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Crimean-Congo haemorrhagic fever outbreak in Middle Anatolia: a multicentre study of clinical features and outcome measures

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A Crimean-Congo haemorrhagic fever (CCHF) outbreak emerged from 2001 to 2003 in the Middle Anatolia region of Turkey. This study describes the clinical characteristics and outcome features of CCHF patients admitted to four tertiary care hospitals in Turkey. Definitive diagnosis was based on the detection of CCHF virus-specific IgM by ELISA or of genomic segments of the CCHF virus by RT-PCR. Related data were collected by a retrospective chart review. Hospital costs were extracted from the final discharge bills. Univariate and multivariate analyses were conducted to determine the independent predictors of mortality. CCHF virus-specific antibodies or genomic segments were detected in the sera of 99 cases. Seven cases that were treated with ribavirin were excluded from the study. Cases were mostly farmers (83 cases, 90 %), and 60 % had a tick-bite history before the onset of fever. Impaired consciousness and splenomegaly were independent predictors of a fatal outcome.

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INTRODUCTION

Viral haemorrhagic fever syndrome is a serious clinical entity caused by a number of distinct viruses from several RNA virus families with zoonotic origins. The distribution of these viruses is generally limited and the viruses are therefore endemic in certain areas of the world. Changing social, economic and climatic conditions such as increased travel, bioterrorism and ecological disruption may increase the possibility of the introduction of VHF-associated viruses into new areas or increase the incidence in endemic areas (Crowcroft *et al.*, 2002; Isaacson, 2001; Papa *et al.*, 2002; Sidwell & Smee, 2003).

In 2001, a cluster of unidentified haemorrhagic fever cases was seen in a tertiary care hospital in Turkey. All the patients were from a small region of Middle Anatolia. In 2002, more cases were admitted to additional tertiary care hospitals; however, the disease was not noted as a common infectious entity until the second half of 2003. At this time, infectious disease and clinical microbiology units of four tertiary care hospitals declared the existence of an outbreak. Once an outbreak was noted, the Turkish Health Ministry took prompt action, and other infectious disease groups and related health care scientists joined the investigation.

This study aimed to analyse the clinical and outcome features of Crimean-Congo haemorrhagic fever (CCHF) cases in Turkey.

METHODS

Setting and study design. Infectious Diseases and Clinical Microbiology units at a teaching hospital and three university hospitals participated in this study. These hospitals were located in distinct regions surrounding the outbreak area.

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Abbreviations: CCHF, Crimean-Congo haemorrhagic fever; LOS, length of hospital stay.

Clinical and outcome data were collected retrospectively by chart review in the hospitals. Hospital costs were extracted from the discharge billing records obtained from the financial departments of these hospitals.

Diagnostic studies. Stored sera from suspected CCHF patients were collected by an authorized laboratory of the Turkish Health Ministry and sent to reference centres collaborating with the World Health Organisation. Briefly, sera were tested for specific CCHF IgM and IgG antibodies by ELISA and CCHF virus RNA by RT-PCR and direct sequence analysis at two specialized centres: the Special Pathogens Branch of CDC in Atlanta, Georgia, USA and the National Reference Center for Arbovirus and Viral Haemorrhagic Fevers, Pasteur Institute, Lyon, France.

After considering possible circulating haemorrhagic fever viruses in the region, IgM and IgG ELISA studies, using inactivated native CCHF virus (strain IbAr 10200) antigens grown in Vero E6 cells, were performed on serum samples (Bryan *et al.*, 1996). The primers and PCR methods for amplification of the nucleocapsid-coding sequence (S segment) were as previously described (Burt *et al.*, 1998) except for slight modifications. Separate reverse transcription was performed using CCHF-F2 primer at 42 °C for 1 h. Subsequently, 10 µl of the reverse transcription reaction was used in a 50 µl PCR reaction using FastStart Taq DNA Polymerase with GC-rich solution (Roche) and primers CCHF-F2 and CCHF-R3. The temperature profile for the PCR reaction was as follows: 2 min at 95 °C; 36 cycles of 1 min at 95 °C, 1 min at 50 °C and 2 min at 72 °C; and a final elongation of 10 min at 72 °C. Amplified DNA fragments were analysed using a 1 % low-melt agarose gel, and bands corresponding to 536 bp products were purified using the Qiagen Gel Extraction Kit (Qiagen). Sequencing of both DNA strands was performed using primers CCHF-F2 and CCHF-R3 in a BigDye Terminator v3.1 reaction on a 3100 Genetic Analyzer (Applied Biosystems). The obtained sequences were analysed using Sequencher (Gene Codes).

