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Crimean-Congo Hemorrhagic Fever in Albania, 2001

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Abstract During the spring and summer of 2001, an outbreak of eight cases of Crimean-Congo hemorrhagic fever (CCHF) occurred in Albania. The epidemiological investigation, the clinical presentation of the cases, and the course of the disease are described. Seven of the cases were laboratory confirmed. A nosocomial infection and a cluster of cases within a family were observed. Genetic analysis of the CCHF virus strain that caused the outbreak showed that it was clustered together with other European CCHF virus strains except the Greek one (strain AP92). The Greek strain, which forms an independent clade, differed from the causative strain by 25.3% at the nucleotide level.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) virus belongs to the genus *Nairovirus* in the *Bunyaviridae* family and causes severe disease in humans, with resultant mortality reaching up to 30%. The virus can be transmitted to humans either by bites of ixodid ticks (mostly of the *Hyalomma* genus) or by contact with blood or tissue from CCHF patients or viremic livestock. Cases of the disease have been reported from different parts of southeastern Europe, Africa, and Asia [1, 2, 3, 4, 5]. Like other nairoviruses, CCHF virus is a single-stranded negative-sense RNA virus and has a tripartite genome consisting of small (S), medium (M), and large (L) segments, which encode for the nucleocapsid protein, the envelope glycoproteins, and the putative RNA-dependent polymerase, respectively [6].

Albania is situated in the southwestern part of the Balkan Peninsula. Since 1986, when the first CCHF case was recognized in this country, a number of cases have been reported each year. Although the cases are generally spread throughout the country, including areas of Tirana, Mirdita, Lezha, Gjirokastra, and Skrapar, most are observed in the Kukes area, in the northeastern part of Albania. In most cases, patients present with severe disease, and many cases are fatal [7]. Acute gastroenteritis, influenza, viral hepatitis, leptospirosis, acute leukemia, and hemorrhagic abnormalities are among the syndromes included in the differential diagnosis of the disease. Person-to-person infections have been documented in Albania. Moreover, in one study, naturally occurring antibodies to CCHF were found in 1.3% of the subjects tested [7].

During the spring and summer months of 2001, an outbreak of 69 cases of suspected CCHF occurred in the neighboring area of Kosovo, 18 of which were laboratory or clinically confirmed and 6 of which were fatal [http://www.who.int/disease-outbreak-news/n2001/june/29june2001.html]. In the same time period, an outbreak of CCHF also was observed in Albania. The epidemiological characteristics, the clinical presentation of the cases, and the course of the disease, as well as the genetic analysis of the CCHF virus strain that caused the outbreak, are described in this report.

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Patients and Methods

Patients

From the end of May to September 2001, eight cases of CCHF were identified in Albania. Patient 1 was a resident of Koder Luma village in the Kukes area, a 66-year-old female housekeeper

who tended livestock and spent much time outdoors in the fields. She was admitted to the hospital on 22 May 2001 with malaise, nausea, vomiting, frontal headache, high fever, chills, abdominal pain, purpura, hematemesis, and gingival bleeding. She mentioned a tick bite; ticks were found on her body as well as on the animals she tended.

Patient 2, a 28-year-old male nurse who had come in contact with the infected blood of patient 1, felt ill on 26 May, with fever, abdominal pain, headache, and hiccups. Two days later he was admitted to the same hospital in Kukes. His general condition deteriorated (neck rigidity, extreme gingival bleeding, epistaxis, and massive ecchymoses and petechiae in the lumbar region and both arms bilaterally), and he was transferred to the University Hospital in Tirana. On day 5 of hospitalization, the laboratory findings included leukopenia (leukocyte count of 2,000 cells/µl), thrombocytopenia (platelet count of 16,000/µl), and elevated serum alanine transaminase (ALT of 400 U/ml). The leukocyte count comprised 47% lymphocytes, 39% segmented neutrophils, and 14% mononuclear cells. A sternal bone marrow biopsy was negative for myeloproliferative process, but excessive postbiopsy bleeding and delayed hemostasis were present. By day 14 the patient had almost fully recovered, except for profound fatigue and malaise.

