FATAL MALARIA INFECTION IN TRAVELERS: NOVEL IMMUNOHISTOCHEMICAL ASSAYS FOR THE DETECTION OF PLASMODIUM FALCIPARUM IN TISSUES AND IMPLICATIONS FOR PATHOGENESIS

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Abstract. Plasmodium falciparum is a significant cause of morbidity and mortality in travelers to areas where the parasite is endemic. Non-specific clinical manifestations may result in failure to recognize malaria until autopsy, when it is often too late to obtain whole blood for microscopic evaluation. The use of immunohistochemical (IHC) assays in the detection of three P. falciparum antigens, histidine rich protein-2 (HRP-2), aldolase, and Plasmodium lactate dehydrogenase (pLDH), was evaluated in formalin-fixed paraffin-embedded autopsy tissues from five travelers to malaria-endemic areas, whose deaths were initially suspected to have been caused by other bacterial or viral hemorrhagic fevers. The HRP-2 assay was specific for P. falciparum, whereas the aldolase and pLDH assays also reacted with P. vivax. Immunostaining patterns were predominately cytoplasmic and membranous. P. falciparum antigens were detected in a variety of organs but were most abundant in the blood vessels of brain, heart, and lung tissues.

INTRODUCTION

The intra-erythrocytic protozoan Plasmodium falciparum is the etiologic agent of severe malaria, a systemic illness characterized by acute respiratory distress syndrome, renal insufficiency, and central nervous system involvement that can be confused clinically with viral or bacterial hemorhagic fevers. P. falciparum causes the majority of the 300–500 million acute infections and 1–3 million deaths from malaria worldwide each year. Ninety percent of global malaria mortality occurs in sub-Saharan Africa, where P. falciparum and its primary mosquito vector, Anopheles gambiae, predominates; however, international travel permits the importation of P. falciparum into countries where the parasite is not endemic. Of the 25–30 million travelers who visit malaria-endemic countries annually, ~10,000 return with malaria, often caused by P. falciparum. In the United States, most cases of imported malaria are caused by failure to take appropriate chemoprophylaxis.

The lack of clinical suspicion in non-endemic countries may result in the underdiagnosis and omission of malaria from a differential diagnosis that includes other viral and bacterial hemorrhagic fevers. A review of malaria deaths among US travelers from 1963 through 2001 found that diagnostic delays were common among fatal cases. When untreated, P. falciparum infection can progress rapidly to severe malaria, often leading to coma and death. Case fatality ratios for imported malaria range from 1% to 3.6% in industrialized countries.

Traditional diagnostic techniques, such as the Giemsa-stained blood smear, rely on the detection of parasites by trained microscopists; however, blood samples are not often available for travelers when diagnosis of malaria is not considered before death. Other diagnostic techniques, such as the routine hematoxylin-eosin (H&E) stain, have also limited use in the diagnosis of fatal malaria because P. falciparum parasites are difficult to identify with certainty in tissue samples.

Several genus- and species-specific proteins produced by P. falciparum parasites during intra-erythrocytic growth have been characterized. These proteins are currently used in whole blood rapid detection kits for non-microscopic malaria diagnosis with high sensitivities and specificities. To our knowledge, immunohistochemical (IHC) detection of these proteins has not been previously described in the diagnosis of P. falciparum infection. The following report describes the development of novel IHC assays targeting P. falciparum histidine rich protein-2 (HRP-2), Plasmodium aldolase, and Plasmodium lactate dehydrogenase (pLDH). The IHC assays were used to diagnose and study the pathogenesis of P. falciparum in tissues from five travelers whose deaths were initially suspected to have been caused by other bacterial or viral hemorrhagic fevers.

MATERIALS AND METHODS

Patient samples. Formalin-fixed autopsy tissue samples from five cases of suspect hemorrhagic fevers were submitted to the Centers for Disease Control and Prevention (CDC) between 1996 and 2005 for diagnostic consultation. The patients had a recent travel history to malaria-endemic regions. Histopathology was reviewed by routine H&E stain. At the time of submission, the primary clinical diagnoses made by submitters, which included Ebola, Lassa fever, dengue, and leptospirosis, were excluded by IHC assays using a modified immunoaalkaline phosphatase technique. Representative tissues from each of the five cases were tested and evaluated with P. falciparum IHC assays using three antibodies (HRP-2, aldolase, and pLDH). Interpretation of the IHCs included identification of antigen localization in the different tissues and intra-erythrocytic immunostaining patterns.