Statistical methods. Software NCSS version 2004 (Number Cruncher Statistical Systems) was used for statistical analysis. The differences between the patients who recovered and patients who died were compared using Fisher's exact test for dichotomous variables, or Student's *t* test or Mann-Whitney U test for continuous variables. For the predictors of mortality a logistic regression model was developed. Variables selected by the multivariate variable selection routine of the software, depending on the R^2 changes, were analysed with the forward hierarchical method.

A value of $P < 0{\cdot}05$ was considered statistically significant, and evaluations were always two-tailed.

RESULTS

Patients admitted to tertiary care hospitals with fever (oral temperature >38 °C) and thrombocytopenia (platelet count $< 150 \times 109\,l^{-1}$) were accepted as potentially having a viral haemorrhagic disease. The sera from 113 such patients had been stored and were available for testing. ELISA tests for specific CCHF IgM antibodies were positive in 96 of the 113 cases; among these, 47 were also positive for CCHF-specific IgG. Follow-up samples of IgG-negative patients were not available. CCHF RT-PCR was performed on 29 acute samples; of these, 11 were negative. All of these RT-PCR-negative samples were positive for specific IgM and seven were positive for IgG of CCHF virus as well. Three patients were positive by RT-PCR test only. One PCR product was sequenced, confirming CCHF virus identity. Eventually, 99 patients obtained a definitive diagnosis of CCHF. Seven of these were treated with ribavirin. These had been evaluated in another study and so were not included here (Ergonul *et al.*, 2004).

The cases were admitted to hospital between April and September of 2003. They were from the rural areas of 11 cities of the central and eastern part of Anatolia. It is noteworthy, however, that 80 % of the cases were concentrated in the rural areas of three bordering cities (Sivas, Tokat and Yozgat). Nosocomial transmission of CCHF was not detected in the centres studied.

Several demographic and clinical characteristics of patients who recovered and patients who died are presented in Table 1. In addition, there was no difference in the occurrence of high fever (73 % of cases), malaise (86 %), headache (80 %), nausea (75 %), vomiting (68 %), diarrhoea (33 %), conjunctival injection (42 %), heart murmur (4·9 %), cough (29 %) and rales (16 %) between patients who recovered and those who died. Striking features of the outbreak were an equal male to female ratio, reflecting the culture and lifestyle of Anatolian families, and a relatively low mortality rate (12 %) compared to reports from other countries (Khan *et al.*, 1997; Mardani *et al.*, 2003). Haemorrhagic manifestations were detected in 48 % of patients. These were epistaxis (17·4 %), haematemesis (7·6 %), melena (1·0 %) and multiple forms (21·7 %).

Mental status had been assessed on a clinical basis by physicians and any alteration from disorientation to coma was accepted as impaired consciousness. Results of key laboratory tests performed at the time of admission of patients are shown in Table 2. International normalized ratio, aspartate aminotransferase, lactic dehydrogenase and creatine phosphokinase values were significantly higher among the patients who died.

The following variables were entered into a hierarchical logistic regression model: impaired consciousness, splenomegaly, hepatomegaly, leukopenia and prolonged international normalized ratio (>1). Impaired consciousness and splenomegaly were selected as independent predictors of mortality by Wald probabilities. The model correctly predicted the deaths of seven of the 11 cases who died (sensitivity 63·6 %), and the survival of 64 of the 81 patients who survived (specificity 79 %). However, the sensitivity and specificity as well as the sample size are low, and accordingly, the significance should be evaluated with caution.