Patient 3, a 9-year old boy who also resided in Koder Luma village in the Kukes area, felt seriously ill and was admitted to the hospital. Massive hemorrhages were present.

Patients 4 and 5, the father and sister of patient 3, were tested as "contacts" for possible infection. The family tended livestock.

Patient 6, an 8-year-old boy who resided in Nang Bicaj village in Kukes, presented with high fever, fatigue, muscle pain, and petechiae and was admitted to the hospital. His family tended livestock.

Patient 7, a 28-year-old woman who resided in Shtiqen village in Kukes, was also severely ill with high fever, chills, fatigue, petechiae, and conjuctival hyperemia. She did not mention a tick bite, but she had been living in the fields.

Patient 8, a 24-year-old female housekeeper who resided in Helshan in the Has region, was admitted to the hospital with high fever, chills, lower back pain, vomiting, nausea, and fatigue. She had been tending livestock at her home. An on-the-spot investigation showed ticks on the body of the cows.

In all cases, general symptoms at initial presentation were high fever, headache, lower back pain, vomiting, malaise, and fatigue. Five patients had petechiae and severe hemorrhagic manifestations. The median age of the patients was 28 years (range, 8–66 years). The disease lasted 5–15 days. The treatment was supportive; none of the patients received ribavirin. None of the cases were fatal.

Serological Investigations

Indirect immunofluorescent assay was used for the detection of specific CCHF antibodies. Sera were tested in twofold dilutions (initial dilution 1:8) with fluorescein-labeled goat IgG and IgM anti-human immunoglobulin (Gibco Diagnostics, Madison, Wis., USA) on spot slides containing Vero E6 cells (ATCC CRL 1586), with approximately 50% of the cells infected with the 10200 IbAr strain of the prototype CCHF virus. Titers were recorded as the greatest dilution of serum at which characteristic cytoplasmic immunofluorescence was detected.

RNA Extraction, Amplification, and Sequencing

Total RNA was extracted from whole blood and serum samples of the patients using the RNaid kit (Bio 101, La Jolla, Calif., USA). After reverse transcription, a 260-bp fragment of the small (S) genome segment was amplified by nested polymerase chain reaction (PCR) using primers from the small RNA segment of CCHF virus [8]. Purified DNA products were sequenced in the OpenGene DNA sequencing system (OpenGene; Visible Genetics, Toronto, Canada) using the same primers as for the nested PCR.

Phylogenetic Analysis

Sequences determined in this study were aligned with respective CCHF sequences obtained from the GenBank database using Clustal W, and phylogenetic inference analysis was performed using the PHYLIP package version 3.57c [9]. DNADIST (with Kimura's 2-parameter method) and NEIGHBOR programs were used to construct trees, and SEQBOOT to generate bootstrap data files (100 replicates). CONSENSE was used to compute the consensus phylogenetic tree.

Results

Specific IgG and IgM antibodies to CCHF virus were detected in all patients except patient 3. Although no specific antibodies were detectable in this patient (the 9-year-old boy), the diagnosis was established on the basis of the clinically compatible syndrome and the fact that CCHF was confirmed by serological and/or molecular methods in members of his family (cases 4 and 5)

Table 1 Percentages of nucleotide and amino acid genetic differences between CCHF, Dugbe, and Hazara virus strains

