

## ***Clostridium difficile* Infection Caused by Binary Toxin–Positive Strains**

**To the Editor:** With interest, we read the article by Bacci et al. in which they conclude that *Clostridium difficile* strains containing the binary toxin gene were associated with a higher case-fatality rate after 30 days, even when the analysis was stratified for PCR ribotype (1). Although this was an appealing conclusion, in our opinion the study was severely limited by selection bias and confounding by underlying diseases. First, in Danish patients with a *C. difficile* infection (CDI), isolates were characterized only if they were isolated during outbreaks or from patients with severe disease or if isolates were found to be moxifloxacin resistant. Therefore, selection bias was likely to occur. Second, adjustment for concurrent conditions was not performed. This adjustment was especially warranted because outbreaks on specific hospital wards (e.g., intensive care units) could have influenced the all-cause mortality rate. Last, the selection of specific patients and strains questions the generalizability of the authors' conclusion.

In an approach to confirm the findings of Bacci et al. (1), we used data from a cohort study conducted during 2006–2009 in 13 Dutch hospitals (2). A total of 1,350 consecutive hospitalized

patients with unformed feces and a positive *C. difficile* toxin test result were included in the study. We checked the 30-day survival for study patients in the Dutch Civil Registration System. For 626 (46%) of the patients, a *C. difficile* strain was available for PCR ribotyping and binary toxin gene characterization. Patient data (e.g., age, sex, hospitalization, and antimicrobial drug use in the 3 months before onset of diarrhea) were collected by review of electronic and paper patient charts and by contacting the treating physician. Underlying diseases present at hospital admission were classified into 7 disease categories (Table, footnote). In addition, during at least 6 months, the Charlson comorbidity index at admission was determined in 9 of the 13 hospitals (total of 357 CDI patients). Proportional hazards modeling was used for survival analysis. The Medical Review Ethics Committee of the Leiden University Medical Center approved this study

During the study period, CDI was endemic in all hospitals in the cohort study (13 cases/10,000 admissions). The all-cause risk for dying within 30 days was 22% (12/55) for persons infected with binary toxin–positive 027 strains, 15% (15/100) for those infected with binary toxin–positive non-027 strains, and 11% (50/471) for those infected with binary toxin–negative strains (Table). Selection bias (e.g., by primarily characterizing isolates of patients with severe disease) was

unlikely because the number of deaths among CDI patients without strain characterization (100/724 [14%]) was similar to that among patients with a characterized strain (77/626 [12%];  $p = 0.41$ ). Thirty-day mortality rates were significantly higher among patients with CDI due to type 027 strains than among patients with binary toxin–negative strains (hazard ratio [HR] 2.2); additional adjustment for age and concurrent condition(s) resulted in a relatively constant HR of 2.0–2.4. Patients with CDI due to binary toxin–positive non-027 strains did not have a substantially higher 30-day mortality rate (HR 1.5); additional adjustment for age and concurrent condition(s) lowered the HR to 1.1–1.4, depending on the method of adjustment.

In accordance with findings in the Danish study, we observed a high 30-day mortality rate among persons infected with type 027 isolates. The 30-day mortality rate was lower among persons infected with non-027 binary toxin–positive isolates, especially after correction for concurrent condition(s); however, confidence intervals overlapped with those for type 027. Therefore, we cannot statistically contradict the conclusion of Bacci et al. (1). Nevertheless, because mortality rates in our study among patients with non-027 type CDI strongly resembled mortality rates among patients with CDI caused by binary toxin–negative isolates and because the Danish study was prone to bias and

Table. Thirty-day mortality rates, stratified by binary toxin status, for persons with *Clostridium difficile* infection, the Netherlands, 2006–2009\*

Binary toxin status of infecting strain	Absolute 30-day mortality		Relative 30-day mortality, HR (95% CI)†				
	No.	% (95% CI)	No adjustment	Adjusted for age	Adjusted for age and concurrent condition		
					Method 1	Method 2	Method 3
Positive							
027, N = 55	12	22 (13–35)	2.2 (1.2–4.2)	2.0 (1.1–3.8)	2.4 (1.1–5.5)	2.0 (0.8–5.4)	2.0 (0.7–5.5)
Non-027, N = 100	15	15 (9–23)	1.5 (0.8–2.6)	1.4 (0.8–2.5)	1.3 (0.6–2.7)	1.1 (0.5–2.8)	1.1 (0.4–2.9)
Negative, N = 471‡	50	11 (8–14)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Unknown, N = 724§	100	14 (11–17)	1.3 (0.9–1.9)	1.3 (0.9–1.9)	1.4 (0.9–2.2)	1.5 (0.9–2.3)	1.3 (0.7–2.4)

\*HR, hazard ratio; ref, reference.

†In the model, age and Charlson index were added as continuous variables; all others were dichotomous. Method 1, adjusted for age and history of admissions and antimicrobial drug use in the prior 3 mos. Method 2, adjusted for age; diseases of the respiratory, digestive, circulatory, and genitourinary systems; endocrine diseases; neoplasms and other diseases; history of admissions and antimicrobial drug use in the prior 3 mos. Method 3, adjusted for age; history of admissions; antimicrobial drug use in the prior 3 mos.; and Charlson comorbidity index.

‡Binary toxin–positive non-027 strains belonged to 8 PCR ribotypes (76% type 078).

