS OF CERTAIN TRANSMISSIVE
VIRAL CAUSATIVE AGENTS

Following is the translation of an article by S. A. Ananyan and M. Ye. Karpoza in the Russian-language book "Kleschchevoy entsefalit i virusnyye geliot-
raidskiye likhoradki" (Tick-borne Encephalitis and
Viral Hemorrhagic Fevers), Materials of a Conference

Institute of Poliomyelitis and Viral Encephalitis
of the Academy of Medical Sciences USSR

During 1961-1963, serological and virological examination
of wild and domestic birds, and also samples of mosquitoes col-
lected in the Bol'shenaryamskiy and Kurchumskiy Rayons in Kazakh-
stanskaya Oblast by the A and B virus groups of transmissive in-
fecions using the reaction of suppressed hemagglutination (RSH)
and using the neutralization reaction.

Native unheated avian sera were used in the neutralization
reaction. Before the hemagglutination reaction was begun, the avian
sera were treated with acetone, since kaolin treatment does not
eliminate the inhibiting properties of avian sera.

Organs of birds brought down by shooting were preserved en
route in 50% buffered glycerine. Subsequently, a 20% suspension
of organ fragments in physiological solution containing penicillin,
streptomycin, and 0.75% bovine serum was prepared. After standing
for two-three days the centrifuge suspension diluted to 1:100 was
used to inoculate three tube tissue cultures of chick embryo fibro-
blasts. After two-three passages, the presence or absence of TsTp
[?] In order to isolate the virus newborn white mice were ino-
culated intracerebrally with each culture sample. Observations were
conducted for twelve days. In the absence of clinical manifestations
of infection, an additional passage in sucklings was made.
The mosquito samples (40-100 specimens per sample) after careful washing out glycerine with physiological saline solution were ground in a mortar in 1-2 ml of physiological solution, the centrifugate was used to inoculate tissue culture, and then white mice.

The transmissive character of the isolated viral causative agents was determined by their sensitivity to sodium deoxycholate and ether.

The isolated viral causative agents passed through asbestos Zeitz filters, are penicillin-, streptomycin-, and glycerine-resistant, are inactivated by formaline, well tolerate freezing and drying, are sensitive to ether and sodium deoxycholate, and many of them exhibit cytopathogenic properties. Most of the viruses isolated exhibit hemagglutinating properties at lower pH: 5.8-6 and 6.0-6.4.

Studies showed that in the Vostochno-Kazakhstanskaya Oblast causative agents of the tick-borne encephalitis group in birds and mosquitoes are found in individual cases only, while among wild and domestic birds and also among mosquitoes there is a prevalence of causative agents from virus group A of transmissive infections. Identification of the newly isolated viral causatives is underway.
FACTORS UNDERLYING CHANGE IN POPULATION SIZE OF TICK-CARRIERS AND CHANGE IN INFECTIVITY BY VIRUSES OF TICK-BORNE ENCEPHALITIS AND OMSK HEMORRHAGIC FEVER


A most important indicator of the habitat conditions of tick-borne encephalitis (KE) virus and Omsk hemorrhagic fever (OGL) virus in a given nidus is the extent of virus infectivity of the Ixodes tick population, their number, and the amplitude of their possible variation.

Our problem consisted in tracing the effect of periodic changes in numbers of the pre-imaginal stages of Ixodes and their hosts on the dissemination of the virus in the adult tick population in nidii of two arboviral infections of Western Siberia -- KE and OGL.

In these nidii the main vectors of infection are the ticks Ixodes persulcatus and Dermacentor pictus. Observations were made in Toguchinskiy Rayon of Novosibirskaya Oblast from 1960 to 1963 and in Tyukalinskiy Rayon of Omskaya Oblast (1959-1962). Tyukalinskiy Rayon is located in typical forest-step of Western Siberia, where beach-aspen copses (kolki) and a large number of lakes are characteristic. Intercopse spaces are for the most part plowed. The background species of Ixodes ticks is the Dermacentor pictus. Toguchinskiy Rayon is a cultivated territory, which changes into the taiga of the slopes of the Salyirskiy range in the southern portion. Numerous woodlands (conifer, deciduous, and mixed) representing relics of the reduced taiga are found on the
cultivated territory among elements of secondary forest-step. The main bulk of such plots is situated in the northern half of the Bayon intersecting the Ima River valley. The main species of Ixodidae throughout all landscape types is Ixodes persulcatus, only in the Ima River valley -- along the flood plain and river terraces -- does Dermacentor pictus dominate.

The main factor governing size of adult ixodidae population is the intensity at which their larvae and nymphs are able to be supplied with hosts. In its turn, the intensity of hosting depends on equal measure both on the abundance of larvae and nymphs (their average number per a single animal-host) as well as on the number of hosts in the given territory (statistically expressed, this function serves as an indicator of hosting).

By knowing the intensity at which larvae and nymph of some tick species hosts and characteristics of the species' biology, we can predict the population size of a given species for the following year.

