

# Field Evaluation of Capillary Blood Samples as a Collection Specimen for the Rapid Diagnosis of Ebola Virus Infection During an Outbreak Emergency

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**Background.** Reliable reverse transcription polymerase chain reaction (RT-PCR)-based diagnosis of Ebola virus infection currently requires a blood sample obtained by intravenous puncture. During the current Ebola outbreak in Guinea, we evaluated the usability of capillary blood samples collected from fingersticks of patients suspected of having Ebola virus disease (EVD) for field diagnostics during an outbreak emergency.

**Methods.** A total of 120 venous and capillary blood samples were collected from 53 patients admitted to the Ebola Treatment Centre in Guékédou, Guinea, between July and August 2014. All sample specimens were analyzed by RT-PCR using the RealStar Filovirus Screen RT-PCR Kit 1.0 from Altona Diagnostics (Germany). We compared samples obtained by venipuncture and those obtained by capillary blood sampling absorbed onto swab devices.

**Results.** The resulting sensitivity and specificity of tests performed with capillary blood samples were 86.8% (95% confidence interval [CI], 71.9%–95.6%; 33/38 patients) and 100% (95% CI, 84.6%–100%; 22/22 patients), respectively.

**Conclusions.** Our data suggest that capillary blood samples could serve as an alternative to venous blood samples for the diagnosis of EVD in resource-limited settings during a crisis. This can be of particular advantage in cases when venipuncture is difficult to perform—for example, with newborns and infants or when adult patients reject venipuncture for cultural or religious reasons.

**Keywords.** Ebola virus; Ebola virus disease; field diagnostic; capillary blood.

The current Ebola virus outbreak in West Africa was first described in March 2014 in Guinea [1]. Since then, Ebola

has widely spread to the neighboring countries of Liberia and Sierra Leone. Although intense and widespread transmission of the current Ebola outbreak has been limited to these 3 countries, cases were also reported in Mali, Nigeria, and Senegal as well as in Europe and the United States [2, 3]. As of 11 March 2015, the World Health Organization (WHO) reported a total of 24 282 cases of Ebola virus disease (EVD) with 9976 deaths [4], making the ongoing outbreak in West Africa by far the geographically most widespread and most complex Ebola outbreak since Ebola virus was first discovered in 1976 [5]. Reported EVD case fatality rates vary from 31% to 74% in the main affected West African countries [6–11].

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Effective outbreak containment measures include the strict isolation of EVD patients, which requires reliable diagnosis. Current methods to diagnose suspected Ebola virus infection include reverse transcription polymerase chain reaction (RT-PCR), antigen-capture enzyme-linked immunosorbent assay (ELISA), and immunoglobulin M (IgM) and immunoglobulin G (IgG) ELISA [12–19]. While detection of viral RNA or viral antigen has been proven effective for diagnosis of Ebola virus infections from the early until late stage of illness, serological detection of specific IgM and IgG antibodies can only be used for diagnosis of EVD at the late stage in the acute phase of the disease and during convalescence.

All current techniques for the reliable laboratory diagnosis of Ebola virus infection require whole blood, plasma, or serum collected by venipuncture. In addition, the use of oral fluid specimens has been suggested as a noninvasive, sensitive method for the detection of Ebola virus infections during outbreak investigations [20].

Venous blood sampling requires trained and skilled medical personnel, bears high risk for needlestick injuries, and is especially difficult to perform with newborns and infants. Moreover, patients in African settings often refuse venipuncture due to cultural and religious beliefs. To overcome these problems, we evaluated the suitability of capillary blood sampling from fingersticks in the diagnosis of EVD. The main objective of this study was to compare the sensitivity and specificity of tests that used capillary blood samples with samples obtained by venous puncture during an outbreak emergency. Furthermore, we assessed in a limited study the stability of capillary blood swab samples when stored at room temperature vs 4°C.

## MATERIALS AND METHODS

### Ethical Approval

The National Committee of Ethics in Medical Research of Guinea approved the use of diagnostic leftover samples for this study (permit number 11/CNERS/14). As the samples had been collected as part of the public health response to contain the outbreak in Guinea, informed consent was not obtained from patients.