§Binary toxin–negative strains belonged to 64 different PCR ribotypes (23% type 014).

lacked adjustment for confounding, we think that the results of Bacci et al. (1) should be interpreted with caution. Furthermore, a large clinical study from 2008 concluded that *C. difficile* type 078, which is the most frequently found binary toxin positive non-027 strain, was not associated with a high all-cause mortality rate (3). A more recent publication confirmed this finding (4). Therefore, in our opinion, there is currently no convincing epidemiologic proof that binary toxin is a marker for infection with virulent *C. difficile*.

This work was supported by a grant from ZonMw (the Netherlands Organization for Health Research and Development; grant 4726).

### Marjolein P.M. Hensgens and Ed J. Kuijper

Author affiliation: Leiden University Medical Center, Leiden, the Netherlands

DOI: <http://dx.doi.org/10.3201/eid1909.110814>

### References

1. Bacci S, Mølbak K, Kjeldsen MK, Olsen KEP. Binary toxin and death after *Clostridium difficile* infection. *Emerg Infect Dis*. 2011;17:976–82. <http://dx.doi.org/10.3201/eid1706.101483>
2. Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BHB, Kuijper EJ. All-cause and disease specific mortality in hospitalized patients with *Clostridium difficile* infections; a multicenter cohort study. *Clin Infect Dis*. 2013 Jan 13 Epub ahead of print.
3. Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW, et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin Infect Dis*. 2008;47:1162–70. <http://dx.doi.org/10.1086/592257>
4. Walk ST, Micic D, Jain R, Lo ES, Trivedi I, Liu EW, et al. *Clostridium difficile* ribotype does not predict severe infection. *Clin Infect Dis*. 2012;55:1661–8. <http://dx.doi.org/10.1093/cid/cis786>

Address for correspondence: Ed J. Kuijper, Department of Medical Microbiology, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, the Netherlands; email: [e.j.kuijper@lumc.nl](mailto:e.j.kuijper@lumc.nl)

## Spread of Kyasanur Forest Disease, Bandipur Tiger Reserve, India, 2012–2013

**To the Editor:** Kyasanur Forest disease virus (KFDV; family *Flaviviridae*, genus *Flavivirus*) was first recognized in 1956 in Shimoga District, Karnataka State, India (1). The natural cycle of KFDV involves 2 monkey species—black-faced langurs (*Semnopithecus entellus*) and red-faced bonnet monkeys (*Macaca radiata*)—and various tick species (genus *Haemaphysalis*). Monkeys become infected with KFDV through the bite of infected ticks; the virus is then transmitted to other ticks feeding on infected monkeys. KFDV infection causes severe febrile illness in some monkeys. When infected monkeys die, ticks drop from the body, thereby generating hot spots of infectious ticks that further spread the virus. In the enzootic state, KFDV circulates through small mammals (e.g., rodents, shrews, ground birds) and ticks (2).

Humans can also be infected with KFDV. In humans, the disease causes high fever, frontal headache, and severe myalgia, followed by bleeding from the nasal cavity, throat, gingivae, and, in some cases, gastrointestinal tract (3). In the natural KFDV cycle, humans are dead-end hosts.

KFD is unique to 5 districts (Shimoga, Chikkamagalore, Uttara Kannada, Dakshina Kannada, and Udupi) in the Malnad region of Karnataka State, India, where each year during January–May, 100–500 persons are affected by the disease (2,4). During December 2011–March 2012, a total of 215 suspected KFD case-patients were identified in 80 villages in Shimoga District; laboratory testing confirmed that 61 (28%) were infected with KFDV (5).

In November 2012, the deaths of 12 monkeys in Bandipur National Park, Chamarajanagara District,

Karnataka State, were reported. At the same time, 6 humans from Mole Hole village and Madhur colony in the Bandipur Tiger Reserve who handled and incinerated the sick monkeys were reported to have clinical signs and symptoms typical of KFD (online Technical Appendix Figure 1, [wwwnc.cdc.gov/EID/article/19/9/12-1884-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/19/9/12-1884-Techapp1.pdf)). The monkey handlers (20–55 years of age) were admitted to the local hospital in Gundlupet Taluk. Monkey autopsy specimens, serum samples from suspected human case-patients, and tick pools were collected by staff from the Virus Diagnostic Laboratory in Shimoga. The samples were sent to the National Institute of Virology in Pune for determination of the etiologic agent. Additional samples from humans with suspected KFDV infection, monkeys, and tick pools were received from Chamarajanagar District and adjoining border areas of Tamil Nadu State and Kerala State (Table).

Monkey brain and liver and tick pools were sonicated in 600 mL of Minimum Essential Media (GIBCO/BRL, Life Technologies, Grand Island, NY, USA), and 400 mL of media was added to the homogenate. TriPure Isolation Reagent (Roche Diagnostics, Indianapolis, IN, USA) was used to perform RNA extraction as described (6).

Samples were tested for KFDV by nested reverse transcription PCR (RT-PCR) and real-time RT-PCR as described (6); 12 of 21 human samples and 4 monkey samples were positive (Table). Two of 14 tick pools screened for KFDV by real-time RT-PCR were positive; however, 1 was weakly positive (Table). The PCR-amplified products were purified by using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) and then sequenced. KFDV sequences from the samples showed 95.8%–98.1% similarity with prototype strain KFDV P9605. This finding supports the earlier conclusion that a high level of conservation exists for KFDV sequences (7). The phylogenetic tree formed 2 clades: the first