Strain	AL/ Kukes	Kos/ 9553	Kosovo	Drosdov	8402	66019	ArTeh	ArMg	UGANDA	ARD	SPU415	10200	AP92	Dugbe	Hazara
AL/Kukes	–	0.7	2.3	4.8	10.8	12.7	18.1	13.7	15.6	17.4	18.4	17.8	25.3	64.6	75.2
Kos/9553	–	–	1.5	4	10.8	12.7	17.1	13.7	15.6	17.4	17.4	16.8	24.3	64.6	75.2
Kosovo	–	–	–	3.1	11.3	14	18.6	13.2	16.7	16.9	18.5	18.4	23.6	62.9	76.2
Drosdov	0.6	0.6	0.6	–	11.3	14.9	19	15.5	15.7	17.4	18.9	18.8	22.7	61.7	77.6
8402	5.8	5.8	5.8	4.6	–	4.4	17.7	15	14.2	13.7	15.2	17.9	25.3	62	75.8
66019	7	7	7	5.8	1.1	–	17.2	17.4	15.2	15.6	16.6	18	26.4	62.5	75.4
ArTeh	6.9	6.9	6.9	5.7	7	8.2	–	21.4	18.8	19.1	17.6	23.1	24	59.8	67.5
ArMg	10	10.5	10.5	9.3	9.4	8.1	14.2	–	20	18	18.1	19	26.5	63.2	69.4
UGANDA	1.4	1.4	1.4	1.7	2.3	3.5	5.8	9.4	–	18.7	19.2	19.4	26.7	65.7	71.8
ARD	6.9	6.9	6.9	5.7	5.7	6.9	5.7	12.9	4.6	–	5.2	11.3	27.1	63.1	71.9
SPU415	4.6	4.6	4.6	3.4	3.4	4.6	1.3	10.5	2.3	2.3	–	9.5	25.5	61.7	70
10200	5.8	5.8	5.8	4.6	4.6	4.6	7	9.4	3.5	5.8	3.4	–	25.5	69.1	71.9
AP92	11.8	11.8	11.8	9.3	11.8	10.6	11.7	12.9	10.7	12.9	10.5	9.4	–	52.1	66.4
Dugbe	65.5	65.5	65.5	65.3	65.2	63.1	68	64.8	64.5	72.5	68.6	67.6	58.5	–	64.3
Hazara	64.4	64.4	64.4	65.3	67.7	66.8	67.2	61	69.4	69.8	65.8	67.8	64.2	69.9	–

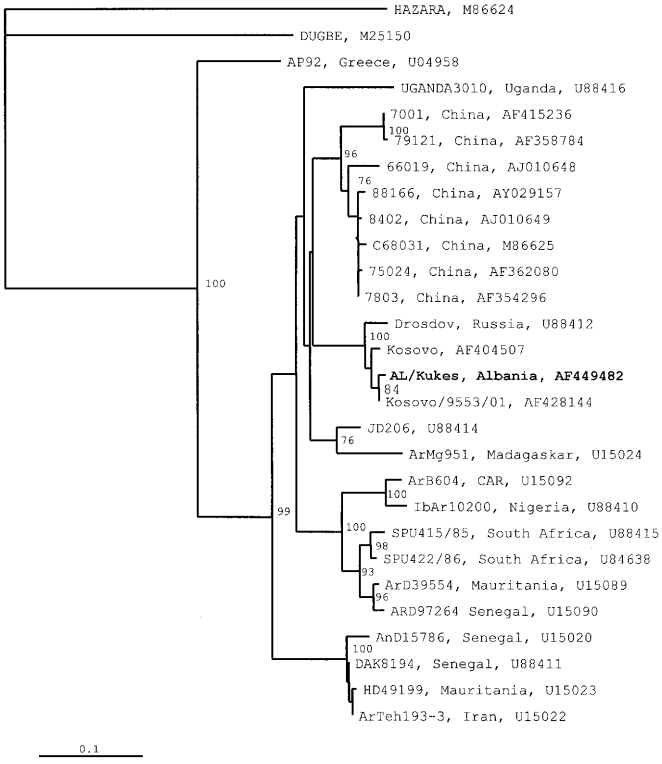

Fig. 1 Phylogenetic tree based on a 255-nt fragment from the S RNA segment showing the clustering of the sequence obtained from this study and respective representative CCHF strains from the GenBank database. Sequences of two other nairoviruses, Dugbe and Hazara, were included; Hazara virus was used as an outgroup. The numbers indicate percentage bootstrap replicates (of 100); values below 70% are not shown. Horizontal distances are proportional to the nucleotide differences. The scale bar indicates 10% nucleotide sequence divergence. Vertical distances are for clarity only. Sequences used in the analysis are shown on the tree as strain, country, GenBank accession number. CAR, Central African Republic