### Sample Collection

The study was conducted during the current outbreak of EVD in Guinea between July and August 2014. The study included 53 patients presenting with symptoms compatible with the WHO case definition for EVD [21]. Clinical samples were collected by Médecins sans Frontières (MSF) medical health personnel at the Ebola Treatment Centre (ETC) in Guéckédou, Guinea, wearing personal protective equipment following MSF safety guidelines.

For each patient, whole blood obtained by venipuncture and capillary blood samples taken from fingersticks were collected at the same time. Venipuncture whole blood samples were

collected using 6.0 mL BD Vacutainer collection tubes containing ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson, East Rutherford, New Jersey). Capillary whole blood samples were collected from fingerpricks using BD Microtainer contact activated lancets, type 21G (Becton Dickinson, East Rutherford, New Jersey). Collected capillary blood was adsorbed on viscose swab collection applicators (Sarstedt, Germany) and immediately placed into a labeled sterile collection tube without viral transport medium. Samples were stored at room temperature (the mean temperature in Guéckédou is 23.8°C in July and August) until they were transferred to the laboratory for analysis. All samples were packaged with a triple packaging system following WHO safety recommendations for sample shipment. All sample tubes, plastic containers, and sealed plastic bags used for transport were surface disinfected with a 0.5% hypochlorite solution, and transported to the European Mobile Laboratory in Guéckédou, where they arrived on average within 1 hour after collection. Matching venipuncture blood sample and fingerstick sample specimen from the same patient were labeled with the same unique laboratory identification number and an additional mark for capillary blood samples. All sample specimens were subjected to the same handling and storage conditions. One hundred twenty venous and capillary blood samples from 53 patients with suspected EVD were obtained.

### Laboratory Tests

Infectious sample specimens were handled in the European Mobile Laboratory in Guéckédou, Guinea, by qualified and trained laboratory personnel using a mobile safety glovebox cabinet, equipped with P3 and ABC filters and operated with negative pressure. All venous blood samples and capillary blood samples were examined for detection of Ebola virus RNA by RT-PCR using RealStar Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics, Germany) [22], specifically targeting the L gene as described previously [15]. Negative and positive controls were included in every batch of samples analyzed. To ensure the integrity of Ebola virus-specific real-time RT-PCR results by indicating potential RT-PCR inhibition, an internal control was analyzed in parallel for each patient sample. RT-PCR was performed using the SmartCycler technology (Cepheid). To this end, aliquots (140 µL) from each sample were inactivated by adding 560 µL of the guanidine thiocyanate lysis buffer AVL. Following 10 minutes of incubation, 560 µL of absolute ethanol was added to the aliquots. All sample tubes were surface disinfected using 0.5% hypochlorite solution for 5 minutes prior to release from the glovebox. For RT-PCR analysis of venipuncture blood samples, 50 µL of EDTA whole blood (plus 90 µL of nuclease-free water; Qiagen, Germany) or 140 µL plasma was used for RNA extraction using the QIAmp Viral RNA kit (Qiagen, Germany).

Plasma was obtained by centrifugation of EDTA whole blood at 3500 rpm for 10 minutes. Comparison of Ebola viral loads

using RT-PCR in 50  $\mu$ L of EDTA whole blood or 140  $\mu$ L plasma revealed similar results (unpublished field data). Swabs were swirled in 300  $\mu$ L of nuclease-free water (Qiagen, Germany) in a 1.5-mL Eppendorf tube to redissolve biological material, and 140  $\mu$ L of supernatant was used for RNA extraction with the QIAmp Viral RNA kit. Extraction and purification of RNA from venous and capillary blood samples was done according to the manufacturer's instructions with an additional second wash step using AW2 buffer. For differential diagnosis, admitted patients in the ETC were tested for malaria infections using venous EDTA whole blood for the qualitative detection of *Plasmodium* species antigens in a commercially available in vitro immunochromatographic assay using BinaxNOW Malaria (Alere).

### Statistical Analysis

To determine sensitivity and specificity of the capillary blood sample tests, RT-PCR results were compared to those obtained with venous blood samples. Sensitivity and specificity of the tests were calculated using a 2-by-2 contingency table.