Plasmodium spp. controls. Plasmodium ovale and Plasmodium vivax were grown in vivo in Aotus and Saimiri sp. monkeys and P. falciparum was cultured in vitro according to the methods established by Trager and Jensen. Infected erythrocytes and uninfected erythrocytes (negative controls) were fixed in formalin and embedded in paraffin for IHC testing.

Immunohistochemical assays. Formalin-fixed paraffin-embedded tissues were cut at 3 μm, deparaffinized in xylene, and rehydrated in a graded alcohol series. Hematoxylin pigment was removed by soaking tissue sections in alcoholic picric acid solution (Newcomber, Middleton, WI) overnight. To ex-

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pose malarial antigens, sections were subsequently pretreated in one of two ways: 1) antigen retrieval was performed by incubating sections submerged in Antigen Retrieval Citra Solution (BioGenex, San Ramon, CA) at 98°C in a steamer for 10 minutes or 2) sections were digested in 0.1% proteinase K (Boehringer Mannheim, Indianapolis, IN) solution for 15 minutes. Sections were blocked with 20% normal sheep serum in Tris-saline-Triton (NSS/TST) and incubated with monoclonal antibodies against malaria proteins (below) for 60 minutes on the Dako Autostainer (DakoCytomation, Carpinteria, CA). Detection of the bound antibody was performed by using the LSAB2 universal alkaline phosphatase system (DakoCytomation) that uses 15-minute sequential incubations at room temperature in a secondary biotinylated anti-mouse/rabbit link antibody, followed by alkaline phosphatase–conjugated streptavidin, and then in a 0.2 mg/mL naphthol phosphatase-fast red chromogen reagent. Slides were rinsed, counterstained for 3 minutes in Mayer hematoxylin (Biomed, Foster City, CA), and mounted with aqueous mounting medium.

**Monoclonal P. falciparum antibodies.** Optimal working dilutions for antibodies were determined by testing infected red blood cells (RBCs) with an IgG1, κ anti-HRP-2 antibody (cat. no. C03400M; dilution 1:1,000; Biodesign, Saco, ME), an IgG1 anti-aldolase antibody (dilution 1:50; donated by Dr. Norm Moore, Binax, Scarborough, ME), and an IgG1 anti-pLDH antibody (dilution 1:200; donated by Dr. Michael Makler, Flow, Portland, OR). Negative controls for each slide consisted of sequential tissue sections incubated with normal mouse serum and an irrelevant, but isotype-identical primary antibody. The specificity of the primary antibodies was evaluated by testing cross-reactivity with other Plasmodium species (P. ovale, P. vivax) and human tissues infected with Babesia microti, Toxoplasma gondii, Trypanosoma cruzi, Rickettsia rickettsii, Leptospira species, and dengue, Ebola, Lassa, and lymphocytic choriomeningitis (LCM) viruses.

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**RESULTS**

**Clinical and epidemiologic findings.** Clinical and epidemiologic data for the five patients are summarized in Table 1. All five men traveled to Africa, South America, or the Caribbean region where *P. falciparum* is endemic and had not taken malaria chemoprophylaxis during their travel. Cases 1 and 2 were Romanian shipmates who made port in South Africa and several West African cities before becoming sick and dying in Conakry, Guinea. It was feared that the men had acquired Ebola in Libreville, Gabon, 1 week earlier because the stop there coincided with an Ebola outbreak occurring northeast of the capital. Their ship was quarantined off the coast of Guinea until a definitive diagnosis could be made. In 2004, CDC received Case 3, a 36-year-old second mate from Rhode Island who had made port calls in Freetown, Sierra Leone; Abidjan, Ivory Coast; and Lagos, Nigeria. Ten days after leaving Nigeria, Case 3 experienced headache, fever, chills, myalgia, and bloody urine, and died 1 week after onset of symptoms, before his ship could reach Galveston, TX. Weeks before this man’s death, a fatal case of Lassa fever was confirmed in a New Jersey man with a similar travel history. As a result, the ship underwent voluntary quarantine, anchored in the Houston channel off the coast of Galveston until autopsy tissues could be examined and Lassa fever could be ruled out. Case 4 was a 32-year-old reporter, native to Guyana, who had traveled to the city of Demerara to report on flooding in the area. After complaining of headache and recurrent fever with diarrhea and vomiting, Case 4 deteriorated into hepaticorenal failure and coma and died. Because of an ongoing outbreak of leptospirosis, local health officials suspected that this patient had acquired leptospirosis during his assignment.

