MAYO CLINIC

ORIGINAL ARTICLE

Clinical Findings and Diagnosis in Human Granulocytic Anaplasmosis: A Case Series From Massachusetts

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Abstract

Objective: To describe clinical findings and the use of a tick-associated pathogen panel in a series of patients with human granulocytic anaplasmosis (HGA) at a suburban Boston hospital.

Patients and Methods: Medical records were reviewed for inpatients and outpatients at Newton-Wellesley Hospital with a positive polymerase chain reaction (PCR) result for *Anaplasma phagocytophilum* during the study period March 1 through November 30, 2009. A PCR panel was used to test for tick-borne pathogens. Postal zip code data from the patients' areas of residence were used to estimate the area of disease transmission.

Results: Thirty-three cases were confirmed during the 2009 transmission season, and 14 of these patients (42%) required hospitalization. Thrombocytopenia and/or leukopenia were observed at the time of presentation in 25 of 30 patients (86%) in whom both white blood cell and platelet counts were determined, and 28 of 33 patients (85%) reported fever. Rash occurred in only 2 of the 33 patients (6%), and 25 (76%) reported one or more respiratory or gastrointestinal symptom. Cases were geographically distributed diffusely throughout the hospital catchment area, with one possible focus of infection identified in Weston, MA. Due to a lack of clinical data reporting to the Massachusetts Department of Public Health, only 20 of 32 HGA cases (63%) fulfilled the case confirmation criteria.

Conclusion: Diagnosis of HGA requires a high suspicion for infection even in endemic areas. Use of a tick-associated pathogen panel that includes PCR assays for several organisms could improve detection of underrecognized tick-borne diseases in endemic areas. Lack of epidemiological follow-up to confirm corroborating clinical findings prevents accurate case reporting and assessment of the true HGA burden.

 ■ Mayo Clin Proc. 2012;87(3):233-239

Human granulocytic anaplasmosis (HGA; formerly, human granulocytic ehrlichiosis or HGE), is an emerging tick-borne disease caused by the bacterium *Anaplasma phagocytophilum*. Human granulocytic anaplasmosis causes a range of clinical syndromes from asymptomatic infection to multisystem organ failure.[1] Subclinical infection is common.[2-4] In the eastern and north central United States, *Ixodes scapularis* ticks serve as the vector for *A phagocytophilum* and other tick-borne pathogens, including *Borrelia burgdorferi* and organisms in the genus *Babesia*. In California, the primary vector of *A phagocytophilum* is the *Ixodes pacificus* tick.[5] Coinfection can occur when multiple organisms infect a tick, and transmission events of more than one tick-borne pathogen via one tick bite are well described.[6,7] Transmission through blood transfusion also occurs.[8]

Reported cases of HGA have increased in recent years.[9-11] Human granulocytic anaplasmosis infections are known to occur throughout the United States, with a focus in the northeast and north central states.[12] In New England, the risk of Lyme disease is well recognized by the general public and by clinicians, partially as a result of public health awareness campaigns.[13,14] The risk of HGA and babesiosis may be underappreciated due to the more recent emergence of these infections and the lower incidence relative to Lyme disease. Testing for tick-borne infections requires separate serology or polymerase chain reaction (PCR) assays for each pathogen. In order to improve diagnosis of acute non-Lyme tick-borne disease in areas of endemic tick-borne disease transmission, several hospitals in suburban Boston, MA, use a tick-associated pathogen panel (TAPP) that includes a PCR assay for detection of the organisms that cause HGA, other human ehrlichioses, Lyme disease, and babesiosis. We describe a cohort of 33 patients diagnosed with HGA using a PCR TAPP at one hospital during the 2009 transmission season.

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PATIENTS AND METHODS

Study Design

Our study population included all patients who had one or more blood sample sent for a PCR TAPP from the Newton-Wellesley Hospital laboratory in Newton, MA, during the study period, including outpa-

tients seen at primary care clinics affiliated with the hospital. Patients with a positive PCR result for *A phagocytophilum* between March 1 and November 30, 2009, were included in the study. This time period encompassed the expected 2009 season of transmission for tick-borne disease in Massachusetts. Patient data were collected by retrospective chart review. This study was approved by the Human Research and Investigation Committee of Newton-Wellesley Hospital.