## RESULTS

During the Ebola outbreak in Guinea, 53 Guinean patients presenting with clinical symptoms of EVD were included in the study. Among the 53 patients, 18 (33.9%) were men and 35 (66.1%) were women. The mean age was 27.4 years (range, 1–80 years). Venous and capillary blood samples were collected simultaneously from each patient and analyzed for the presence of Ebola virus RNA by real-time RT-PCR. Of the 60 capillary blood samples analyzed, 33 tested positive and 27 negative for EVD. For the 60 corresponding venous blood samples, 38 tested positive and 22 negative for EVD. Identical results between capillary and venous samples were observed for 55 samples, while 5 samples revealed false-negative results using capillary blood samples. For the 55 capillary blood samples with identical results, 33 tested EVD positive and 22 tested EVD negative. In our study, no false-positive results were observed using capillary blood samples as a diagnostic specimen. The sensitivity and the specificity of tests performed with capillary blood samples absorbed onto swab devices compared to tests performed with venous blood samples were 86.8% (95% confidence interval [CI], 71.9%–95.6%; 33/38 patients) and 100% (95% CI, 84.6%–100%; 22/22 patients), respectively (Table 1). Table 2 summarizes the comparative analysis of RT-PCR-based EVD detection in venous blood and capillary blood samples according to the time of sampling after onset of clinical symptoms. With the exception of 1 sample pair, pairwise comparison of venous and corresponding capillary blood samples revealed lower levels of Ebola RNA in capillary blood samples, regardless of the time to sampling after symptom onset. False-negative RT-PCR results caused by RT-PCR

**Table 1. Sensitivity and Specificity of Tests Performed on Capillary Blood Specimens for the Diagnosis of Ebola Virus Infection**

Ebola Virus Detection With Capillary Blood Samples	Ebola Virus Detection With Standard Venous Blood Samples		
	Positive	Negative	Total
Positive	33	0	33
Negative	5	22	27
Total	38	22	60

Data are No. of samples. Results of Ebola virus detection in capillary finger-stick samples were compared with samples obtained by venous blood puncture using real-time reverse transcription polymerase chain reaction. The sensitivity for detection of Ebola virus in capillary blood samples was 86.8% (95% confidence interval [CI], 71.9%–95.6%), the specificity was 100% (95% CI, 84.6%–100%), and the negative likelihood ratio was 0.13 (95% CI, .06–.30).

inhibition have been reported for patients with viral hemorrhagic fevers [23]. However, analysis of cycle threshold (Ct) values for the corresponding internal control for each individual patient sample indicated that a higher Ct value or even a negative RT-PCR result for Ebola virus-specific RNA in capillary blood samples was not due to severe RT-PCR inhibition (Supplementary Tables 1 and 2, Supplementary Figure 1).

The discrepancy in Ct values may stem from differences in recovery efficiency of viral RNA between both sample specimen types. Alternatively, differences in viral load levels in capillary and venous blood may account for the observed Ct variations, particularly in the early phase of symptomatic patients. Sequential sampling of an individual patient allowed us to monitor Ebola viral load levels in venous and capillary blood samples during the course of clinical illness (Table 3). This patient, suspected of having EVD based on clinical symptoms, was admitted 1 day after the onset of symptoms to the ETC. At the day of admission, laboratory results were negative for EVD in both sample types. RT-PCR testing was repeated 48 hours later and the patient tested positive for Ebola RNA in the venous blood sample but remained Ebola RNA negative in the capillary blood sample. On day 4, both sample types tested EVD positive, with the venous blood sample showing a higher viral load compared with the capillary blood sample. On day 7, EVD-positive test results revealed similar Ct values for capillary blood and venous blood, indicating comparable viral load. These data indicate potential differences in viremia between venous and capillary blood according to time of disease onset. This might also explain the 2 additional discordant results between venous blood and capillary blood from day 2 and day 3 after onset of symptoms (Table 2, samples 7 and 19). Yet with low numbers of patients, further studies are needed to compare the kinetics of viral load in venous and capillary blood during the course of infection.

