HUMAN LABORATORY ACQUIRED ARBO-, ARENA-, AND HANTAVIRUS INFECTIONS

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ABSTRACT

A total of 78 cases of laboratory acquired infections occurred at D. I. Ivanovsky Institute of Virology, M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis and Rostov na Donu Institute of Epidemiology, Microbiology and Hygiene. All these cases were caused by accidental infection of the staff with the following viruses: Venezuelan equine encephalomyelitis (n=34), Kyasanur forest disease (n=1), Omsk hemorrhagic fever (n=1), Crimean-Congo hemorrhagic fever (CCHF) (n=2), Dhori (n=5), vesicular stomatitis (n=1), Machupo (n=3), and hemorrhagic fever with the renal syndrome (n=31). The majority of cases were caused by inhalation of virus aerosols. All cases were a result of accidents or neglect of safety measures. Diagnoses were confirmed by virus isolation and/or detection of specific antibodies in convalescent serum. With the exception of one lethal case of CCHF, patients recovered without disability. The pathogenicity of Dhori virus in man was discovered as a result of laboratory infection of 5 staff members.

Key Words: laboratory acquired infection, arboviruses, arenaviruses, hantaviruses, nairoviruses, Dhori virus.

INTRODUCTION

Many workplaces and occupations have unique worker associated risks. In microbiological and clinical laboratories the risk posed to workers manifests as the potential for contracting a laboratory acquired infection (LAI). Over the past decades numerous papers have been published describing LAI resulting from exposure to a variety of classes of infectious agents and their routes of transmission (11,12 13,16). The collection and publication of this information provides important insights regarding (a) safety practices which prevent disease transmission, (b) the development and use of primary barriers that protect the worker, (c) specialized aspects of facility design and construction which facilitate the safe containment of pathogens in the laboratory, and (d) the identification of agents that are transmissible to humans.

This paper provides data describing 78 cases of laboratory acquired infection of the staff members of D. I. Ivanovsky Institute of Virology, M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis, and Rostov na Donu Institute of Epidemiology, Microbiology and Hygiene. These diseases were caused by accidental infection with the following viruses: Venezuelan equine encephalomyelitis (VEE), Kyasanur forest disease (KFD), Omsk hemorrhagic fever (OHF), Crimean-Congo hemorrhagic fever (CCHF), Dhori, Vesicular stomatitis (VS), Machupo, and hemorrhagic fever with renal syndrome (HFRS). A summary of this information is provided in Table 1.

This paper includes published (1, 2, 3, 4, 7, 10, 14, 15) and unpublished data. Data from publications have only been available in the Russian literature, and mostly in Proceedings of local workshops (non-peer reviewed Journals). This information has been previously unavailable to the majority of scientists. This paper provides a compendium and original analysis of LAI associated with several Russian Research Institutes.

CASE HISTORIES

Venezuelan Equine Encephalomyelitis Virus (Togaviridae, Alphavirus)

VEE virus is endemic in the Central part of the American continent. In nature it is transmitted by mosquitoes. Human infections occur during epidemics of horses and mules. We observed 34 cases of laboratory acquired VEE infection from 1956-1975. All of these cases were caused by accidents or neglect of safety measures. An accident which occurred at D. I. Ivanovsky Institute of Virology on May 3, 1956 led to the infection of 22 staff members. As a result of negligence of a laboratory assistant, several ampoules, each containing 0.5 ml 5% lyophilized brain suspension from mice in-
fected with VEE virus (strain Trinidad) dropped and broke on the staircase. The titer of the virus in ampoules was very high: 10-11 LD50/ml. The resultant aerosol disseminated in the direction of the air flow along a 30 meter passage towards an open air vent. Five subjects who disinfected the site of the accident and 17 subjects passing the zone were infected. Three subjects walking closer to the site of the accident (than some of the 17 infected subjects), were not infected because they were upwind of the accident, with directional air in the facility flowing from their location toward the accident site.

The onset of the disease was acute, which helped to precisely determine the duration of the incubation period. In one case it was 28 h, in nineteen cases it was 50 h, and in two cases about 3 days. Blood specimens, throat washings, urine and fecal specimens from 16 patients were examined by virological methods (by infecting young albino mice weighing 5-6 g). The results of virus isolations were positive with the material collected from 8 patients during fever within 48-72 h from the onset of disease. In two patients VEE virus was detected only in the blood, in five only in throat washings, and in one in the blood and throat washings. All isolated strains were identified as VEE in the virus-serum mouse neutralization test. Irrespective of virological findings, specific neutralizing antibodies were detected in convalescents’ blood after 2-3 weeks (14).

