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References

1. Baldwin A, Loughlin M, Caubilla-Barron J, Kucerova E, Manning G, Dowson C, et al. Multilocus sequence typing of *Cronobacter sakazakii* and *Cronobacter malonaticus* reveals stable clonal structures with clinical significance which do not correlate with biotypes. BMC Microbiol. 2009;9:223. http://dx.doi.org/10.1186/1471-2180-9-223
2. Joseph S, Desai P, Ji Y, Cummings CA, Shih R, Degoricij L, et al. Comparative analysis of genome sequences covering the seven Cronobacter species. PLoS ONE. 2012;7:e49455. http://dx.doi.org/10.1371/journal.pone.0049455
3. Joseph S, Cetinkaya E, Drahovska H, Levican A, Figueras M, Forsythe SJ. *Cronobacter condimenti* sp. nov., isolated from spiced meat and *Cronobacter universalis* sp. nov., a novel species designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water and food ingredients. Int J Syst Evol Microbiol. 2012;62:1277–83. http://dx.doi.org/10.1099/ijs.0.032292-0
4. Joseph S, Sonbol H, Hariri S, Desai P, McClelland M, Forsythe SJ. Diversity of the *Cronobacter* genus as revealed by multi locus sequence typing. J Clin Microbiol. 2012;50:3031–9. http://dx.doi.org/10.1128/JCM.00905-12
5. Joseph S, Forsythe SJ. Predominance of *Cronobacter sakazakii* ST4 in neonatal infections. Emerg Infect Dis. 2011;17:1713–5. http://dx.doi.org/10.3201/eid1709.110260
6. Centers for Disease Control and Prevention. CDC Update: investigation of *Cronobacter* infections among infants in the United States. January 13, 2012 [cited 2012 Aug 22]. http://www.cdc.gov/foodsafety/diseases/cronobacter/investigation.html
7. Kucerova E, Joseph S, Forsythe S. The *Cronobacter* genus: ubiquity and diversity. Quality Assurance and Safety of Crops & Foods. 2011;3:104–22. http://dx.doi.org/10.1111/j.1757-837X.2011.00104.x

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Seroprevalence of Crimean-Congo Hemorrhagic Fever Virus, Bulgaria

To the Editor: Crimean-Congo hemorrhagic fever (CCHF) is endemic in southern Russia, southeastern Europe, Africa, the Middle East, and southwestern Asia (*1*). The incidence and spread of the disease have increased in recent years. In Bulgaria, located on the Balkan Peninsula, CCHF is endemic. The disease was first described in the country in 1952 (*2*). Since then, a mandatory reporting system has been introduced. Most of Bulgaria is an ecologically favorable environment for CCHF virus (CCHFV) circulation in nature. In the 1970s, numerous virologic and serologic studies were performed by Vasilenko et al., who showed that the most affected age group was 21–50 years and that most of those with CCHF were male (65%) (cited in [*3*]). A genetic study showed that CCHFV strains in Bulgaria cluster together with strains from other Balkan countries and Russia (*2*). A vaccine consisting of chloroform-inactivated CCHFV was developed in 1974, and the currently used vaccine strain, isolated from a Bulgarian patient, was characterized genetically (*4*).

In the last 10 years, <10 CCHF cases have been registered annually in Bulgaria. Although the number of cases is lower than previously, the disease has spread into new areas (southeast, northeast, south-central provinces). In 2008, a cluster of cases was observed in southwestern Bulgaria (Blagoevgrad district), a low-risk CCHF area (*5*). Since then, a substantial number of cases have been reported in this district. During the past 4 years (2008–2011), 30 CCHF cases have been registered in Bulgaria, 12 from Blagoevgrad district, 8 from Burgas district, 4 each from Haskovo and Sliven districts, and 1 each from Kardjali and Shumen districts.

To estimate the current situation on CCHFV seroprevalence in both disease-endemic and -nonendemic areas in Bulgaria, we tested serum samples for CCHFV IgG antibodies using a commercially available ELISA kit (Vector Best, Novosibirsk, Russia). The serum samples were collected prospectively during 2011 from 1,018 healthy persons (50.2% male) from 13 districts: Sofia (n = 116), Blagoevgrad (n = 100), Pazardjik (n = 52), Stara Zagora (n = 36), Smolyan (n = 46), Yambol (n = 60), Haskovo (n = 108), Kardjali (n = 50), Sliven (n = 50), Burgas (n = 200), Shumen (n = 50), Ruse (n = 100), and Pleven (n = 50); they were then tested for CCHFV IgG antibodies with a commercially available ELISA kit (Vector Best). The median age of participants was 48 years (range 2–89 years). Persons previously vaccinated against CCHFV were excluded from the study.