The blood fractions used in the treatment of the patients are shown in Table 3. Of the patients who died, seven had received fresh frozen plasma and five had received platelet suspensions, but none had received erythrocyte suspensions. Of the four patients who did not receive any blood products, three died soon after admission and one stayed in hospital for 11 days and did not receive any blood products but eventually died.

Three outcome measures were compared between patients who recovered and patients who died, namely, hospital discharge costs, length of hospital stay (LOS) and time

Table 1. Comparison of demographic and clinical characteristics between patients who recovered and patients who died

Variable	Non-fatal cases ($n = 81$)*	Fatal cases ($n = 11$)*	All cases*	P†
Male sex	42 (52)	4 (36)	46 (50)	0·52
Age	40·5 (16·8)	43·7 (15·6)	40·9 (16·6)	0·56
Farmer‡	74 (91)	9 (82)	83 (90)	0·29
Tick-bite history	50 (62)	5 (45)	55 (60)	0·34
Myalgia	59 (73)	10 (91)	69 (75)	0·28
Impaired consciousness	7 (9)	6 (55)	13 (14)	$< 0·01$
Lymphadenopathy	12 (15)	0 (0)	12 (13)	0·35
Splenomegaly	10 (12)	3 (27)	13 (14)	0·18
Hepatomegaly	27 (33)	1 (9)	28 (30)	0·16
Maculopapular rash	29 (36)	7 (64)	36 (39)	0·10
Haemorrhagic manifestations	37 (46)	7 (64)	44 (48)	0·34
Thrombocytopenia§	81 (100)	11 (100)	92 (100)	1·0
Leukopenia§	66 (80)	7 (64)	73 (78)	0·31
Anaemia§	27 (33)	1 (11)	28 (32)	0·26

*Values are presented as number of patients (%) except for age, which is presented as mean years (SD).

†P values obtained by Fisher's exact test for dichotomous variables and Student's t test for the continuous variable, age.

‡Farmers have close contact with livestock and are involved in slaughtering activity.

§Thrombocytopenia, platelet count $< 150 \times 10^9$ l^{-1}; leukopenia, leukocyte count $< 4 \times 10^9$ l^{-1}; anaemia, haemoglobin < 120 g l^{-1}.

Table 2. Medians and ranges of important laboratory tests

Test*	Median (range)			
	Non-fatal cases ($n = 81$)	Fatal cases ($n = 11$)	All cases	P†
INR	1 (0·84–1·69)	1·18 (1–2·39)	1·01 (0·84–2·39)	0·006
ALT	131·5 (18–2550)	244 (53–1958)	148 (18–2550)	0·18
AST	120·5 (14–6770)	898 (125–3460)	146 (14–6770)	0·001
LDH	1135·5 (262–11700)	4062 (600–25380)	1164 (262–25380)	0·044
CPK	355·5 (35–3117)	1214 (267–7590)	401 (35–7590)	0·009
Platelet	55·5 (5–134)	20 (6–82)	53 (5–134)	0·08
WBC	2·2 (0·7–11)	2·9 (1·5–4·2)	2·4 (0·7–11)	0·199

*INR, international normalized ratio; ALT, alanine aminotransferase (normal range, 7–56 U l^{-1}), AST, aspartate aminotransferase (normal range, 5–40 U l^{-1}); LDH, lactic dehydrogenase (normal range, < 200 U l^{-1}); CPK, creatine phosphokinase (normal range, < 170 U l^{-1}); platelet count (normal range, 150–450 $\times 10^9$ l^{-1}); WBC, white blood cell count (normal range, 4–11 $\times 10^9$ l^{-1}).

between the onset of the disease and the admission to a hospital. The difference in hospital discharge costs between patients who recovered (median, $1199; confidence interval, 1015·1–1517·4) and those who died (median, $2142·3; confidence interval, 271·5–5341·6) was not significant; whereas the difference in the LOS between patients who recovered (median, 10; range, 8–10) and those who died (median 2·5; range 0–9) was significant. Most of the patients were living in rural areas and so, depending on transportation problems, a delay in admission to central hospitals could have been a cause for adverse outcomes. However, the comparison of the time between the onset of the disease and admission to a hospital between patients who recovered and patients who died was not significant, such that the median (and range) values were 5 (5–6) and 5 (3–7) days, respectively.