The high incidence of virus isolation from throat washings may be due to the respiratory route of VEE infection in this outbreak. Despite the presence of the virus in the throat, the infection was not transmitted from patients to healthy subjects at home or in laboratory (15). It is likely the virus titers in the throat were below the infective threshold dose. In addition, none of the patients coughed or had rhinitis, which might have further facilitated virus transmission through the air.

In 1967 a laboratory assistant homogenizing the brains of mice infected with VEE virus was infected in a laboratory room along with three other subjects who were working in the same room without a protective face shield. In 1969 and 1975 eight subjects were infected with VEE at different times under unknown circumstances. The clinical picture in these cases was influenza-like, and VEE was retrospectively diagnosed by testing blood sera collected from these patients before and after disease. One of these patients was infected with VEE during the 8th month of pregnancy, after which she gave birth to a healthy child, currently a healthy adult.

All VEE cases we observed had an acute onset: high fever (39.0-39.5°C), headache, myalgia, sometimes vomiting. After 3-5 days the temperature decreased and asthenia ensued, which lasted for 2-3 weeks. No signs of CNS focal lesions or respiratory symptoms were observed, except a slight throat hyperemia in several patients. Convalescence was complete, without any residual symptoms. Specific antibodies to VEE virus were detected in all convalescents by the hemagglutination inhibition test, radial hemolysis in gel, and enzyme immunoassay for up to 23 years (5,6).

Kyasanur Forest Disease Virus (Flaviviridae, Flavivirus, Tick-Borne Encephalitis Antigenic Complex)

KFD virus is endemic for a specific territory in India (Shimoga state, Mysore province). Ecologically the virus is associated with Ixodidae, mainly Haemaphysalis ticks. Natural infection with this virus causes a severe disease with the hemorrhagic syndrome in humans and monkeys.

In 1988 a scientist at D. I. Ivanovsky Institute of Virology was infected as a result of an iatrogenic wound to the finger through the glove with a broken ampoule containing the virus. The incubation period was 4 days. The disease was characterized by an acute onset, 40°C fever, sharp headache, nausea, lymphadenopathy, and hemorrhagic syndrome which manifested by repeated nasal bleedings and stomorrhagia. Blood was collected on day 2 of disease, and virus identical to KFD virus (according to the biological neutralization test) was isolated by infecting suckling mice. One month after disease onset, specific antibodies were detected in the blood by neutralization, hemagglutination inhibition, complement fixation, and immunofluorescence tests. The patient recovered, but asthenia persisted for more than 2 months (7).

Omsk Hemorrhagic Fever Virus (Flaviviridae, Flavivirus, Tick-Borne Encephalitis Antigenic Complex)

In 1970 a scientist from Institute of Poliomyelitis and Viral Encephalitis was infected, probably via aerogenic route, while preparing concentrated culture antigens of several OHF strains isolated in West Siberia. The onset was acute, and the disease ran a grave course with high fever, severe head-
ache, anorexia, hyperemia of the face, scleritis, and conjunctivitis, but no hemorrhages. Typical OHF manifestations included leukopenia, thrombocytopenia, and pneumonic infiltration. The fever lasted for 7 days. Infiltration resolved after 3 weeks, and the patient recovered completely. The etiology of the disease was estimated by isolating of the virus from the blood and detecting seroconversion of specific antibodies to OHF virus in paired sera in the neutralization, hemagglutination inhibition, and agar diffuse precipitation tests.

Crimean-Congo Hemorrhagic Fever Virus (Bunyaviridae, Bunyavirus, CHF-Congo Antigenic Group)

Under natural conditions CCHF virus is transmitted through bites of Ixodidae ticks, mainly Hyalomma. Humans may be infected through contact with the blood of patients or animals with the disease.