**Table 2. Comparison of Ebola Virus Load Between Venous Blood and Capillary Blood Samples Using Real-time Reverse Transcription Polymerase Chain Reaction**

No. of Tested Sample	Patient No.	Day of Sampling After Symptom Onset	EVD Detection in Venous Blood (Ct Value)	Semiquantitative Viral Load Result <sup>a</sup>	EVD Detection in Capillary Blood (Ct Value)	Semiquantitative Viral Load Result <sup>b</sup>	Ct Difference Between Venous and Capillary Blood Samples <sup>c</sup>
1	Patient 1	Day 1	Positive (22.47)	+++	Positive (23.44)	+++	+0.97
2	Patient 2	Day 1	Positive (15.49)	+++	Positive (18.42)	+++	+2.93
3	Patient 3	Day 1	Positive (25.59)	++	Positive (29.26)	++	+3.67
4	Patient 4	Day 2	Positive (20.31)	+++	Positive (28.28)	++	+7.97
5	Patient 5	Day 2	Positive (21.38)	+++	Positive (26.39)	++	+5.01
6	Patient 6	Day 2	Positive (26.03)	++	Positive (30.47)	++	+4.44
7	Patient 7	Day 2	Positive (30.48)	++	Negative (–)	–	NA
8	Patient 8	Day 3	Positive (16.42)	+++	Positive (18.27)	+++	+1.85
9	Patient 9	Day 3	Positive (18.10)	+++	Positive (19.65)	+++	+1.55
10	Patient 10	Day 3	Positive (28.08)	++	Positive (21.01)	+++	–7.07
11	Patient 11	Day 3	Positive (23.39)	+++	Positive (26.46)	++	+3.07
12	Patient 12	Day 3	Positive (18.77)	+++	Positive (21.45)	+++	+2.68
13	Patient 13	Day 3	Positive (23.94)	+++	Positive (28.65)	++	+4.71
14	Patient 14	Day 3	Positive (24.32)	+++	Positive (27.26)	++	+2.94
15	Patient 15	Day 3	Positive (22.64)	+++	Positive (26.42)	++	+3.78
16	Patient 16	Day 3	Positive (20.74)	+++	Positive (25.32)	++	+4.58
17	Patient 17	Day 3	Positive (19.48)	+++	Positive (20.60)	+++	+1.12
18	Patient 18	Day 3	Positive (26.17)	++	Negative (–)	–	NA
19	Patient 19	Day 3	Positive (27.03)	++	Negative (–)	–	NA
20	Patient 20	Day 4	Positive (15.31)	+++	Positive (20.47)	+++	+5.16
21	Patient 18	Day 4	Positive (22.23)	+++	Positive (28.41)	++	+6.18
22	Patient 21	Day 4	Positive (20.19)	+++	Positive (25.87)	++	+5.68
23	Patient 22	Day 4	Positive (21.46)	+++	Positive (24.36)	+++	+2.90
24	Patient 23	Day 4	Positive (17.71)	+++	Positive (20.29)	+++	+2.58
25	Patient 24	Day 5	Positive (19.01)	+++	Positive (26.12)	++	+7.11
26	Patient 25	Day 5	Positive (21.12)	+++	Positive (23.74)	+++	+2.62
27	Patient 4	Day 5	Positive (21.02)	+++	Positive (28.10)	++	+7.08
28	Patient 26	Day 5	Positive (21.99)	+++	Positive (23.72)	+++	+1.73
29	Patient 27	Day 6	Positive (17.06)	+++	Positive (21.33)	+++	+4.27
30	Patient 28	Day 6	Positive (21.84)	+++	Positive (27.45)	++	+5.61
31	Patient 29	Day 6	Positive (22.04)	+++	Positive (28.05)	++	+6.01
32	Patient 30	Day 6	Positive (21.74)	+++	Positive (26.00)	++	+4.26
33	Patient 18	Day 7	Positive (16.00)	+++	Positive (17.00)	+++	+1.00
34	Patient 31	Day 9	Positive (25.04)	++	Positive (30.01)	++	+4.97
35	Patient 32	Day 15	Positive (31.77)	++	Negative (–)	–	NA
36	Patient 33	Day 17	Positive (17.04)	+++	Positive (19.43)	+++	+2.39
37	Patient 34	Day 18	Positive (24.35)	+++	Positive (25.60)	++	+1.25
38	Patient 35	Not known	Positive (30.61)	++	Negative (–)	–	NA

Abbreviations: Ct, cycle threshold; EVD, Ebola virus disease; NA, not applicable.