With respect to Ixodes persulcatus such prediction is grounded on a comparison of the total of nymph hosting indices for May-June-July with similar indices of the past year. The rise in this indicator points to the probability of increased imago population size by next year.

Similarly, a prediction of the intensity of nymph hosting is formulated. The total of larvae hosting indices for May-June-July is compared with the similar sum for last year. In addition, an assumed number of host-animals during the season of the following year is taken into account.

With an increase in larvae hosting intensity and the high population size of small mammals anticipated the year ahead, the intensity of nymphal hosting most probably will rise. We must, however, note that prediction of nymph population from larval hosting is less precise, since larvae are more subject to environment.

When we speak of predicting population of Dermacentor pictus, we must note that the spring population consists only of wintered ticks. This means that when their numbers increased during autumn, we must also expect an increase during the spring of the next year, and vice versa.

The greater the autumn peak prevails over the spring, the more sharply will tick population increase the following year. In its turn, the height of the autumn peak depends on the intensity of the larval and nymphal hosting during the season.

During the study the extent of virus infection of the population of both tick species was systematically determined. Significant fluctuations in the numbers affected by virus during several years were established and their statistical reliability demonstrated. We can explain these fluctuations by analyzing the change in age composition and numbers of host-animals (mouse-like rodents and
shrews). Under the condition that a large portion of larvae and nymphs host on young (nonimmune) animals, the virulence of the imagos developing from these nymphs becomes enhanced. As to Dermacentor pictus this regularity is traced more clearly, since larvae and nymphs host during a single season practically on a uniform host population.

When we consider Ixodes persulcatus this regularity is complicated by the fact that larvae and nymphs host in different years, that these two forms differ in numbers of nonimmune young in the host population. As a result, the fluctuations in virulence can rise or fall.

When we take the above factors affecting numbers and virus infection of Ixodes ticks into account, a method of predicting these two most important indices of the relative strength of natural foci of tick-borne encephalitis and Omsk hemorrhagic fever.

Such predictions are being prepared annually for Toguchinskiy Rayon and verification has shown the validity of using the regularities enumerated here.
In studying the avian carrier status of tick-borne encephalitis virus in foci of this disease in Eastern Siberia, we selected for fixed observation points the taiga section in Tayshetskiy Rayon, Irkutskaya Oblast. The frequency at which strains of this virus were isolated from ticks, rodents, and birds in this area is significant. Tick-borne encephalitis virus was isolated from birds of twenty-six species, and for birds of another seventeen species the fact of virus encounter was established serologically.

Fairly recently thrushes have become of research interest in different foci as virus-carriers (Moskvin, 1940; Tatarinova, 1957; Zakorkina, 1958; Sotnikova and Ambarnikov, 1959; Sotnikova and Soldatov, 1962; Sotnikova, 1963, and several others). We succeeded in the Tayshetskiy focus in isolating tick-borne encephalitis virus from thrushes: redwing \( \text{belobrovik} \) (two strains), fieldfare \( \text{ryabinnik} \) (one strain), pale thrush \( \text{blednyy droz} \) (one strain) and blackbill thrush \( \text{chernozobyy droz} \) (two strains). A significant percentage of these bird species and also the song thrush revealed antibodies to tick-borne encephalitis virus.

We must note particularly that the virus and antibodies were discovered in fairly newly hatched nestlings of the redwing (one strain), and also in two broods of thrush nestlings not determined as to species (from five nestlings of the first brood, three strains were isolated, and from six of the second -- three strains).

The virus was isolated from the blood, brain, and liver of the nestlings. Similar data was obtained in Primorskiy Kray (Sotnikova and Soldatov, 1962) where the infection of the young was even higher than of adult birds, and also in several other foci.
It is known that endogenic infection of embryos is found in many bacterial and viral diseases of birds, such as: pullorum disease, paratyphoid, tuberculosis, brucellosis, spirochetosis, laryngotracheitis, leukemia, plague, viral hepatitis, etc.

The significant adaptiveness of tick-borne encephalitis virus to parasitism in the bird organism is evidenced by the large number of bird species from which the virus can be isolated, by the ability of the parasite to reside protractedly in the avian brain, to circulate in its blood, and to be excreted with the droppings (Sotnikova, 1963; and also our data in this publication). Accordingly, it is highly probable that transmission of tick-borne encephalitis virus from adult birds to fledglings through the egg does exist.

In order to verify this possibility we began during the season of 1963 (end of May) a virological and serological examination of the eggs of various species of birds in the Tayshtetakiy focus of tick-borne encephalitis. In all a total of 37 eggs of birds of four species were investigated. These included 28 thrush eggs. Six strains of tick-borne encephalitis virus were isolated from the brains of thrush embryos. Three strains were isolated from the eggs of the red wing, of which two strains were from one nest; one strain was isolated from fieldfare eggs, and two strains from four blackbill thrush eggs.

No antibodies were found to tick-borne encephalitis virus in the perivisceral fluids of the infected eggs (allantoid and amniotic fluids were examined in complement fixation and hemaglutination inhibition reactions). Complement-binding antibodies were found in a one: ten titer in the perivisceral fluids of eight uninfected eggs of the blackbill fieldfare, and redwing thrushes. Positive hemaglutination inhibition reaction was obtained with the perivisceral fluids of two eggs of the redwing.