In summer of 1968 in Rostov-na-Donu while visiting the Institute of Epidemiology, Microbiology and Hygiene, a laboratory assistant from Institute of Poliomyelitis and Viral Encephalitis was infected while processing a blood specimen from a patient with CCHF (centrifuging and preparation of plasma for infecting mice). This case was an example of a typical and very grave course of CCHF. A laboratory assistant had a high fever and markedly expressed viremia and developed the hemorrhagic syndrome, which involved hemorrhage in the gastrointestinal system and uterus along with, nasal bleedings, and hematomas. Anemia, leukopenia, and thrombocytopenia were noted. The convalescence was characterized by stubborn (up to 6 months) asthenia, loss of hair, and undue fatigability.

The titer of CCHF virus in the blood on day 1 of disease was at least 4.5 lg LD₅₀ /ml. Testing of paired sera in the complement fixation and precipitation tests showed clear-cut seroconversion of specific antibodies to CCHF virus.

On February 20, 1970, at Rostov-na-Donu Institute of Epidemiology, Microbiology, and Hygiene one more laboratory assistant fell ill. She and 3-4 staff members worked with live CCHF virus 10 days before the onset of the disease. A flask with highly active virus-containing material was broken in a centrifuge rotor, which probably led to infection through aerosol, although other workers present in the same room did not fall ill.

The disease involved an extremely severe hemorrhagic syndrome. The patient died on February 27, 1970, despite numerous blood transfusions, injections of convalescent serum and plasma, and therapy with vascular and tonic drugs. Chronic liver disease (hepatocolecystitis), which the patient had suffered for 6 years, may have contributed to the lack of effectiveness of the extensive treatment protocols (1).

Dhori Virus (Orthomyxoviridae)

Ecologically the Dhori virus is associated with ixodicid ticks. The geographic region of the virus includes India, Egypt, Southern regions of European Russia, Kirghizia, Armenia, Azerbaijan, and Portugal. The pathogenicity of the virus for man was discovered in 1978 and 1979 as a result of an accidental laboratory infection of five staff members of Institute of Poliomyelitis and Viral Encephalitis. One of the authors of this paper (A.M.B.) and a laboratory assistant were infected in June and July of 1978, while working with strain GI1313 from Arbovirus Center (YARU), New Haven, USA. One more scientist and two laboratory assistants fell ill in March and April, 1979. They were infected while working with a strain isolated from the blood of a patient who fell ill in 1978. In both cases the route of infection was aerosol inhalation caused by opening a 1.5-liter flask containing a cell culture infected with Dhori virus.

Dhori virus was isolated from the blood of one of the three patients examined. During convalescence the titers of specific antibodies increased in all five patients. Two patients were hospitalized. The incubation period was 2-4 days.

In inpatient AMB the disease manifested with a fever of 38-39°C, chills, diffuse myalgia, and headache. Although the temperature normalized after 3 days, the patient exhibited giddiness, eyeball pain, and rhythmic hyperkinetic twitching of the right limbs. The other inpatient developed fever, conjunctivitis, scleritis, moderate throat hyperemia, and bradycardia. Nervous symptoms were slight radicular syndrome, painful trigeminal points, and decreased abdominal reflexes. No visceral disorders were detected. Regional lymph glands were not enlarged. In the outpatient the disease ran a less severe course with acute febrile symptoms and 2-4-day fever. The period of convalescence was associated with long asthenia in all 5 patients (2).

Vesicular Stomatitis Virus (Rhabdoviridae, Vesiculovirus, Vesicular Stomatitis Antigenic Group)
Under natural conditions, vesicular stomatitis (VS) virus is pathogenic for horses, cattle, swine, and other domestic animals and for humans. Humans are infected during contact with sick animals or through the bite of mosquitoes (VS Indiana strain), Simulidae moths, or Culicoides biting midges belonging to Heleidae family (VS New Jersey strains).

In March 1964 a laboratory assistant was infected while dismantling a centrifuge vessel, when droplets of culture fluid containing VS virus (Indiana strain) splashed on his cheek and upper lip. In less than 2 days he developed fever of 39°C, chill, strong headache, spinal myalgia, pain in the eyeballs, and photophobia. Examination revealed pronounced injection of the conjunctival vessels, hyperemia of the throat and tonsils, and nasal mucosa infiltration. Submandibular and anterior cervical lymph nodes were moderately painful. On day 3 of disease the temperature was subfebrile, abundant vesicles filled with transparent fluid appeared on the skin of the upper lip and tip of the nose; on day 4 vesicles appeared on the wings of the nostrils and solid whitish eruptions emerged in the oral cavity. On day 6 roentgenogram showed pneumatic foci. After several additional days the patient recovered, but asthenia persisted for two more weeks. No complications were recorded.