Twenty-eight persons (2.8%) had IgG antibodies to CCHFV. The highest seroprevalence was observed in Burgas (7.6%), followed by Kardjali (6%), Pazardjik (5.8%), and Haskovo (4.6%) districts (Figure, Appendix, wwwnc.cdc.gov/EID/article/19/1/12-0299-F1.htm). Low seroprevalence levels were detected in Sliven (2%), Blagoevgrad (1%), and Ruse (1%) districts. Generally, these results are consistent with the number of reported cases in different districts. Notably, Kardjali and Pazardjik districts showed high CCHFV seroprevalence but single reported cases in the last

Table. Univariate and multivariate regression analysis of CCHFV seropositivity in human population, Bulgaria*

Variable	No. (%) IgG positive, n = 28	No. (%) IgG negative, n = 990	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p value	OR (95% CI)	p value
Age, y			1.00 (0.99–1.03)	0.494		
Median	46	48				
Range	20–83	2–89				
Sex				0.456		
M	16 (3.1)	495 (96.9)	1.33 (0.62–2.85)			
F	12 (2.4)	495 (97.9)	Ref			
Tick bite				<0.001		<0.001
Yes	15 (9.3)	147 (90.7)	6.62 (3.09–14.19)		5.40 (2.47–11.84)	
No	13 (1.5)	843 (98.5)	Ref			
Animal contact				0.253		
Yes	11 (3.7)	290 (96.3)	1.56 (0.72–3.38)			
No	17 (2.4)	700 (97.6)	Ref			
Farming				0.001		0.012
Yes	13 (6.7)	182 (93.3)	3.85 (1.80–8.23)		2.76 (1.25–6.08)	
No	15 (1.8)	808 (98.2)	Ref			

*CCHFV, Congo-Crimean hemorrhagic fever; OR, odds ratio; Ref, reference.

years. However, these regions were among the main endemic foci in the past. In contrast, the low seroprevalence rate found in district of Blagoevgrad conflicts with the high number of diagnosed CCHF cases, but this district has been at low risk for many years.

Multivariate analysis showed that having a former tick bite and farming were significant risk factors, while age and sex were not related to seropositivity (Table). Although no significant difference was seen among age groups, none of the samples from persons 0–19 years of age were seropositive, whereas seroprevalence levels were increasing in those 20–59 years (2.65%) and 60–89 years (3.37%). This increase would be expected because the probability of contacting the virus increases with age. The main risk factor for the 20–29 year age group was the tick bite, and farming and contact with animals were incriminated in the older age groups.

A similar study conducted in Greece, a neighboring country, showed an overall seroprevalence of 4.2%; slaughtering and agricultural activities were significant risk factors for CCHFV seropositivity (*6*). Notably, the seroprevalence levels in the Greek districts Rodopi and Evros (4.95% and 4.49%, respectively) were similar to those in neighboring Bulgarian districts Kardjali and Haskovo (6% and 4.6%, respectively).

We found that the risk for seropositivity was increased 5.4-fold in persons bitten by ticks. Increased tick aggressiveness in years that have favorable climatic conditions results in high rates of attacks on humans and an increased number of tick-borne diseases (*7*). A recent survey for CCHFV in ticks in Haskovo, Kardzhali, and Stara Zagora districts showed that 4.83%, 2.09%, and 1.46%, respectively, were infected by CCHFV, and that the most infected tick was *Hyalomma marginatum* (*8*). These results coincide with those of the current study because Kardzhali and Haskovo were among the districts with the highest seropositivity.

Because of the increasing spread of CCHFV in new foci, public health awareness of this problem is essential. Studies giving information about the spread and ecology of the virus can provide the necessary data for risk assessment analysis and even for prediction of epidemics.

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References

1. Papa A. Crimean-Congo hemorrhagic fever and hantavirus infections. In: Maltezou H, Gikas A, editors. Tropical and emerging infectious diseases. Kerala (India): Research Signpost; 2010. p. 49–73.
2. Papa A, Christova I, Papadimitriou E, Antoniadis A. Crimean-Congo hemorrhagic fever in Bulgaria. Emerg Infect Dis. 2004;10:1465–7. http://dx.doi.org/10.3201/eid1008.040162
3. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979;15:307–417.
4. Papa A, Papadimitriou E, Christova I. The Bulgarian vaccine Crimean-Congo haemorrhagic fever virus strain. Scand J Infect Dis. 2011;43:225–9. http://dx.doi.org/10.3109/00365548.2010.540036
5. Christova I, Di Caro A, Papa A, Castilletti C, Andonova L, Kalvatchev N, et al. Crimean-Congo hemorrhagic fever, southwestern Bulgaria. Emerg Infect Dis. 2009;15:983–5. http://dx.doi.org/10.3201/eid1506.081567