Molecular Testing

In acute illness, laboratory diagnosis of HGA is often conducted by PCR assay on DNA extracted from a whole blood sample.[1] All patients were tested with a PCR TAPP for organisms that cause HGA and other human ehrlichioses (*A phagocytophilum*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii*), in addition to *B burgdorferi* and *Babesia microti*, the causative agents of Lyme disease and babesiosis, respectively. Polymerase chain reaction testing was conducted at the Mayo Clinic reference laboratory (Rochester, MN).[15] The PCR target for detection of *A phagocytophilum* is *HSPD1*, an open reading frame gene segment of the heat shock protein operon (*groESL*) that detects and differentiates between *A phagocytophilum*, *E chaffeensis*, and *E ewingii*.[16,17] This PCR assay was shown to be equivalent to individual PCR tests that separately detect these pathogens.[16] The TAPP was approved for use by internal validation at the Mayo Clinic laboratories; however, there are no published reports of the specificity and sensitivity using this TAPP in human populations.

Epidemiological Case Definition

National case definitions for infectious diseases under public health surveillance set forth clinical and laboratory criteria that, if met, define a confirmed case. These definitions are not intended for clinical diagnostic purposes. The surveillance data are useful for comparing standardized burdens of disease between geographic areas. The current national case definition for HGA was developed in 2008.[18] A confirmed case is an individual with fever and 1 or more of the following clinical signs/symptoms: headache, myalgia, anemia, leukopenia, thrombocytopenia, or any transaminase elevation, in addition to at least 1 of the following laboratory criteria: (1) serologic evidence of a 4-fold change in IgG-specific antibody titer to *A phagocytophilum* antigen by indirect immunofluorescence assay in paired serum samples (one taken in the first week of illness and a second 2-4 weeks later); (2) detection of *A phagocytophilum* DNA in a clinical specimen via amplification of a specific target by PCR assay; (3) demonstration of *A phagocytophilum* antigen in a biopsy/autopsy sample by immunohistochemical methods; or (4) isolation of *A phagocytophilum* from a clinical specimen in cell culture.

Statistical Analyses

Statistical testing for independence was conducted by χ^2 testing with Yates correction for large values. The *t* test was used to compare mean values, with a predetermined cutoff of $P<.05$ indicating a statistically significant difference. Analyses and figure preparation were performed on GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA) and Stata version 9.0 (Stata Corp, College Station, TX).

RESULTS

During the study period, 692 samples were sent for testing using a PCR TAPP, and 33 of the 692 patients (4.8%) had an HGA-positive result. Each of the patients with a positive result presented to a health care provider with symptoms compatible with *A phagocytophilum* infection. Patient demographics and relevant exposures are shown in Table 1. The duration of symptoms before presentation was re-

TABLE 1. Patient Characteristics and Presenting Signs and Symptoms in 33 Cases of Human Granulocytic Anaplasmosis[a]

Demographics	
Female	10 (30)
Mean age (y) (range)	53 (13-94)
Known tick bite in the previous 2 mo (n=17)[b]	11 (65)
Report of frequent outdoor activity (n=21)[b]	19 (90)
Signs and symptoms at presentation[c]	
Fever	28 (85)
Myalgia	24 (73)
Headache	14 (42)
Cough/dyspnea	10 (30)
Nausea	9 (27)
Diarrhea	9 (27)
Vomiting	8 (24)
Abdominal pain	6 (18)
Rash	2 (6)
Confusion	1 (3)

[a] Data are presented as No. (percentage) of patients unless indicated otherwise.
[b] Denominators vary due to differences in clinical histories.
[c] Twenty-eight of the 33 patients presented with multiple symptoms.