<sup>a</sup> Ct values are classified in subsequent categories of 0–24 (highly positive), 25–34 (positive), and 35–40 (weak positive) and correspond with +++, ++, and + results, respectively.

<sup>b</sup> Negative real-time reverse transcription polymerase chain reaction result is indicated by “–.”

<sup>c</sup> For Ct differences, “+” indicates higher viral load in venous blood sample, whereas “–” indicates higher viral load in capillary blood sample.

For differential diagnostic, 52 out of 53 newly admitted patients were tested for malaria infections (*Plasmodium* species) using an antigen-based rapid diagnostic test. Among the patients

who tested positive for EVD, 13 patients scored positive and 21 patients scored negative for malaria. For the patients who tested EVD negative, 8 patients scored positive and 10 patients scored

**Table 3. Real-time Reverse Transcription Polymerase Chain Reaction Results From an Individual Patient in the Course of a Follow-up Study During His Stay at the Ebola Treatment Center, Guéckédou, Guinea, August 2014**

Day of Sampling After Symptom Onset	EVD Detection in Venous Blood (Ct Value)	Semiquantitative Viral Load Result <sup>a</sup>	EVD Detection in Capillary Blood (Ct Value)	Semiquantitative Viral Load Result
Day 1	Negative (–)	–	Negative (–)	–
Day 3	Positive (26.17)	++	Negative (–)	–
Day 4	Positive (22.23)	+++	Positive (28.41)	++
Day 7	Positive (16.00)	+++	Positive (17.00)	+++

Abbreviations: Ct, cycle threshold; EVD, Ebola virus disease.

<sup>a</sup> Ct values are classified in subsequent categories of 0–24 (highly positive), 25–34 (positive), and 35–40 (weak positive) and correspond with +++, ++, and + results, respectively. Negative real-time reverse transcription polymerase chain reaction result is indicated by “–.”

negative for malaria. Comparison of results between matched capillary blood and venous blood samples and malaria coinfections showed that malaria coinfections had no influence on the sensitive and specific detection of EVD when using capillary blood samples (data not shown).

Due to the favorable location in Guéckédou with the European Mobile Laboratory adjacent to the MSF ETC, blood samples collected from patients, including the capillary blood specimens, could be treated for laboratory diagnosis within 1 hour to a few hours. However, in other settings, timely delay between specimen collection and laboratory analysis might be much greater. In cases where EVD diagnostics can not be performed within 24 hours after sample collection, the WHO recommends a storage temperature between 0°C and 5°C for sample specimens to maintain sample preservation [24]. In a limited test, we addressed sample stability under field conditions and observed rapid sample degradation when capillary blood swab samples were stored for >24 hours at room temperature compared with corresponding samples stored at 4°C, indicating an impact of storage conditions on sensitive Ebola nucleic acid detection by RT-PCR using capillary blood swab samples (data not shown).

## DISCUSSION

Efficient interruption of Ebola virus transmission chains critically depends on reliable and rapid laboratory diagnosis of patients suspected of having EVD. The laboratory results are needed to confirm suspected cases and to execute subsequent isolation measures. The high numbers of patients with suspected EVD admitted to ETCs in the affected countries Liberia, Sierra Leone, and Guinea often requires large-scale sampling of patients. A major challenge is sampling of venous blood in patients with suspected EVD, posing a serious risk to healthcare workers due to accidental needlestick injuries [25]. The less invasive collection of capillary blood samples could be an alternative approach, strongly reducing the risk for medical personnel. In addition, capillary blood samples might be useful for monitoring viral

load during the course of disease where repeated sampling by venous puncture would be too much of a strain for severely suffering EVD patients. The aim of this study was therefore to evaluate the feasibility of using capillary blood samples from fingertips for detection of Ebola virus and to compare the results with blood samples obtained by venipuncture. Analyzing capillary blood specimens collected from 53 patients with suspected EVD, we demonstrate for the first time the applicability of blood samples derived from fingersticks for the detection of EVD by RT-PCR.

