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# Crimean-Congo hemorrhagic fever disease due to tick bite with very long incubation periods

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SUMMARY

*Background:* Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease with a high mortality rate, and is one of the viral hemorrhagic fever syndromes. The average mortality rate of CCHF is 3–30%. Research indicates that the longest incubation period after a tick bite is 12 days in CCHF disease. However, in clinical practice, we encounter patients with CCHF as a result of tick bites with much longer incubation periods (max. 53 days) than those reported in the literature. We present herein CCHF cases presumably infected through tick bites and having incubation periods longer than the upper limit reported in the literature.

*Methods:* We analyzed the cases of the 825 CCHF patients admitted to our hospital from 2007 to 2010 and found that 312 of them had undoubtedly been bitten by a tick. We searched the patient records for information on the incubation period and found that 12 patients had experienced an incubation period of over 12 days, which is the longest incubation period stated in the literature for patients definitely bitten by a tick.

*Results:* A total of 12 patients (eight males and four females, with a mean age of 45 years) were recruited into this study. Five (41.7%) of the 12 patients had positive CCHF virus-specific IgM antibodies, three (25%) had a positive reverse transcription polymerase chain reaction test for CCHF virus, and four (33.3%) had positive results in both tests during the acute and/or convalescent phase of the disease. In these cases, the interval between tick bite and the onset of symptoms was a mean of 23.6 days (range 13–53 days).

*Conclusion:* Physicians serving in endemic regions should be aware of these longer incubation periods after a tick bite. It is suggested that they perform more follow-ups on clinically and serologically highly suspected patients than they currently do.



## 1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease that has a high mortality rate, and is one of the viral hemorrhagic fever syndromes. The disease is caused by the CCHF virus belonging to the genus *Nairovirus* in the *Bunyaviridae* family. The average mortality rate of the disease is 3–30%.[1] CCHF came to the attention of modern medical science and was first described as a clinical entity in 1944–1945, and it remains an endemic disease in different regions of Africa, Asia, and Eastern Europe.[2]

Sporadic cases or epidemics can be seen in human beings.[3] Humans become infected by tick bites, crushing infected ticks, contact with a CCHF patient during the acute phase of infection, or by coming into contact with blood or tissues from viremic livestock.[1] Nosocomial transmission is a major mode of acquisition of CCHF infection and accounts for a significant portion of CCHF cases and outbreaks worldwide.[4] In general, the course of CCHF infection has four distinct phases: incubation, pre-hemorrhagic, hemorrhagic, and convalescence periods.[1] After a short incubation period, the CCHF reveals itself with generalized pain, myalgia, fever, nausea, vomiting, abdominal pain, diarrhea, ecchymoses, bleeding, and non-specific laboratory findings such as elevated liver enzymes, thrombocytopenia, and leukopenia.[1,5,6] The incubation period after a tick bite is usually 3–7 days,[7] but longer incubation periods than usual – 9 and 12 days – have also been reported.[8,9] The incubation period may differ depending on several factors, including viral dose and route of exposure.[1] However, it is difficult to obtain precise data on the length of the incubation period after a tick bite in CCHF disease.[1,10,11]

In clinical practice, we sometimes encounter patients with CCHF following a tick bite who have had incubation periods much longer than the longest periods reported in the literature. When

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this issue caught our attention, we reviewed the literature in order to determine whether similar cases exist or not. However, we found no studies conducted on CCHF patients presumably infected by tick bite that investigated whether these longer incubation periods existed or not. Hence the aim of this study was to report CCHF cases presumably infected through tick bite with incubation periods of longer than the upper limit reported in the literature.

## 2. Materials and methods

We analyzed the cases of the 825 CCHF patients admitted to our hospital from 2007 to 2010. Three hundred and twelve of these patients were undoubtedly bitten by a tick. We searched the patient records for information regarding the incubation period and found that 12 of the patients (3.8%) had experienced an incubation period of over 12 days, which is the longest incubation period reported in the literature among those patients undoubtedly bitten by a tick.

The study protocol was approved by the local human ethics committee. Ten of 12 patients had been followed up by the infectious diseases and clinical microbiology department, while two patients had been followed up by the pediatrics department of our center.

Tick bite was confirmed when tick removal had been performed by a doctor. Tick removal was documented following tick removal performed by the patient him/herself. Charts of all hospitalized CCHF patients were reviewed with respect to age, sex, history of tick bite or history of removing a tick, other risk factors for CCHF transmission, and interval between the tick bite and the onset of symptoms. Outcomes and clinical and laboratory findings were recorded for each patient. With the exception of tick bite, patients with other risk factors for CCHF transmission were excluded from the study. Patients who had removed the tick by themselves were excluded, because the history of the patient or of their relatives with regard to the timing of tick removal might have been unreliable. Patients with a history of tick bite, those knowing the exact date of the tick bite, and those with clinical complaints starting 13 days or more after the bite (by taking into consideration the longest period reported in the literature) were included in the study.