6. Sidira P, Maltezou HC, Haidich AB, Papa A. Seroepidemiological study of Crimean-Congo haemorrhagic fever in Greece, 2009–2010. Clin Microbiol Infect. 2012;18:E16–9. http://dx.doi.org/10.1111/j.1469-0691.2011.03718.x
7. Papa A, Chaligiannis I, Xanthopoulou K, Papaioakim M, Papanastasiou S, Sotiraki S. Ticks parasitizing humans in Greece. Vector Borne Zoonotic Dis. 2011;11:539–42. http://dx.doi.org/10.1089/vbz.2010.0036
8. Gergova I, Kunchev M, Kamarinchev B. Crimean-Congo hemorrhagic fever virus–tick survey in endemic areas in Bulgaria. J Med Virol. 2012;84:608–14. http://dx.doi.org/10.1002/jmv.23214

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Primary Multidrug-Resistant Leprosy, United States

To the Editor: Since the initiation of multidrug therapy for leprosy (Hansen disease) in the 1980s by using rifampin, dapsone, and clofazimine, resistance to rifampin and dapsone has been observed worldwide and is still prevalent (*1*,*2*). Because few alternative effective antileprosy drugs exist, resistance to these first-line drugs could seriously affect leprosy control programs. We report a documented case of primary multidrug-resistant (MDR) leprosy in the United States.

A man from American Samoa migrated to Hawaii at age 25 years and, at age 41 years, first sought care for generalized erythematous papules and plaques. A skin biopsy showed borderline lepromatous (BL) leprosy (Figure, panel A). He had no prior history of leprosy and no prior treatment. He was treated for 44 months with a daily regimen of dapsone (100 mg), clofazimine (100 mg), and rifampin (600 mg). He appeared to comply with this regimen, and the lesions slowly resolved. He remained free of any new lesions until 4 years after completing treatment, when multiple brown hyperpigmented patches appeared on his lower legs. A skin biopsy showed only hemosiderin deposition but no organisms.

At 51 years of age, 6 years after completing treatment, the man again sought care for a 2-week history of multiple generalized erythematous papules and plaques on his face, trunk, and extremities. Some lesions were pruritic but nontender. A skin biopsy showed chronic inflammatory infiltrates with numerous acid-fast bacilli (Figure, panel B). Clinically considered to have relapsed BL leprosy, he was again treated daily with dapsone (100 mg), clofazimine (50 mg), and rifampin (600 mg). After 1 month of this regimen, no clinical improvement was observed.

Real-time PCR using the *Mycobacterium leprae*–specific repetitive element assay (*3*) confirmed the presence of *M. leprae* in biopsy specimens taken at the initial diagnosis and at relapse. Molecular genotyping of these samples with a panel of single-nucleotide polymorphism (SNP) and variable number of tandem repeat (VNTR) markers (*4*) showed that both biopsy specimens harbored *M. leprae* with the identical SNP subtype 3I and VNTR profile. PCR/DNA sequencing of the drug resistance–determining regions of *M. leprae* from these samples showed mutations within codon 53 of the *folP1* gene (ACC→GCC) and in codon 425 of the *rpoB* gene (TCG→TTG). These mutations have been characterized to induce high-level resistance to dapsone and rifampin, respectively (*5*,*6*). Careful evaluation of electropherograms of these drug resistance–determining regions showed only the resistant alleles in both strains.

These data indicated that this patient had been infected with MDR *M. leprae* before his initial treatment for leprosy. Therefore, when he was initially treated with leprosy multidrug therapy, he was essentially given clofazimine monotherapy. This treatment appears to have resulted in a slow, temporary clinical improvement. After relapse, he was placed on a daily regimen of clofazimine (100 mg), clarithromycin XL (500 mg), and minocycline (100 mg). The lesions clinically improved within 2 weeks, and the patient no longer noted any pruritus or tenderness in the lesions.

This report documents a case of primary MDR leprosy in the United States. In evaluating several previous biopsy samples from other patients in Hawaii, we have not seen any rifampin-resistant or MDR isolates. Health officials in American Samoa, the patient's country of origin, indicated that they were not aware of drug-resistant *M. leprae* among their patients (D. Scollard, pers. comm.). The patient reported no family history of leprosy, and no other contact could be identified. The origin of the MDR *M. leprae* in this case cannot be definitively determined.

Drug-resistant leprosy, including dapsone- and rifampin-resistant and MDR leprosy, has been reported in other parts of the world, usually in association with relapse after insufficient therapy (*1*,*2*). Relapses in leprosy are not usually seen until many years after completion of treatment (*7*,*8*). In the United States, among patients treated for 2 years with a multidrug protocol involving daily rifampin, no relapses were observed after 10–15 years' follow-up (*9*). Most new or worsening skin lesions clinically suspected to be relapses are actually leprosy reactions (*10*),