As shown in Table 2, pairwise comparison of venous blood samples and capillary blood samples revealed in general a good correlation of test results for patients who were found to be positive for EVD. Compared to venous blood samples, capillary blood samples from fingerpricks revealed a sensitivity of 86.8% (95% confidence interval [CI], 71.9%–95.6%; 33/38 patients) and a specificity of 100% (95% CI, 84.6%–100%; 22/22 patients), respectively. Our findings thus support the use of capillary blood samples as an alternative diagnostic specimen to venous blood samples in an outbreak situation.

Due to the observed 100% specificity of test results in our field study, we consider a positive EVD laboratory result from capillary blood samples valid for reliable diagnostic evaluation. Capillary blood sampling may therefore represent a suitable diagnostic approach for initial rapid screening of a large number of patients in the midst of an outbreak, allowing accelerated EVD patient management and isolation measures. Suspected cases testing negative for EVD using capillary blood samples should be additionally tested using venous blood samples, the current gold standard specimen for RT-PCR-based EVD diagnosis. Similarly, at present, venous-derived blood is recommended for testing patients during the convalescent phase to enhance decision making on their discharge and reintroduction in the community.

Those cases when capillary blood samples were less sensitive or tested negative compared with venous blood samples may relate to the stage of disease. At present, no information is available regarding Ebola viral load kinetics in capillary blood during

the course of infection. Future studies should address the viremia in the capillary blood according to the stage of clinical illness to ensure valid interpretation of diagnostic results.

Alternatively, the differences in viral load between the 2 specimen types might be due to inherent sampling and collection procedures (Supplementary Figure 2). Although introducing identical blood volumes (eg, from whole blood vacutainers into sample preparation) is easy to achieve, adsorption and desorption of capillary blood from swab fingerpricks might vary, thus explaining the observed differences in Ct values. Further studies should address standardization of collection procedures and sampling volumes for the swab approach.

Finally, in this study we compared EDTA-anticoagulated blood collected by venipuncture and capillary blood samples that were deposited onto viscose swab devices, exhibiting no anticoagulation activity. Protective effects of EDTA on stability of pathogen-derived RNA in blood samples have been reported [26]. Despite this fact, we have chosen these swab devices for our comparison study, as the European Mobile Laboratory units in Guéckédou, Guinea, and Foya, Liberia, received a considerable number of capillary blood samples absorbed onto viscose swabs as a diagnostic sample specimen from both infants and adults for EVD testing (unpublished observation). Although these types of swabs are routinely used for sensitive detection of Ebola virus in oral fluids in the current EVD outbreak in West Africa [27, 28], at present very little is known about the stability of Ebola virus RNA or potential degradation effects in capillary blood samples absorbed onto swabs. Rapid RNA degradation might result in lower detection sensitivity or even render a test falsely negative. Thus, the value of swabs as a collection and storage device for capillary blood samples for diagnostic purposes needs to be further evaluated and compared with capillary blood samples collected into a microcollection container that has EDTA as an anticoagulant.

Capillary blood sampling offers some advantages over blood sampling by venipuncture. Sampling can be done by medical health personnel other than experienced and trained phlebotomists. Furthermore, for patient convenience and in some circumstances for cultural reasons and religious beliefs, this procedure may be more acceptable than venous blood draw. Thus, the less invasive collection of samples eliminates the anxiety associated with venipuncture and may lead to higher acceptance of blood testing in settings where cultural traditions otherwise might interfere with effective outbreak management. Moreover, capillary blood collection is the preferred method of blood specimen collection for newborns and infants. These practical reasons might outbalance the higher sensitivity observed in some cases for venous blood analysis, particularly in the critical situation of an outbreak.

Reliable detection and laboratory confirmation of Ebola virus is key to successfully controlling the current EVD outbreak in

West Africa. The WHO has identified an ongoing need to develop sensitive and specific tests for diagnosis of EVD using accessible point-of-care (POC) technologies, including POC fingerstick tests that are capable of providing early diagnosis of EVD and Ebola viral load measurement. Our report is the first study that compares the sensitivity of RT-PCR-based EVD tests on capillary vs venous blood samples. Although a more extensive evaluation of capillary blood specimens under field conditions is still needed, our results point toward the use of capillary blood samples as an appropriate collection specimen for sensitive and specific diagnosis of EVD. In conclusion, this study demonstrates the value of capillary fingerstick blood as a clinical sample specimen for virologic diagnosis of Ebola virus infection during an outbreak emergency.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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