All serum specimens obtained for the definitive diagnosis of CCHF were stored at −70 °C until testing. Acute and convalescent phase serum samples were sent to the Virology Laboratory of Refik Saydam Hygiene Center in Ankara, Turkey for serological and virological analyses. The definitive diagnosis of CCHF infection was made based on typical clinical and epidemiological findings and the detection of CCHF virus-specific IgM by ELISA or of genomic segments of the CCHF virus by reverse transcription polymerase chain reaction (RT-PCR), either in the acute and/or convalescent phase of the disease.[1,12] Since there was no case reported with leptospirosis or hanta virus infection in our region, we did not rule out these infections in our cases.

## 3. Results

A total of 12/312 patients (3.8%), including nine males and three females, with a mean (range) age of 45 (15–77) years, were recruited into this study. The mean incubation period in these CCHF patients was 23.6 days (range 13–53 days).

Table 1 presents selected demographic, clinical, and laboratory data of the patients with CCHF grouped according to the incubation period (long incubation period $n = 12$; normal incubation period $n = 300$). There were significant differences between the study groups with regard to the incubation period (days) and in the percentages of patients with the most common symptom (fever), leukopenia, elevated alanine aminotransferase (ALT), a long activated partial thromboplastin time (aPTT), and death ($p < 0.05$). The percentages of patients with myalgia, headache, conjunctival hyperemia, rash, bleeding, thrombocytopenia, and elevated aspartate aminotransferase (AST) were comparable between those with a long incubation period and those with a normal incubation period ($p > 0.05$).

**Table 1**
Selected demographic, clinical and laboratory data of patients with Crimean-Congo hemorrhagic fever grouped according to the length of the incubation period

| | Long incubation period ($n = 12$) | Normal incubation period ($n = 300$) | $p$-Value |
|---|---|---|---|
| Age, years, mean (range) | 45 (15–77) | 45 (5–67) | NS |
| Sex, $n$ (%) | | | |
| Female | 4 (33.3) | 112 (37.3) | NS |
| Male | 8 (66.7) | 188 (62.7) | NS |
| Incubation period (days), mean (range) | 23.6 (13–53) | 5 (3–12) | <0.05 |
| Most common symptoms, $n$ (%) | | | |
| Myalgia | 4 (33.3) | 94 (31.3) | NS |
| Headache | 4 (33.3) | 98 (32.7) | NS |
| Fever | 8 (66.7) | 272 (90.7) | <0.05 |
| Physical findings, $n$ (%) | | | |
| Fever[a] | 4 (33.3) | 225 (75) | <0.05 |
| Conjunctival hyperemia | 5 (41.7) | 123 (41) | NS |
| Rash | | | |
| Maculopapular | 3 (25) | 91 (30.3) | NS |
| Petechiae/ecchymosis | 1 (8.3) | 30 (10) | NS |
| Bleeding | | | |
| Hematuria | 2 (16.7) | 59 (19.7) | NS |
| Hematemesis | 1 (8.3) | 22 (7.3) | NS |
| Selected laboratory tests, $n$ (%) | | | |
| Thrombocytopenia[b] | 12 (100) | 289 (96.3) | NS |
| Leukopenia[c] | 7 (58.3) | 251 (83.7) | <0.05 |
| Elevated AST (IU/l) | 10 (83.3) | 275 (91.7) | NS |
| Elevated ALT (IU/l) | 7 (58.3) | 247 (82.3) | <0.05 |
| Long aPTT (s) | 4 (33.3) | 195 (65) | <0.05 |
| Death | 0 (0) | 20 (6.7) | <0.05 |

NS, not significant; AST, aspartate aminotransferase; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time.
[a] Axillary, ≥38 °C.
[b] Thrombocytopenia, platelet count $<150 \times 10^9$/l.
[c] Leukopenia, leukocyte count $<4 \times 10^9$/l.