Case 5, a 43-year-old man from Virginia, traveled to the Central African Republic and the Ivory Coast during a 17-day mission trip in December 2005. On his return, he complained

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**Table 1**

Epidemiologic and clinical information on five cases sent to the Centers for Disease Control and Prevention (CDC), 1996–2005

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Year submitted</th>
<th>Travel history</th>
<th>Clinical and epidemiologic information</th>
<th>Disease(s) initially suspected by history and clinical manifestations</th>
<th>IHC testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>1996</td>
<td>South Africa, Gabon, and Guinea</td>
<td>Ages unknown; male shipmates from Romania. Limited clinical information available, but concomitant Ebola outbreak in north Gabon. 400 km from Libreville.</td>
<td>Ebola</td>
<td>Dengue virus, Ebola virus, Lassa virus <em>P. falciparum</em></td>
</tr>
<tr>
<td>3</td>
<td>2004</td>
<td>Sierra Leone, Ivory Coast, and Nigeria</td>
<td>36-year-old man; shipmate from the United States. Headache, fever (&gt; 103°F), chills, myalgia, and sore throat, nausea, vomiting, jaundice, pain, “bloody” and “rust-tinged” urine. Malaria chemoprophylaxis was found among his belongings, but it was not thought to have been taken.</td>
<td>Lassa fever</td>
<td>Dengue virus, Ebola virus, Lassa virus, <em>Leptospira spp.</em>, <em>Rickettsia rickettsii</em>, Yellow fever virus, <em>P. falciparum</em></td>
</tr>
<tr>
<td>4</td>
<td>2005</td>
<td>Guyana (western region)</td>
<td>32-year-old man; reporter from Guyana. Headache and recurrent fever with diarrhea and vomiting, hepaticorenal failure, coma. Reported on flooding in western Guyana, where an outbreak of leptospirosis occurred. No malaria chemoprophylaxis was taken.</td>
<td>Leptospirosis</td>
<td><em>Leptospira spp.</em>, <em>P. falciparum</em></td>
</tr>
<tr>
<td>5</td>
<td>2005</td>
<td>Central African Republic, Ivory Coast</td>
<td>43-year-old man; missionary from the United States. Unproductive cough, fever and chills, muscle and joint aches, dark urine. Malaria chemoprophylaxis was not taken during the 2-week trip to Africa.</td>
<td>Yellow fever, dengue virus</td>
<td>Yellow fever virus, dengue virus, <em>P. falciparum</em></td>
</tr>
</tbody>
</table>
of fever, chills, dizziness, joint aches, and dark urine and died 10 days later. Clinicians suspected dengue and yellow fever because outbreaks of the viruses were occurring in the areas visited.

Assessment of *P. falciparum* IHC assays. The HRP-2 antibody was specific for *P. falciparum* (Figure 1), whereas the aldolase and pLDH antibodies reacted with both *P. falciparum*– and *P. vivax*–infected erythrocytes (pRBCs). Depigmentation dramatically improved visualization of parasites in H&E-stained sections and increased antigen exposure in IHC assays; therefore, depigmentation was routinely performed on tissues (Figure 2A–D). Other IHC pretreatment conditions

**Figure 1.** *P. falciparum* HRP-2 assay applied to positive and negative control cells. A, Immunostaining of *P. falciparum*-infected erythrocytes (pRBCs), (parasitemia 2.8%; original magnification ×100). Immunostaining pattern of positive control cells was similar with aldolase and pLDH assays. B, *P. ovale* infected erythrocytes, (parasitemia 1%; original magnification ×100).

**Figure 2.** Immunohistochemical development techniques. A–D, Depigmentation removes hemozoin pigment in *P. falciparum*-infected spleen sections (Case 4). A, Hemozoin pigment (arrow) in a routine H&E stain of spleen section without depigmentation (original magnification ×100) B, HRP 2 immunohistochemical assay on *P. falciparum*-infected spleen section without depigmentation; diffuse and abundant hemozoin pigment (original magnification ×50). C, Routine H&E with depigmentation (original magnification ×100). D, HRP-2 immunohistochemical assay on spleen section after overnight depigmentation (original magnification ×50). E–H, *P. falciparum* HRP-2 immunohistochemical assay using different pretreatment, based upon duration of formalin-fixation (Case 1). E, Lung vessel of tissue sample fixed in formalin for 1 month using proteinase K and F, antigen retrieval (original magnification ×50). G, Liver vessels of tissue stored for 9 years treated with proteinase K and H, antigen retrieval (original magnification ×50).
were determined for each antibody on a case by case basis; overall, antigen retrieval was optimal in exposing parasite antigens on tissues with longer formalin fixation times (Figure 2E–H). Proteinase K could be used in the HRP-2 IHC assay on tissues that were stored in formalin from days to several weeks.