who were taking care of him and came in close contact with him.

A 260-bp PCR product was obtained from whole blood or serum samples from patients 1,2, and 4. Nucleotide sequence analysis of the PCR products confirmed that the causative agent was CCHF virus. As expected, the three sequences were identical. The nucleotide sequence obtained from patient 2 (AL/Kukes/3/01) has been submitted to GenBank and assigned the accession number AF449482. Phylogenetic analysis revealed that the sequences of the Albanian strain are clustering together with the respective sequences of the Kosovo strain, as well as with sequences of the Drosdov strain from Russia (Fig. 1). Table 1 shows the genetic distances between the Albanian CCHF sequence and other respective CCHF virus sequences. For comparison, distances between the Albanian CCHF virus sequence and the sequences of two other nairoviruses, Dugbe and Hazara, are included.

Discussion

CCHF is endemic in the Balkan Peninsula, and sporadic cases as well as outbreaks have been reported during the last three decades in Albania, Bulgaria, and the former Yugoslavia [7, 10, 11, 12]. It usually presents in a severe form and is associated with high mortality. During the spring-summer period of 2001, an outbreak of eight CCHF cases occurred in Albania. Most of the cases (7 of 8) were recorded in the Kukes area, in the northeastern part of Albania, as occurred in previous years, suggesting that the endemicity of CCHF is highest in this region of the country.

All patients with primary cases of CCHF reported either a tick bite or a lifestyle that included the tending of livestock; when the latter was reported, the presence of ticks on the patients was confirmed. The tick population was extremely high and active that year in Albania due to supportive climatic conditions such as a mild winter, which increases the chance of tick survival, and the early arrival of spring, which enables ticks to become active earlier.

In addition to being transmitted by tick bites, CCHF can be spread from person to person via contact with infected blood, excreta, or tissues. Nosocomial infections caused by CCHF virus have been reported in several countries [1, 13, 14, 15]. In our study, one nosocomial infection was observed, in the male nurse (patient 2) who was infected through contact with the blood of patient 1. A cluster of cases within a family was also present, in that the father (patient 4) and sister (patient 5) of the 9-year-boy from Kukes (patient 3) were infected. Interestingly, a blood sample from the father drawn during the incubation period, before the onset of the disease, was tested to investigate the father as a possible “contact case”, and it was found to be CCHF positive by PCR, before a detectable amount of specific antibodies had developed, while a blood sample from the sister was found positive by indirect immunofluorescent assay.

High fever, chills, fatigue, and malaise were the initial symptoms in all cases. Nausea and vomiting were also frequent symptoms at the initial phase, suggesting early involvement of the gastrointestinal tract. None of the patients had a pneumonia-like syndrome. All patients presented with a severe form of the disease; most of them displayed marked thrombocytopenia with hemorrhagic manifestations. While the mortality rate of the disease in the years 1985–1987 was as high as 43%, no fatal case was reported in this 2001 outbreak. The improvement in survival compared with previous cases suggests that early recognition as well as initiation of good supportive care is critical, and/or that there is a probable difference in the virulence and pathogenicity of the causative strain.