VS virus was isolated by infecting chick embryo fibroblasts and young albino mice with preparations from throat and nasal washings and from vesicular fluid collected on days 3, 4, and 5 of disease. The results of blood examination were negative. Complement-fixing and neutralizing antibodies to VS virus were present in the blood serum starting from day 11 (4).

**Machupo Virus ( Arenaviridae, Tacaribe Antigenic group)**

The natural source of Bolivian hemorrhagic fever agent (Machupo virus) is Calomys callosus, a hamster-like synanthrope rodent.

In 1972 the laboratory of Hemorrhagic Fevers at Institute of Poliomyelitis and Viral Encephalitis was engaged in isolation of the virus from rodents brought from Bolivia, the preparation of antigens, and studies of the isolated strains. Three subjects (a scientist and two laboratory assistants) were infected under unknown circumstances. The disease was severe in one case and moderately severe in the other two. The virus was isolated from one patient; seroconversion of specific antibodies was observed in all of the convalescents (10).

The disease onset was acute and the symptoms progressed over a week. Fever lasted for 7-8 days with temperature fluctuations of 1.5-2°C and chill. All patients complained of myalgia and arthralgia, dryness in the mouth, anorexia, and vomiting. Blood pressure decreased, and hemorrhagic symptoms presented as a petechial rash. The most pathognomonic signs were involvement of the buccal mucosa, fibrinous exudation on the tonsils, and polyadenitis, which presented mainly as enlarged and painful submandibular end cervical lymph nodes. In one case the disease was virtually asymptomatic. Nervous symptoms included irritability, disorientation regarding time, and loss of memory of recent events. Blood analysis showed leukopenia with a shift to the left, thrombocytopenia, and atypical mononucleocytes. Liver function was affected, hyperglycemia and hypocoagulation were observed. The convalescence period was characterized by rapid anorexia, weakness, loss of hair. All patients recovered.

**Hemorrhagic Fever with the Renal Syndrome (Bunyaviridae, Hantavirus)**

Hantaviruses causing HFRS were not yet isolated by the time of the events we describe, and therefore, the disease was diagnosed on the basis of clinical and epidemiological data.

In October 1958, a total of 235 small rodents of several species (Clethrionomys glareolus, Apodemus flavicollis, Apodemus agrarius, and Microtus arvalis) were brought to Institute of Poliomyelitis and Viral Encephalitis from a natural focus of HFRS in the Tula region. Before working in the vivarium with these animals, the workers put on face shields made of 4 layers of gauze, two coveralls, caps, and gloves. The first HFRS case was recorded 10 days after the animals were brought to the vivarium. A total of 12 subjects fell ill before December 28, 1958. Four of the infected individuals did not directly handle the animals, they were working in rooms adjacent to the vivarium.

The incubation period varied from 17 to 30 days. The onset was acute with high fever, which lasted for 4-8 days and was paralleled by myalgia, lumbar pain, and sometimes epigastrial pain. The hemorrhagic syndrome was manifested by nasal bleedings. The course of the disease was moderately severe in some patients, and less severe in others. All the patients recovered (3).

Another outbreak occurred at D. I. Ivanovsky
Institute of Virology in autumn 1967, when 19 subjects were infected. In this case three pairs of bank voles were brought from the Kirov region (700 km North-East of Moscow). The animals were housed in a room with an anteroom which provided a double door entry. While the doors were always maintained in the closed position, they did not provide an airtight seal. Additionally, in retrospect, it is possible there was not adequate directional airflow from the clean corridor into the room to prevent the escape of airborne microorganisms. The staff started falling ill 10 days after the animals were brought to the room. The first patients were two workers who directly handled the animals. HFRS ran a very severe course in them. The other 17 people were infected while passing by the vivarium doors. They developed moderately severe HFRS. All patients recovered.

A similar (by epidemiological and clinical characteristics) outbreak of HFRS occurred in N. F. Gamaleya Institute of Epidemiology and Microbiology in Moscow, when 113 subjects were infected (8).