**Histopathology and IHC detection of *P. falciparum* antigens.** Heart, lung, and liver tissues were available for all patients; other tissues (no. of patients) included spleen (4), kidney (3), and brain (2). The following tissues were available from only one patient: tongue, trachea, thyroid, adrenal gland, gall bladder, and testes. Viral and/or bacterial hemorrhagic fever pathogens suspected at death or autopsy were ruled out by IHC on tissues from the five cases (Table 1).

The histopathologic features of infection were similar in all cases. No significant inflammation was observed in liver, kidney, and spleen. Abundant hemoglobin pigment was observed as small black discrete granules within erythrocytes or as diffuse deposits throughout the parenchyma (Figure 2A). Parasitized erythrocytes (pRBCs) generally contained one distinct hemoglobin granule or were heme-depleted (ghost erythrocytes). Kupffer cells and other tissue macrophages also contained fine brown hemoglobin pigment (Figure 3A).

After depigmentation, parasites were evident inside RBCs in systemic blood vessels. Blood vessels were generally packed with pRBCs and non-pRBCs. Congestion in spleen, liver, and kidney sections was common (Figure 3A, C, and E), and erythrocytagnosis by macrophages was also observed in these tissues. In kidney sections, there were varying degrees of cast formation in tubules and glomerular edema (Figure 3E). In the lungs, intra-alveolar edema and hyaline membranes composed of fibrin and proteinaceous material were observed (Figure 4A and B), typical of lung injury and early diffuse alveolar damage. In all cases where tissue was available, aggregation of pRBCs was most prominent in the capillaries of heart and brain (Figure 4C–F).

In general, HRP-2 immunostaining was greatest in representative tissues, followed by aldolase and pLDH. All representative tissues from study cases were positive by HRP-2 and aldolase assays; the pLDH assay, however, did not produce immunostaining in all tissues from Case 2 and in the renal tissues of Case 3—perhaps because of severe autolysis. HRP-2 immunostaining revealed pRBCs in hepatic and splenic sinusoids (Figure 3B and D); immunostaining was abundant in the vasculature of lung, heart, and central nervous system tissues (Figure 4B, D, and F). Kupffer cells and other tissue macrophages also showed granular antigen staining. In spleen sections, there was immunostaining of discrete extracellular “dots,” representing parasites (Figure 3D). In the RBCs, there were two predominant immunostaining patterns: punctate and specific for the intraerythrocytic parasite or membranous (Figure 4D). Staining of the intraerythrocytic parasite was observed with or without erythrocyte membrane staining, and peripheral membrane staining highlighted hemoglobin-depleted erythrocytes. HRP-2 and aldolase antigens were detected in the endothelium of various organs, as well as in renal tubular epithelium and casts (Figure 3F and G), whereas the pLDH immunostaining pattern was predominately cytoplasmic and specific for intra-erythrocytic parasites (Figure 4D).

**DISCUSSION**

In this study, three novel IHC assays targeting HRP-2, aldolase, and pLDH were developed and confirmed severe *P. falciparum* infection in five travelers whose death was initially suspected to be caused by other infectious agents of hemorrhagic fevers. Malaria is the most common cause of fever in travelers returning to industrialized countries from malaria-endemic countries. However, the clinical features of disease are non-specific, and in countries where *P. falciparum* is not endemic, fatal malaria is often not suspected. The pathologic features of malaria resemble many other viral, rickettsial, and bacterial infections; as a result, an unequivocal diagnosis can be made only by laboratory testing.

IHC assays and histopathologic review confirmed *P. falciparum* infection in study cases. As previously reported, abundant hemoglobin pigment, a byproduct of parasite metabolism, was distributed diffusely throughout peripheral tissues and in blood vessels in the central nervous system, findings consistent with reports of hemoglobin localization. The density of hemoglobin increases in proportion to the duration of falciparum infection and decreases with appropriate therapy. In this context, the quantity of hemoglobin deposits observed in each of the study cases strongly suggested that none had taken malaria chemoprophylaxis during their travel.

