Case Report

Fatal *Plasmodium falciparum*, *Clostridium perfringens*, and *Candida* spp. Coinfections in a Traveler to Haiti

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Malaria is one of the most common causes of febrile illness in travelers. Coinfections with bacterial, viral, and fungal pathogens may not be suspected unless a patient fails to respond to malaria treatment. Using novel immunohistochemical and molecular techniques, *Plasmodium falciparum*, *Clostridium perfringens*, and *Candida* spp. coinfections were confirmed in a German traveler to Haiti. *Plasmodium falciparum*-induced ischemia may have increased this patient’s susceptibility to *C. perfringens* and disseminated candidiasis leading to his death. When a patient presents with *P. falciparum* and shock and is unresponsive to malaria treatment, secondary infections should be suspected to initiate appropriate treatment.

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1. Introduction

*Plasmodium falciparum*, an intraerythrocytic parasite that causes the most severe form of human malaria, is endemic to Haiti where it caused 32,739 infections and contributed to an estimated 741 malaria deaths in 2006 [1–3]. Malaria infection poses a risk to the 140,000 travelers from nonendemic countries that visit Haiti each year [4]. Progression to severe illness from initial symptoms of fever, headache, chills, and myalgia occurs rapidly through the phenomenon of sequestration, in which *P. falciparum*-infected erythrocytes attach to blood vessels and impede normal blood flow, particularly in the brain [5]. Poor perfusion leads to tissue ischemia, which may subsequently increase a patient’s susceptibility to secondary infections [5, 6] such as *C. perfringens*, and *Candida* spp. *C. perfringens* is an anaerobic spore-forming bacillus that produces a virulent hemolytic alpha toxin [7] and causes gangrene, a potentially deadly infection characterized by fever, pain, edema, myonecrosis, and gas production [8]. And in a setting of tissue ischemia and necrosis, endogenous gut microflora such as Candida may translocate across the epithelial border and gain access to systemic circulation resulting in disseminated candidiasis [9].

Malaria coinfections with *Leptospira* spp., *Coxiella burnetii*, *Brucella melitensis*, and *Streptococcus pneumoniae* as well as with enteric bacteria (*Escherichia coli* and *Salmonella*, and *Acinetobacter baumannii*) have previously been reported [10–14]. To our knowledge, this report describes the first fatal coinfection of *P. falciparum*, *C. perfringens*, and *Candida* spp.

2. Materials and Methods

2.1. Case Presentation. On October 29th 2005, a 56-year-old German tourist living in Haiti, with a history of heavy alcohol use and 2 malaria infections treated with chloroquine, developed symptoms of fever, headache, nausea, and diarrhea. On November 3rd he visited a local hospital in Côtes des Arcadins and was diagnosed with *P. falciparum* infection by peripheral blood smear. The patient was treated with chloroquine and doxycycline the same day but developed altered mental status and was hospitalized. Despite falling
parasite counts, the patient’s neurological condition continued to worsen and he was mechanically ventilated. Seizures, bloody stools, hematemesis, and decreased urinary output were also documented. Due to his worsening condition, he was transferred to a hospital in Miami the following day, November 4th. At the time of hospital admission in Miami, the patient had thrombocytopenia, diffuse intravascular coagulation, electrolyte imbalance, and acute renal failure. Blood smears showed few malaria parasites, but treatment regime was modified for the FFPET sections of small intestine using the QIAamp DNA minikit (Qiagen, Valencia, Calif, USA), following the tissue extraction protocol. A C. perfringens-specific PCR assay was performed using the High Fidelity PCR kit (Roche Diagnostics, Indianapolis, Ind, USA) according to the manufacturer’s instructions to amplify 283-bp fragment of the phospholipase C (alpha toxin) gene. For further evaluation, two P. falciparum-specific PCR assays targeting the 18S rRNA gene were performed. Primers used in PCR assays were published previously [17, 18] and are described in Table 1. PCR assays were modified for the FFPET. For each primer set, annealing temperature was adjusted accordingly. PCR was carried out on a GeneAmp PCR System 9700 thermocycler (Perkin-Elmer).