CONCLUSION

This review of the history of studies of zoonotic viral diseases provides numerous examples of human disease resulting from infection in the laboratory. By the year 1994 out of 537 agents recorded in the International Catalogue of Arboviruses 71 were identified as causes of such diseases. The greatest number of cases were caused by VEE (150 cases) and KFD (133 cases) viruses (9). Studies of laboratory cases of these infections provided useful information on the probable routes of infection other than natural exposure, duration of the incubation period, clinical features, possible treatments and the outcome of the diseases. The pathogenicity of many viruses infecting humans was proven for the first time only as a result of studies of laboratory acquired disease.

The human laboratory acquired infections described in this paper were observed and studied personally by the authors. One of us (AMB) even contracted Dhori virus as a result of a laboratory acquired infection (2). Viruses which caused the illness described in this paper are categorized as Biosafety Level 3 and 4 agents, with only VSV belonging to Biosafety Level 2 (9). Work with those viruses was conducted in a special isolated laboratory. Personal protective equipment and clothing included the use of double medical gowns, cap, protective face shield, rubber gloves, and boots. Accidents which have taken place were the result of negligence of safety procedures and practices by a few members of the staff. The majority of the cases were associated with inhalation of the virus aerosols. The diagnoses were confirmed by virus isolation and/or antibodies conversion in convalescent sera. Patients were treated symptomatically. One patient who contracted CCHF died. One patient (AMB) who contracted Dhori infection suffers hyperkinetic twitching of the right extremities to date in response to stressful situations.

Data on laboratory acquired infections laid the basis for safety measures in virological laboratories, including containment measures to prevent the release of pathogens into the environment. We hope that the results of our observations will supplement the information about arboviral and other zoonotic viral infections.

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REFERENCES


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</tr>
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<td>VEE</td>
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<td>22*</td>
<td>Aerosol</td>
<td>Back passage of virus in susceptible host, Specific antibodies in convalescent serum</td>
<td>Double contain materials for transport in unbreakable containers</td>
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<tr>
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<td>4*</td>
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<td>VEE</td>
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<td>8*</td>
<td>Unknown</td>
<td>Specific antibodies in convalescent serum</td>
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</tr>
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<td>KFD</td>
<td>Iatrogenic wound</td>
<td>1*</td>
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<td>Back passage of virus contained in patient samples in susceptible host, Specific antibodies in convalescent serum</td>
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<td>OHF</td>
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<td>1**</td>
<td>Aerosol</td>
<td>Specific antibodies in convalescent serum</td>
<td>Provide HEPA filtered respirators (or equivalent), Conduct work inside glovebox or biosafety cabinet</td>
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<td>Centrifugation</td>
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<td>Aerosol</td>
<td>Specific antibodies in convalescent serum, Autopsy results</td>
<td>Provide HEPA filtered respirators (or equivalent), Check equipment before use and replace broken parts.</td>
</tr>
<tr>
<td>CCHF</td>
<td>Broken centrifuge rotor</td>
<td>1***</td>
<td>Aerosol</td>
<td>Specific antibodies in convalescent serum</td>
<td>Provide HEPA filtered respirators (or equivalent), Check equipment before use and replace broken parts.</td>
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<tr>
<td>Dhoric</td>
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<td>Provide HEPA filtered respirators (or equivalent), Conduct work inside glovebox or biosafety cabinet</td>
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<tr>
<td>VS</td>
<td>Dismantling centrifuge</td>
<td>1*</td>
<td>Splash (oral or aerosol)</td>
<td>Back passage of virus contained in patient samples in susceptible host, Specific antibodies in convalescent serum</td>
<td>Use of face shield and HEPA filtered respirator (or equivalent)</td>
</tr>
<tr>
<td>Machu</td>
<td>Unknown (same time while isolating virus)</td>
<td>3**</td>
<td>Unknown (suspected aerosol)</td>
<td>Virus isolation from patient, Specific antibodies in convalescent serum</td>
<td>Use of face shield and HEPA filtered respirator (or equivalent)</td>
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<tr>
<td>HFRS</td>
<td>Unknown (transmission by infected animals)</td>
<td>12**</td>
<td>Suspected aerosol from vivarium with infected animals</td>
<td>Symptomology commensurate with infection and proximity to know infected animals</td>
<td>Use of face shield and HEPA filtered respirator (or equivalent), Maintain sufficient inward airflow to areas housing infected animals; HEPA filter exhaust air; use HEPA filter animal enclosures; room entry through air lock</td>
</tr>
</tbody>
</table>

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