The hallmark of *P. falciparum* infection is sequestration, characterized by adherence of mature stage falciparum pRBCs (trophozoites and schizonts) to endothelial cells of capillaries and venules. Studies in humans and animal models have described sequestration of trophozoites and schizonts in a variety of tissues, including brain, heart, lung, skeletal muscle, and subcutaneous tissue. The ability of mature stage falciparum-infected erythrocytes to evade circulation in the bloodstream underscores the use of tissue-based diagnosis such as IHC, in fatal cases. As a result of sequestration, peripheral blood parasitemia, traditionally evaluated using a Giemsa-stained blood smear, may not necessarily correlate with the severity of clinical illness; thus, the severity of infection may be underestimated.

Sequestration in study case tissues, as evidenced by IHC and histopathologic findings in this report, correlated with the clinical manifestations of multiple organ failure, which is a common feature of severe malaria and is associated with high mortalities. *P. falciparum* infection causes respiratory symptoms that resemble influenza-like illness; in correlation, the study cases described in this study had pulmonary edema with intra-alveolar hyaline membranes and proteinaceous debris associated with malarial antigens. Pulmonary edema is associated with high parasitemias and often leads to acute respiratory distress syndrome.

Rust-tinged urine, described for several of the patients in this study, is associated with hyperbilirubinemia caused by erythrocyte destruction. In this study, malarial antigens were detected in tubular epithelial cells in association with erythrocyte casts. Progression to renal failure is reported in 30% of patients who are admitted to intensive care units with falciparum malaria. The pathologic findings in the kidneys of study cases, including congestion and cast formation in renal tubules, are an important feature of this illness. Other studies have shown that renal mesangial and proliferative changes, in addition to tubular necrosis, are also associated with *P. falciparum* infection. Extracellular “dots” of
parasites were also observed in spleen sections, showing the process of pitting, or removal of the parasite from the RBC (Figure 3D).

Histopathologic review was strongly suggestive of \textit{P. falciparum} infection; however, the IHC assays developed in this report were essential for definitive diagnosis. In severe malaria infections, such as the study cases presented here, the accumulation of hemozoin pigment in tissues often obscures malarial antigens present in erythrocytes. On optimizing the IHC assays, we found that hemozoin pigment obscured antigens present in erythrocytes and regularly performed the de-pigmentation step after deparaffinization using an established method. Pretreatment conditions also took into account the cross-linking property of formalin fixation. In general, antigen retrieval pretreatment was more effective in improving immunostaining than proteinase K; this was especially true for archival tissues stored in formalin for > 1 month.

The HRP-2 antibody used here was specific for \textit{P. falciparum}, whereas the aldolase and pLDH antibodies reacted with both \textit{P. falciparum} and \textit{P. vivax}. Immunostaining patterns were consistent with what is known about the proteins from previous studies. HRP-2 immunostaining was observed within pRBCs, on the pRBC cell membrane, and in endothelial cells. HRP-2 is a 30-kd water-soluble protein localized in the parasite food vacuole and on the erythrocyte membrane, exclusively produced by \textit{P. falciparum}. Because HRP-2 is eventually excreted in blood and accumulates in correlation with disease severity, it has been targeted for rapid detection tests, such as \textit{ParaSightF}. Immunostaining with \textit{P. falciparum} aldolase, a 41-kd tetrameric polypeptide and a key glycolytic enzyme in parasite metabolism, was similar. Intra-erythrocytic immunostaining is supported by studies of recombinant \textit{P. falciparum} aldolase, which suggest that the enzyme associates with the parasite or host cytoskeleton. Aldolase is also detected in the blood and it is another target in rapid tests like the \textit{Now Malaria Test}. HRP-2 and aldolase immunostaining in renal tubules provides further evidence that the proteins are excreted in the urine. Previously, antigenic proteins ranging from 29,000 to 224,000 M\textsubscript{s} were
isolated in urine samples from adults with *P. falciparum* infection. Furthermore, Genton and others showed that urine samples were adequate specimens for the diagnosis of *P. falciparum* using the ParaSight-F, which is usually applied only to whole blood.

pLDH is another soluble glycolytic enzyme, targeted in the OptiMal rapid test. Compared with the HRP-2 and aldolase assays, pLDH immunostaining was less abundant, limited to discrete staining of the parasite. The difference in relative abundance may be caused by a rate of clearance that is higher for pLDH antigens. Overall, the most abundant immunostaining was observed using the HRP-2 assay, which has been shown to clear more slowly from the blood; HRP-2 antigens may persist for up to 2 weeks even after malaria is cured in the patient.