Table 3. Blood fractions that patients received during their hospital stay

Blood fraction*	No. of patients	Mean†	SD	Range
Non-fatal cases ($n = 81$)				
FFP	29	7·3	5·2	1–23
Platelet	34	7·3	5·7	1–24
Erythrocyte	11	3·4	1·7	2–6
Fatal cases ($n = 11$)				
FFP	7	14·1	12·1	2–40
Platelet	5	20	28·3	3–70
Erythrocyte	0			

*FFP, fresh frozen plasma; Platelet, platelet suspension obtained by apheresis; Erythrocyte, erythrocyte suspension.

†Mean number of units received per patient.

DISCUSSION

In 2003 we detected 99 CCHF cases in various hospitals. A chart review in these hospitals discovered 36 more cases with fever and thrombocytopenia in 2002 but only a few in 2001 (data not shown). Retrospective data imply a rapid emergence of CCHF in Middle Anatolia. In other words, the CCHF outbreak is of a new clinical entity in these hospitals.

The mortality rate is lower in this study than previous reports, in which the mortality rates were 30 to 62 % (el-Azazy & Scrimgeour, 1997; Khan *et al.*, 1997; Mardani *et al.*, 2003). A possible explanation for the low mortality rate observed in this study could be the wide availability of blood products and other facilities in Turkish tertiary hospitals.

Ribavirin, a broad-spectrum antiviral agent, is supposed to be a potential therapeutic for CCHF, based on some experimental studies (Tignor & Hanham, 1993), anecdotal case reports (Fisher-Hoch *et al.*, 1995; van Eeden *et al.*, 1985; Watts *et al.*, 1989) and an open study in which the controls were historical (Mardani *et al.*, 2003). Ribavirin, therefore, is expected to improve the outcome variables such as LOS and mortality in Turkish hospitals as well. However, the literature reveals no blinded and randomized clinical trials of ribavirin against CCHF. In other words, reliable data are not available, and ribavirin, ultimately, has not been certified in the treatment of CCHF. On the other hand, the treatment costs are doubled with ribavirin use. This will cause a significant burden considering the already high treatment costs of CCHF in Turkey.

Significant increases in the cost of patient care, adverse effects of the drug and probable unnecessary treatments due to false and/or delayed diagnosis in routine practice complicate the decision over empirical ribavirin use on a routine basis. One strategy to control the costs and other problems could be to stratify the cases depending on the independent variables predicting an adverse outcome and to restrict the utilization of ribavirin to high-risk groups. This study indicated impaired consciousness and splenomegaly as independent predictors of adverse outcome. Nevertheless, the sample size was small. Moreover, the sensitivity and specificity of such discrimination was disappointing. Therefore, the Turkish CCHF study group recommended the use of ribavirin by Turkish hospitals in 2004 without any stratification and comparison of the results with the data from 2003 for future decisions.

Overall, 80 % of the cases in this study were concentrated in an area in Middle Anatolia. However, the source of this outbreak remains obscure. Two recently augmented problems in this region might be significant in this outbreak: (i) increases in uncontrolled animal trading with Iran, where this infection is endemic (Mardani *et al.*, 2003; Saidi *et al.*, 1975), and (ii) increases in the tick population in the region as a consequence of increased wild hosts of the ticks due to the prohibition of hunting in recent years. It is clear, however, that the true bases of this outbreak will remain unidentified until some local data become available through future studies. The most critical strategy against this outbreak would be to increase the preventive measures among the population living in the unsafe area by continuous education programmes and to restore the ecological balance in the tick-infested area.

This investigation demonstrated that infectious diseases restricted to certain areas in the past could rapidly emerge in other areas under the changing conditions of the world. Therefore, today, recommending a joint international effort to monitor and prevent infectious diseases seems to be more essential than ever.

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