**Table 2**
Selected demographic, clinical and laboratory data for the 12 patients with a long incubation period

| Case No. | Age (years)/ gender | Incubation time (day) | Test time (days)[a] | Recovery time (days)[b] | CCHF- IgM | CCHF RT-PCR | PLT count[c] ($\times 10^9$/l) | WBC count[c] ($\times 10^9$/l) | ALT,[c] U/l | AST,[c] U/l | aPTT,[c] s |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 15/F | 15 | 16 | 12 | – | + | 124 | 7.1 | 14 | 28 | 26 |
| 2 | 16/M | 16 | 17 | 9 | – | + | 128 | 3.04 | 58 | 130 | 30 |
| 3 | 50/M | 27 | 30 | 10 | – | + | 72 | 5.3 | 244 | 418 | 41 |
| 4 | 64/F | 53 | 60 | 9 | + | – | 102 | 4.5 | 225 | 339 | 31 |
| 5 | 57/F | 15 | 20 | 9 | + | + | 39 | 2.5 | 167 | 331 | 44 |
| 6 | 71/M | 23 | 30 | 10 | + | – | 110 | 2.6 | 51 | 81 | 32 |
| 7 | 20/M | 13 | 14 | 7 | + | + | 65 | 3.0 | 81 | 157 | 48 |
| 8 | 17/M | 22 | 25 | 7 | + | + | 91 | 1.7 | 26 | 101 | 43 |
| 9 | 43/M | 41 | 45 | 11 | + | – | 89 | 2.1 | 46 | 95 | 47 |
| 10 | 47/M | 24 | 28 | 19 | + | + | 7 | 5.6 | 1451 | 2680 | 68 |
| 11 | 67/M | 14 | 17 | 11 | + | – | 57 | 1.6 | 53 | 115 | 40 |
| 12 | 77/M | 20 | 20 | 11 | + | – | 21 | 1.9 | 81 | 256 | 34 |

CCHF, Crimean-Congo hemorrhagic fever; RT-PCR, reverse transcription polymerase chain reaction; PLT, platelet; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; aPTT, activated partial thromboplastin time; F, female; M, male.
[a] Time between the beginning of symptoms and collection of serum samples for ELISA and PCR.
[b] Time after the onset of symptoms.
[c] At admission.

In the patients with a long incubation period, the incubation period was significantly longer than that of the patients with a normal incubation period (mean 23.6 (range 13–53) and mean 5 (range 3–12) days, respectively) ($p < 0.05$). Serological markers for viral hepatitis A, B and C, human immunodeficiency virus, cytomegalovirus, Toxoplasma, and Epstein–Barr virus were all negative in all patients. Tube agglutination assays for *Brucella spp* were also negative. In our patients, the most common symptom was fever (66.7%), while the most common laboratory finding was thrombocytopenia (100%).

Table 2 presents some of the demographic, clinical, and laboratory data of these 12 patients. The interval between the onset of symptoms and tick bite was 13, 14, 15, 15, 16, 20, 22, 23, 24, 27, 41, and 53 days, respectively. Five (41.7%) of the 12 patients had positive CCHF virus-specific IgM antibodies, three (25%) had a positive RT-PCR test for CCHF virus, and four (33.3%) were positive in both tests during the acute and/or convalescent phase of the disease. Five patients were bitten on the hips, while the other seven patients were bitten on the right hand ($n = 2$), right armpit, right ankle ($n = 2$), left arm, and left inguinal region. None of these patients died during follow-up.

## 4. Discussion

Patients infected with CCHF virus in Turkey were first reported from the provinces of Tokat, Amasya, and Sivas in 2002.[13–15] Since 2002, CCHF has become endemic in our country and large outbreaks have been seen in the service area of our training hospital during the spring and summer seasons. To date, 3135 confirmed CCHF cases have been reported in our country.[16]

The incubation period of CCHF disease depends on how the disease has been acquired. The incubation period is usually 1–3 days (maximum 12 days) in infections following tick bites. If the disease develops via blood or tissue contact, the incubation period is 5–6 days (maximum 13 days).[6,17,18] In our cases, the incubation periods after tick bite were evidently much longer than those reported in the literature.

The longer incubation periods seen in our patients may have occurred for a variety of reasons. Firstly, these patients might have been infected by a different strain that has a longer incubation period. Elevli et al.[19] have recently reported a new CCHF virus strain in Turkey with a longer incubation period. However, we could not perform phylogenetic studies. Secondly, the viral load received during the tick bite might have been lower in these patients; the clinical course of patients with higher viral loads is worse. Viral load is a useful predictor of disease progression and outcomes. A high viral load tends to indicate a fatal outcome.[20,21] The good survival rate for those patients with longer incubation periods may suggest that these patients had a lower viral load.[22,23] In addition, these patients had better laboratory results, including white blood cell counts, ALT, and aPTT, compared to patients with normal incubation periods.

During the summer, hundreds of people are admitted to the local hospitals due to tick bites. In general, physicians follow up their patients for a maximum of 10 days, because it is a presumed fact that the incubation period after a tick bite is usually 1–3 days, but a maximum of 12 days.[6,9,17,18] As we have reported, although infrequent (approximately 4%), the incubation period may be longer than usual – up to 53 days.