Amplified PCR products were separated on 1.8% agarose gel, extracted from the gel by using QIAquick gel extraction kit (Qiagen), and cycle sequenced by CEQ 2000 dye terminator cycle sequencing with quick start kit (Beckman Coulter, Fullerton, Calif, USA) and the respective primers. Postreaction cleanup was done by centri-sep spin
Figure 1: Histopathologic and immunohistochemical findings in representative tissues of study patient. (a) Extensive necrosis of intestinal mucosa with diffuse, extensive, submucosal edema and multifocal inflammatory cell infiltrates (H&E, original magnification X13). (b) Abundant, diffuse immunostaining of Clostridium spp. antigens in intestine (original magnification X13; inset X100). (c) Immunohistochemical detection of P. falciparum HRP-2 antigens in pRBCs in spleen (original magnification X100). (d) HRP-2 antigen immunostaining in endothelium of CNS blood vessels (original magnification X50).

3. Results

No hemozoin pigment was observed by routine hematoxylin-eosin (H&E) stain and no malaria parasites were seen in red blood cells on careful examination of multiple tissues. Histopathology of the colon and small intestine mucosa showed extensive necrosis with diffuse, extensive, submucosal edema and multifocal inflammatory cell infiltrates comprised predominately of neutrophils (Figure 1(a)). The serosa was moderately thickened and contained mixed inflammatory cell infiltrates. The liver showed autolysis with no significant inflammatory cell infiltrates. Spleen sections were congested and necrotic. The mucosal surface of the larynx was also extensively necrotic with abundant neutrophilic infiltrates. There were diffuse autolysis in the kidneys and intra-alveolar edema in the lungs. The heart showed interstitial edema. There were focal hemorrhages in the white matter of the cerebral cortex, but no conspicuous inflammatory cell infiltrates observed in the hippocampus, pons, cerebellum, and spinal cord.

The HRP-2 IHC assay revealed discrete immunostaining of intra-erythrocytic parasites in CNS, kidney, liver, heart, and spleen (Figure 1(c)). HRP-2 antigens were also detected in endothelium of systemic and CNS blood vessels (Figure 1(d)), and in renal tubular epithelium and renal casts. The Clostridia spp. IHC assay revealed abundant immunostaining in necrotic areas of the small intestine (Figure 1(b)); other tissues were negative. Small budding yeasts in the alveolar space of the lung were identified by an IHC stain for Candida spp.

Amplification products of expected sizes were generated by both the C. perfringens and P. falciparum PCR assays using...
DNA extracted from FFPET sections. Sequence analysis of positive amplicons also confirmed the infections of *P. falciparum* and *C. perfringens*.

4. Discussion

Immunohistochemical and molecular analysis of FFPET sections from the study patient confirmed coinfections with *P. falciparum*, *C. perfringens*, and *Candidia spp*. Malaria is one of the most common causes of fever in travelers [19] and nosocomial coinfections may occur with a frequency of 25%, according to a recent study of 96 fatal *P. falciparum* cases [20]. However, malaria coinfections are difficult to diagnose clinically and may only be suspected when a patient fails to respond to malaria treatment [10]. Several observations suggest that the patient described here, who presented to hospital with classic malaria symptoms, was successfully treated for malaria infection with chloroquine and doxycycline: (1) declining parasite counts on peripheral blood smear were documented during his hospital stay; (2) absence of hemozoin, a birefringent pigment produced by plasmodium in correlation with parasite density [21, 22], on H&E evaluation; (3) rare HRP-2 antigens detected by IHC in CNS, heart, lung, and liver sections as described in detail above. Rare staining was anticipated in this patient, considering that HRP-2 antigens are slow to clear from the blood and may persist in treated patients for up to two weeks [23]. The presence of amplification products of *P. falciparum* using PCR assays is not inconsistent with the H&E and immunohistochemical findings of resolving malaria infection. The primers used are highly sensitive, shown to detect as few as 0.7 parasites/mL [18]. In this context, and as our patient illustrates, PCR results must be correlated with the patient’s clinical history.