The IHC assays described here provide further insight of reticuloendothelial interactions through the visualization of pRBCs sequestered in blood vessels and of parasite antigens localized in endothelial cells. Of particular interest in this study was the immunostaining of HRP-2 and aldolase antigens in endothelial cells. The mechanisms of endothelial cell adhesion that allow falciparum parasites to sequester in blood vessels have been studied extensively. Falciparum-infected erythrocytes produce erythrocyte membrane protein 1 (PfEMP-1), which binds to endothelial cell receptors intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet/endothelial cell adhesion molecule 1 (PECAM-1), P-selectin, E-selectin, CD36, thrombospondin, and chondroitin sulfate-A, constitutively expressed on vessel linings. Subsequent to adhesion, the release of soluble parasite proteins and their accumulation in vasculature may cause cytokine recruitment and endothelial cell destruction. In the CNS, it has been hypothesized that activation of cerebral endothelial cells causes a disruption of the blood–brain barrier that could result in the penetration of brain parenchyma by parasite proteins.

**Figure 4.** Pathologic findings in lung, heart and CNS of study cases. A, Intraalveolar edema and intraalveolar macrophages with fine brown hemozoin pigment (Case 4, H&E, original magnification ×50). B, *P. falciparum* HRP-2 immunostaining, showing hyaline membranes (arrow) and pRBCs in vessel (Case 1, original magnification ×100). C, *P. falciparum* HRP-2 immunostaining throughout cardiac vessels (Case 4; original magnification ×100). D, pLDH immunostaining in heart vessel; note discrete parasite (arrow) and membranous (arrowhead) immunostaining of pRBCs (Case 5, original magnification ×153). E, pRBCs throughout CNS vessel in the absence of inflammation (Case 3, H&E, original magnification ×153). F, *P. falciparum* HRP-2 immunohistochemical staining of pRBCs in vessels (Case 3, original magnification ×50; inset, ×158).
As described above, vascular endothelial cell interactions in peripheral and central nervous system tissues are crucial to the survival of *P. falciparum* and contribute to the virulence of infection. The deposition of fibrin and *P. falciparum* IgG and IgM immune complexes along the walls of cerebral vessels has been reported. Furthermore, ultrastructural studies have found that areas of *P. falciparum* sequestration are associated with endothelial cell edema and narrowing of the capillary lumen. The immunostaining of HRP-2 in endothelial cells suggests the protein plays a role in the extracellular environment, perhaps in cytokine recruitment. *In vitro* studies have shown that when the parasitized erythrocyte has reached its final stage of maturation, the erythrocyte bursts and releases ~90% of the HRP-2 content. In *in vitro* culture and affinity chromatography studies describe how soluble parasite antigens from culture supernatants activate neutrophils and monocytes, resulting in the production of oxygen radicals. Both aldolase and HRP-2 are soluble parasite proteins, and their association with endothelial cells seen in IHC assays suggests that they play a role in the cytokine response described above; however, endothelial cell interactions with HRP-2 and aldolase remain to be explored.

While the majority of travelers are aware of the risk of acquiring malaria during international travel, only ~50% comply with an appropriate chemoprophylaxis regimen. In a recent example, five siblings from Chicago, IL, were diagnosed with *P. falciparum* on their return from Nigeria; three of the cases were severe, requiring admission to an intensive care unit. The family assumed incorrectly that malaria drugs were to be taken for treatment only and did not realize that the drugs were to be used for chemoprophylaxis. Nonetheless, the timely exclusion of malaria from a differential diagnosis includes other viral and hemorrhagic fevers is critical to infection control. The quarantine of the crew aboard the ships on which Cases 1, 2, and 4 traveled exemplifies the public health implications for timely diagnosis and rule-out of common etiologies such as malaria among exotic diseases like Ebola that are transmissible from person-to-person. The incidence of other travel-associated hemorrhagic fevers continues (imported yellow fever, typhus, Lassa fever, etc.), and IHC assays described in this report can provide important information with broad implications for public health. When the first clues of malaria infection are found at autopsy, the *P. falciparum* HRP-2, aldolase, and pLDH IHC assays developed in this report may definitively confirm *P. falciparum* and provide insights into the pathogenesis of malaria.

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