The phenomenon of transvarial transmission of tick-borne encephalitis virus found in the birds (blackbill fieldfare, and redwing thrushes) points to the greater significance of birds in foci of Eastern Siberia than has thusfar been assumed, and corroborates the high extent to which the virus has become adapted to avian organisms.
NEW OBSERVATIONS ON THE EPIDEMIOLOGY OF TICK-BORNE ENCEPHALITIS IN THE WESTERN URAIS


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Epidemiological characteristics of tick-borne encephalitis in the Western Urals have been studied by the authors for many years. The investigations revealed that encephalitis virus is broadly disseminated in ticks within the limits of the oblast as a whole. The virus has been isolated in almost any of the rayons studied, although the extent of carrier infectiousness is not the same.

In comparing the extent to which disease-bearing ticks have infected the population three groups of foci can be distinguished. High disease rate among the human population corresponded to the first group of high tick infectivity. A relatively low infectivity and incidence were observed in the foci of the second group. The third, the most interesting group, comprised foci where population morbidity was slight, while tick infectivity remained at a high level. In addition, immunological shifts in the blood of the healthy population in the foci evidenced extensive contact with the encephalitis virus. Sometimes, during individual incidences of clinically pronounced forms which were not recorded yearly, antibodies appeared in almost a third of the population.

The most pronounced disparity between morbidity on the one hand and tick infectivity and immunological shifts on the other was observed in the northern rayons of the oblast.
We conducted investigations in the settlement of Nirob, located 120 kilometers north of sixty degrees North latitude in order to delineate the northern distribution boundary of tick-borne encephalitis. Here cases of encephalitis were not generally recorded either during the season of the investigation, or in previous epidemic seasons.

Out of 50 investigated sera, taken from persons living more than 10 years in this locality, 7 contained antibodies to tick-borne encephalitis in titers from 1:40 to 1:1280. Thus, serological data convincingly evidenced the presence of virus in nature and the contact of population with it. At the same time, clinically pronounced cases of encephalitis were not found.

It seems to us that the absence of a disease incidence rate can scarcely be explained by an existence of an immune layer of population. In this area it is relatively limited. In fact large outbreaks of encephalitis have been observed in other rayons (Dobryanskiy and Oktyabr'skiy) when antibodies were found in almost half of the population.

More probably, the factor behind this phenomenon must be sought for in the properties of the virus itself. It is possible that variants of the virus which have diminished virulence exist. This is suggested also by our experimental data on the isolation from ticks of an encephalitis virus which does not induce death of mice upon first passages, but renders them immune. In fact, attenuation of these strains proved nonpersistent and with further passages reversion set in. In particular, in the northern rayons there may exist specifically the climatic conditions which favor development of such strains. It is also possible that immunity induced by this virus is not always sufficiently intense, but the incorporation into circulation of new high-virulence strains can lead to activation of viruses and outbreaks.
The phenomenon of transvarial transmission of tick-borne encephalitis virus has been discovered by Soviet researchers at the very outset of research on this infection. At the same time, the irregularity of virus transmission to the progeny of infected ticks (Shchubladze and Serdyukova, 1939) was noted. This fact resulted in certain foreign authors regarding as doubtful in general the possibility of transvarial transmission (Sabin, 1958, Eklund et al).

The factual basis of this report is found in materials of eleven years' observations on spontaneous virus carrier status in Jx. ricinus ticks from foci of tick-borne encephalitis in Lenigradskaya Oblast and six years of experimental data on transvarial and interstage transmission of the virus in the forest tick in the Northern Primor'ya (Far East).

During the study, more than 100,000 larvae of 32 individual and group ovipositions were investigated, as well as material from other developmental stages of Ixodes ticks.

Isolation of the tick-borne encephalitis causative agent from ticks and eggs was carried out on white mice, chick embryos, and in tissue culture. During the special experiments on the study of transvarial transmission of virus we had no live virus cultures in the laboratory and did not undertake auxiliary bacterial studies using infected material.
As a result of the experiments, the successive transition of tick-borne encephalitis virus over two complete generations in naturally infected ticks was traced. Penetration of the virus by way of the ovaries occurred in artificially infected individuals not more than in one-third of the experiments, while the interstage transmission of the virus was confirmed only during the lifetime of the first tick generation. However, we did not succeed in any of the experiments in establishing virus through direct examination of the eggs oviposited by infected females. In larvae developing from ticks collected in different geographical zones (Leningradskaya Oblast, Lake Sakhalin, and the region of Soviet Gavan''), the frequency of virus detection was the same. The best conditions for infection of adult ticks were established the virus was obtained in the nymphal phase (up to 50\%) of the interstage transmission of the causative agent to the imago, while the virus lasted from the larvae to the in only 9-10\% of the cases.

Therefore, constant diminution of the virus content in the Ixodes tick population takes place during metamorphosis.