The observation of necrosis in the small intestine supports the finding of *C. perfringens* in blood cultures found on November 4th, (day 7 of illness) after the patient was hospitalized in Miami. *C. perfringens* infection was subsequently confirmed by IHC testing and by PCR and sequencing analysis. *C. perfringens* is associated with several human diseases, including necrotizing enterocolitis [24], gas gangrene [25], antibiotic-associated diarrhea, and food poisoning outbreaks worldwide [26]. The alpha toxin is the most virulent of the 12 toxins produced by *C. perfringens* [9] because it destroys cell membranes, including those of red blood cells, platelets, and muscles. The bacterium also has sphingomyelinase activity that causes damage to the nerve-sheath in the central nervous system [8]. Deaths due to *C. perfringens* infections are rare in humans, and the portals of entry are usually surgical wounds [27]. However concomitant ischemia, or low oxygen tension in necrotic tissue, is a trigger for bacterial spore germination [28], and subsequent toxin production leads to anaerobic cellulitis or myonecrosis (gas gangrene) that rapidly progress to severe sepsis [8]. The source of this patient’s *C. perfringens* infection is unknown as *C. perfringens* is widespread in the environment and can be a component of normal human flora, but broad spectrum antibiotic use is suspected. It is likely that *P. falciparum* infection increased susceptibility to *C. perfringens* and *Candida spp*. in this patient.

*C. perfringens* infection causing intestinal myonecrosis may have begun with tissue ischemia due to *P. falciparum* sequestration, which is characterized by the attachment of parasitized erythrocytes (pRBCs) to endothelial cells lining blood vessels via a variety of constitutive receptors. Sequestration causes sluggish blood flow and disruption of microcirculation [6, 29] leading to ischemia. The infection subsequently leads to hyperlactemia, hypoglycemia, and metabolic acidosis, creating a dependence on anaerobic glycolysis for energy production [30]. Studies suggest that obstruction of the splanchic blood vessels by pRBCs facilitates the entry of endotoxins and bacteria like *C. perfringens* from the digestive tract into the bloodstream [31]. While limited clinical data on this patient is available, this mechanism seems plausible and is supported by the tissue-based and molecular testing performed.

This patient’s chronic alcohol consumption may also have contributed to the severity of multiple infections. Ethanol has been shown to decrease the respiratory burst activity of neutrophils [32], and heavy alcohol consumption (> or = 5 drinks per day) is significantly associated with ICU-acquired bacterial infection, even when controlling for duration of mechanical ventilation and other risk factors [33]. Further, the oral flora of heavy alcohol drinkers has been shown to differ significantly from the flora of nonalcoholics. One study showed that anaerobes, including *Clostridium spp.*, are present in 84.5% of heavy drinkers, compared with 30.5% of nonalcoholics and similarly, *Candida spp.* were found in 34.5% of heavy drinkers whereas only 5.5% of nonalcoholics carried the microbiota [34]. The pathogens detected by bronchoalveolar lavage, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* are particularly common causes of pneumonia in chronic alcoholics; while *Pseudomas aeruginosa* is associated with mechanical ventilation [35].

Disseminated candidiasis contributed to our patient’s demise; *C. tropicalis* was detected by bronchoalveolar lavage, and our IHC analysis revealed *Candida spp.* in FFPET of small intestine. Animal studies have shown that mucosal damage and broad-spectrum antibiotics are important factors in opportunistic candidiasis [30]. In this case, we suggest that the use of broad spectrum antibiotic coverage in the setting of tissue ischemia and mucosal erosion secondary to *P. falciparum* and *C. perfringens* infections facilitated disseminated infection.

To our knowledge, this is the first report of *P. falciparum*, *C. perfringens*, and *Candida spp.* coinfections. In the case presented here, *P. falciparum* may have increased susceptibility to *C. perfringens* infection by inducing a state of hypoxia-ischemia. The patient’s chronic alcohol consumption may have increased his susceptibility to intestinal necrosis and ischemia, which created a suitable environment for translocation and dissemination of *Candida spp.*, particularly in the setting of broad-spectrum antibiotic administration. *P. falciparum* remains a significant threat to travelers to Haiti and other areas where the parasite is endemic. This case
highlights the importance of suspecting bacterial and fungal coinfections in patients refractory to malaria treatment.

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References

[1] T. P. Eisele, J. Keating, A. Bennett, et al., “Prevalence of *Plasmodium falciparum* infection in rainy season, Artibonite Valley, Haiti, 2006,” Emerging Infectious Diseases, vol. 13, no. 10, pp. 1494–1496, 2007.