Phylogenetic analysis showed that the Albanian CCHF virus strain is clustering together with other European CCHF virus strains, including strains from Kosovo (AF404507 and AF428144) and the Drosdov strain from Russia (U88412). It is of interest that the Greek CCHF virus strain (strain AP92), which has not yet been associ-

ated with disease in humans, is extremely different from these strains (25.3% nucleotide difference from the Albanian strain), forming an independent phylogenetic clade. Additional sequences obtained from humans with CCHF as well as from ticks and animals from different parts of the world will elucidate the epidemiology of this life-threatening disease.

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References

1. Suleiman MN, Muscat-Baron JM, Harries JR, Satti AG, Platt GS, Bowen ET, Simpson DI: Congo/Crimean haemorrhagic fever in Dubai. An outbreak at the Rashid Hospital. Lancet (1980) ii:939–941
2. Yen YC, Kong LX, Lee L, Zhang YQ, Li F, Cai BJ, Gao SY: Characteristics of Crimean-Congo hemorrhagic fever virus (Xinjiang strain) in China. American Journal of Tropical Medicine and Hygiene (1985) 34:1179–1182
3. Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, Searle LA, Gill DE: Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. American Journal of Tropical Medicine and Hygiene (1987) 36:120–132
4. Butenko AM, Chumakov MP: Isolation of Crimean-Congo hemorrhagic fever virus from patients and from autopsy specimens. Archives of Virology (1990) Supplement 1:295–301
5. Marriott AC, Polyzoni T, Antoniadis A, Nuttall PA: Detection of human antibodies to Crimean-Congo haemorrhagic fever virus using expressed viral nucleocapsid protein. Journal of General Virology (1994) 75:2157–2161
6. Elliott RM: Molecular biology of the *Bunyaviridae*. Journal of General Virology (1990) 71:501–522
7. Eltari E, Cani M, Cani K, Gina A: Crimean-Congo hemorrhagic fever in Albania. In: Abstracts of 1st International Symposium on Hantaviruses and Crimean-Congo Hemorrhagic Fever Virus, Halkidiki, Greece (1988) pp 34
8. Schwarz TF, Nsanze H, Longson M, Nitschko H, Gilch S, Shurie H, Ameen A, Zahir AR, Acharya UG, Jager G: Polymerase chain reaction for diagnosis and identification of distinct variants of Crimean-Congo hemorrhagic fever virus in the United Arab Emirates. American Journal of Tropical Medicine and Hygiene (1996) 55:190–196
9. Felsenstein J: PHYLIP (Phylogeny Inference Package) version 3.57c. Department of Genetics, University of Washington, Seattle (1993)
10. Vasilenko S, Katsarov G, Bradvarova I: Study on Crimean Congo hemorrhagic fever in Bulgaria. In: Abstracts of 1st International Symposium on Hantaviruses and Crimean-Congo Hemorrhagic Fever Virus, Halkidiki, Greece, (1988) pp 37
11. Heneberg D, Heneberg N, Celina D, Filipovic D, Markovic Z, Zubi D, Zivkovic B, Simic M, Zonjic S, Pantelic M: Krimska hemoragièna groznica u Jugoslaviji (Crimean hemorrhagic fever in Yugoslavia). Vojnosanitetski Pregled (1968) 4:181–184
12. Gligic A, Stamatovic L, Stojanovic R, Obradovic M, Boskovic R: Prva izolacija virusa krimske hemoragiène groznice u Jugoslaviji (The first isolation of the Crimean hemorraghic fever virus in Yugoslavia). Vojnosanit Pregl (1977) 34:318–321
13. Burney MI, Ghafoor A, Saleen M, Webb PA, Casals J: Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever-Congo virus in Pakistan, January 1976. American Journal of Tropical Medicine and Hygiene (1980) 29:941–947
14. van Eeden PJ, Joubert JR, van de Wal BW, King JB, de Kock A, Groenewald JH: A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part I. Clinical features. South African Medical Journal (1985) 68:711–717
15. Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB: Crimean-Congo haemorrhagic fever treated with oral ribavirin. Lancet (1995) 346:472–475