In conclusion, physicians serving in endemic regions should be aware of these longer incubation periods following tick bites, and should follow-up the clinically and serologically highly suspected patients for longer than they currently do.

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*Conflict of interest:* No conflict of interest to declare.

## References

1. Ergonul O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* 2006;**6**:203–14.
2. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res* 2004;**64**:145–60.
3. Alavi-Naini R, Moghtaderi A, Koohpayeh HR, Sharifi-Mood B, Naderi M, Metanat M, et al. Crimean-Congo hemorrhagic fever in southeast of Iran. *J Infect* 2006;**52**:378–82.
4. Vorou R, Pierroutsakos IN, Maltezou HC. Crimean-Congo hemorrhagic fever. *Curr Opin Infect Dis* 2007;**20**:495–500.
5. Ergün H, Çiftçi E. Crimean-Congo haemorrhagic fever. *Türkiye Klinikleri J Pediatr Sci* 2007;**3**:23–6.
6. Kara A. Crimean-Congo haemorrhagic fever. *Turk Arch Ped* 2008;**43**:108–18.
7. Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray MG, Erasmus MJ, et al. Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. *Am J Trop Med Hyg* 1987;**36**:120–32.
8. Republic of Turkey, Ministry of Health, The Basic Health Services General Directorate. Crimean-Congo hemorrhagic fever. 2nd ed. Ankara, Turkey: Onur Publication; 2005.
9. Bossi P, Tegnell A, Baka A, Van Loock F, Hendriks J, Werner A, et al. Task Force on Biological and Chemical Agent Threats, Public Health Directorate, European Commission, Luxembourg. Bichat guidelines for the clinical management of haemorrhagic fever viruses and bioterrorism-related haemorrhagic fever viruses. *Euro Surveill* 2004;**9**:11–2.
10. Hoogstraal H. The epidemiology of tick borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol* 1979;**15**:307–417.

11. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis* 1989;**11**:794–800.
12. Drosten C, Kummerer BM, Schmitz H, Gunther S. Molecular diagnostics of viral hemorrhagic fevers. *Antiviral Res* 2003;**57**:61–87.
13. Bakir M, Ugurlu M, Dokuzoguz B, Bodur H, Tasyaran MA, Vahaboglu H. Crimean-Congo haemorrhagic fever outbreak in Middle Anatolia: a multicentre study of clinical features and outcome measures. *J Med Microbiol* 2005;**54**:385–9.
14. Gozalan A, Akin L, Rolain JM, Tapar FS, Oncül O, Yoshikura H, et al. Epidemiological evaluation of a possible outbreak in and nearby Tokat province. *Mikrobiyol Bul* 2004;**38**:33–44.
15. Çiftçi E. An emerging infectious disease in Turkey: Crimean-Congo hemorrhagic fever. *J Pediatr Inf* 2009;**3**(Suppl 1):86–9.
16. Ministry of Health, Turkey. Reports of the Communicable Diseases Department. Ankara, Turkey: Ministry of Health; 2008.
17. Capua I. Crimean-Congo haemorrhagic fever in ostriches: a public health risk for countries of the European Union. *Avian Pathol* 1998;**27**:117–20.
18. Ergönül O. Viral haemorrhagic fevers. In: Willke TA, Söyletir G, Doğanay M, editors. *Infectious diseases and microbiology*. Istanbul: Nobel Tıp Publication; 2008. p. 1251–65.
19. Elevli M, Ozkul AA, Civilibal M, Midilli K, Gargili A, Duru NS. A newly identified Crimean-Congo hemorrhagic fever virus strain in Turkey. *Int J Infect Dis* 2010;**14**:213–6.
20. Schutten M, Niesters HG. Clinical utility of viral quantification as a tool for disease monitoring. *Expert Rev Mol Diagn* 2001;**1**:153–62.
21. Ergonul O, Celikbas A, Baykam N, Eren S, Dokuzoguz B. Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. *Clin Microbiol Infect* 2006;**12**:551–4.
22. Wölfel R, Paweska JT, Petersen N, Grobbelaar AA, Leman PA, Hewson R, et al. Virus detection and monitoring of viral load in Crimean-Congo hemorrhagic fever virus patients. *Emerg Infect Dis* 2007;**13**:1097–100.
23. Saksida A, Duh D, Wraber B, Dedushaj I, Ahmeti S, Avsic-Zupanc T. Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean-Congo hemorrhagic fever. *Clin Vaccine Immunol* 2010;**17**:1086–93.