[2] “Severe *falciparum* malaria. World Health Organization, Communicable Diseases Cluster,” Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 94, supplement 1, pp. S1–S90, 2000.

[3] World Malaria Report 2008, The World Health Organization, WHO Global Malaria Programme, March 2009, http://www.who.int/malaria/wmr2008.

[4] J. Thwing, J. Skarbinski, R. D. Newman, et al., “Malaria surveillance-United States,” MMWR: Surveillance Summaries, vol. 56, no. 6, pp. 23–40, 2007.

[5] I. W. Sherman, S. Eda, and E. Winograd, “Cytoadherence and sequestration in *Plasmodium falciparum*: defining the ties that bind,” Microbes and Infection, vol. 5, no. 10, pp. 897–909, 2003.

[6] B. M. Cooke, S. Morris-Jones, B. M. Greenwood, and G. B. Nash, “Mechanisms of cytoadhesion of flowing, parasitized red blood cells from Gambian children with *falciparum* malaria,” American Journal of Tropical Medicine and Hygiene, vol. 53, no. 1, pp. 29–35, 1995.

[7] R. S. Cotran, V. Kumar, and S. L. Robbins, Robbins Pathologic Basis of Disease, W.B. Saunders Company, Philadelphia, Pa, USA, 1994.

[8] J. Sakurai, M. Nagahama, and M. Oda, “Clostridium perfringens alpha-toxin: characterization and mode of action,” Journal of Biochemistry, vol. 136, no. 5, pp. 569–574, 2004.

[9] S. Inoue, J. A. Wirman, J. W. Alexander, O. Trocki, and R. R. Cardell, “Candida albicans translocation across the gut mucosa following burn injury,” Journal of Surgical Research, vol. 44, no. 5, pp. 479–492, 1988.

[10] F. Bruneel, B. Gachot, J. F. Timsit, et al., “Shock complicating severe *falciparum* malaria in European adults,” Intensive Care Medicine, vol. 23, no. 6, pp. 698–701, 1997.

[11] R. Gopinath, J. S. Keystone, and K. C. Kain, “Concurrent *falciparum* malaria and salmonella bacteremia in travelers: report of two cases,” Clinical Infectious Diseases, vol. 20, no. 3, pp. 706–708, 1995.

[12] P. Brouqui, J. M. Rolain, C. Foucault, and D. Raoul, “Short report: Q fever and *Plasmodium falciparum* malaria co-infection in a patient returning from the comoros archipelago,” American Journal of Tropical Medicine and Hygiene, vol. 73, no. 6, pp. 1028–1030, 2005.

[13] S. Badiaga, G. Imbert, B. La Scola, P. Jean, J. Delmont, and P. Brouqui, “Imported brucellosis associated with *Plasmodium falciparum* malaria in a traveler returning from the tropics,” Journal of Travel Medicine, vol. 12, no. 5, pp. 282–284, 2005.

[14] C. Wongsrichanalai, C. K. Murray, M. Gray, et al., “Co-infection with malaria and leptospirosis,” American Journal of Tropical Medicine and Hygiene, vol. 68, no. 5, pp. 583–585, 2003.

[15] G. L. Genrich, J. Guarner, C. D. Paddock, et al., “Fatal malaria infection in travelers: novel immunohistochemical assays for the detection of *Plasmodium falciparum* in tissues and implications for pathogenesis,” American Journal of Tropical Medicine and Hygiene, vol. 76, no. 2, pp. 251–259, 2007.

[16] J. Guarner, J. Bartlett, S. Reagan, et al., “Immunohistochemical evidence of *Clostridium sp*, *Staphylococcus aureus*, and group A *Streptococcus* in severe soft tissue infections related to injection drug use,” Human Pathology, vol. 37, no. 11, pp. 1482–1488, 2006.

[17] P. Fach and M. R. Popoff, “Detection of enterotoxigenic *Clostridium perfringens* in food and fecal samples with a duplex PCR and the slide latex agglutination test,” Applied and Environmental Microbiology, vol. 63, no. 11, pp. 4232–4236, 1997.

[18] F. Perandin, N. Manca, A. Calderaro, et al., “Development of a real-time PCR assay for detection of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for routine clinical diagnosis,” Journal of Clinical Microbiology, vol. 42, no. 3, pp. 1214–1219, 2004.

[19] P. Schlagenhaufer and P. Muentener, “Imported malaria,” in *Traveler's Malaria*, P. Schlagenhaufer, Ed., pp. 495–508, Decker, Hamilton, UK, 2001.

[20] F. Legros, O. Bouchaud, T. Ancelle, et al., “Risk factors for imported fatal *Plasmodium falciparum* malaria, France, 1996–2003,” Emerging Infectious Diseases, vol. 13, no. 6, pp. 883–888, 2007.

[21] A. D. Sullivan, I. Ittarat, and S. R. Meshnick, “Patterns of haemoglobin accumulation in tissue,” Parasitology, vol. 112, no. 3, pp. 285–294, 1996.

[22] A. U. Orjih and C. D. Fitch, “Hemozoin production by *Plasmodium falciparum*: variation with strain and exposure to chloroquine,” Biochimica et Biophysica Acta, vol. 1157, no. 3, pp. 270–274, 1993.

[23] M. Mayxay, S. Pukrittayakamee, K. Chotivanich, S. Looareesuwan, and N. J. White, “Persistence of *Plasmodium falciparum* HRP-2 in successfully treated acute *falciparum* malaria,” Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 95, no. 2, pp. 179–182, 2001.

[24] J. Sobel, C. G. Mixter, P. Kolhe, et al., “Necrotizing enterocolitis associated with *Clostridium perfringens* type A in previously healthy north american adults,” Journal of the American College of Surgeons, vol. 201, no. 1, pp. 48–56, 2005.

[25] S. Kuroda, Y. Okada, M. Mita, et al., “Fulminant massive gas gangrene caused by *Clostridium perfringens*,” Journal of Clinical Microbiology, vol. 42, no. 3, pp. 499–502, 2005.

[26] S. G. Sparks, R. J. Carman, M. R. Sarker, and B. A. McClane, “Genotyping of enterotoxigenic *Clostridium perfringens* fecal isolates associated with antibiotic-associated diarrhea and food poisoning in North America,” Journal of Clinical Microbiology, vol. 39, no. 3, pp. 883–888, 2001.
[27] E. S. Caplan and R. M. Kluge, “Gas gangrene: review of 34 cases,” *Archives of Internal Medicine*, vol. 136, no. 7, pp. 788–791, 1976.

[28] C. R. Hitchcock, F. J. Demello, and J. J. Haglin, “Gangrene infection: new approaches to an old disease,” *Surgical Clinics of North America*, vol. 55, no. 6, pp. 1403–1410, 1975.

[29] A. M. Dondorp, “Clinical significance of sequestration in adults with severe malaria,” *Transfusion Clinique et Biologique*, vol. 15, no. 1-2, pp. 56–57, 2008.

[30] N. J. White and M. Ho, “The pathophysiology of malaria,” *Advances in Parasitology*, vol. 31, pp. 83–173, 1992.

[31] I. A. Clark and W. B. Cowden, “The pathophysiology of *falciparum* malaria,” *Pharmacology and Therapeutics*, vol. 99, no. 2, pp. 221–260, 2003.

[32] D. Breitmeier, N. Becker, C. Weilbach, et al., “Ethanol-induced malfunction of neutrophils respiratory burst on patients suffering from alcohol dependence,” *Alcoholism: Clinical and Experimental Research*, vol. 32, no. 10, pp. 1708–1713, 2008.

[33] A. Gacouin, F. Legay, C. Camus, et al., “At-risk drinkers are at higher risk to acquire a bacterial infection during an intensive care unit stay than abstinent or moderate drinkers,” *Critical care medicine*, vol. 36, no. 6, pp. 1735–1741, 2008.

[34] V. Golin, I. M. Mimica, and L. M. Mimica, “Oropharynx microbiota among alcoholics and non-alcoholics,” *São Paulo Medical Journal*, vol. 116, no. 3, pp. 1727–1733, 1998.

[35] E. Bergogne-Bérézin, “Pseudomonads and miscellaneous gram-negative bacilli,” in *Infectious Diseases*, J Cohen and W. G. Powderly, Eds., pp. 2203–2217, Mosby, St. Louis, Mo, USA, 2nd edition, 2004.