Mayo Clin Proc. ■ March 2012;87(3):233-239 ■ doi:10.1016/j.mayocp.2011.09.008
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ported for 26 of the 33 cases (79%). A mean of 3.5 days (range, 1-21 days) of symptoms were reported before presentation. Twelve of the 33 patients (36%) presented in an outpatient setting and 19 (58%) presented to the emergency department; 2 patients (6%) sought attention in an urgent care center. Hospitalization was required for 14 of 33 patients (42%), and the median hospital stay was 4.3 days (range, 1-12 days). Hospitalized patients were significantly older than nonhospitalized patients ($t=-2.1$; $P=.04$). The mean (SD) age was 53 years (14.9 years) for outpatients and 64 years (16.6 years) for inpatients. Mean (SD) platelet counts at presentation for outpatients were 119×10^9/L (60×10^9/L) compared with 76×10^9/L (46×10^9/L) for inpatients, and patients with more profound thrombocytopenia at presentation were more likely to be hospitalized ($t=2.1$; $P=.05$). The degree of leukopenia at presentation was not associated with higher rates of hospitalization ($t=1.2$; $P=.24$). Fifteen of the 33 patients (45%) had peripheral smear examinations at the time of presentation, 5 (33%) of whom had a positive result (identification of inclusion bodies or morulae visualized in neutrophils). Patients with positive smear results were not more likely to be hospitalized ($t=-0.6$; $P=.51$). One patient with serious comorbid conditions was admitted to the intensive care unit for an exacerbation of congestive heart failure during hospitalization for HGA. There were no known in-hospital deaths or clinically significant sequelae of infection; however, patient follow-up varied, and active follow-up to detect possible sequelae was not conducted.

Medical records for 17 of the 33 patients (52%) indicated that clinicians asked the patient if he or she had a recent tick bite, and 11 of the 17 (65%) recalled a tick bite within the previous 2 months. One patient reported travel to California in the month before presentation and denied knowledge of tick bites during or after his trip. No other relevant travel was reported in the medical records for these 33 patients. Twenty-one of the 33 patients (64%) were asked if they spent time outdoors, and 19 (90%) reported that they frequently spent time outdoors (including reports of gardening, walking, and bicycling).

Signs and symptoms are described in Table 1. Fever was the most common chief complaint and was reported in 28 of 33 cases (85%). More than half of the patients reported myalgia at presentation (24 of 33 [73%]), and most patients (25 of 33 [76%]) described one or more respiratory or gastrointestinal symptom including cough, shortness of breath, nausea/vomiting, diarrhea, or abdominal pain. Most patients (28 of 33 [85%]) presented with multiple symptoms. Two of the 33 patients (6%) were noted to have an erythematous, nonpruritic rash on the upper chest and neck at presentation.

Laboratory results are summarized in Table 2. Thirty-two of the 33 HGA cases (97%) had a white blood cell (WBC) measurement at the time of presentation. Most of these patients had leukopenia (22 of 32 [69%]). Platelet counts were determined in 29 of the 33 HGA cases (88%) at the time of presentation, and 20 (69%) had thrombocytopenia. Thirty of the 33 patients (91%) had both platelet and WBC counts measured on presentation, and only 5 (17%) had both a WBC and platelet count in the normal range. Therefore, 83% (25 of 30) of HGA patients demonstrated an abnormality in WBC and/or platelet counts at presentation; 4 of 30 (13%) had thrombocytopenia only, another 4 (13%) had leukopenia only, and more than half (17 of 30 [57%]) had both leukopenia and thrombocytopenia. Among 33 HGA cases, all 5 (15%) who had positive peripheral smear findings were diagnosed by peripheral smear before their PCR test results were received. In 2 of these patients (40%), early diagnoses were made by laboratory technologists who recognized morulae in neutrophils during a routine examination of a peripheral smear for calculation of a leukocyte differential count. Figure 1 shows an example of morulae in one patient's peripheral smear. Subsequent PCR testing on all 5 samples diagnosed by peripheral

TABLE 2. Laboratory Values at Presentation in Patients With Human Granulocytic Anaplasmosis

	Median value	Value range	Normal range	No. of patients tested	No. with abnormal value at presentation (%)
White blood cells ($\times 10^9$/L)	3.2	1.6-9.1	4.0-11.0	32	22 (69)
Platelets ($\times 10^9$/L)	86	31-239	130-400	29	20 (69)
Aspartate aminotransferase (U/L)	76	31-261	6-40	27	20 (74)
Alanine aminotransferase (U/L)	76	17-370	10-49	25	18 (72)
Alkaline phosphatase (U/L)	95	48-263	27-129	26	7 (27)

To convert aspartate aminotransferase to μkat/L, multiply by 0.01667; to convert alanine aminotransferase to μkat/L, multiply by 0.0167; to convert alkaline phosphatase to μkat/L, multiply by 0.0167.

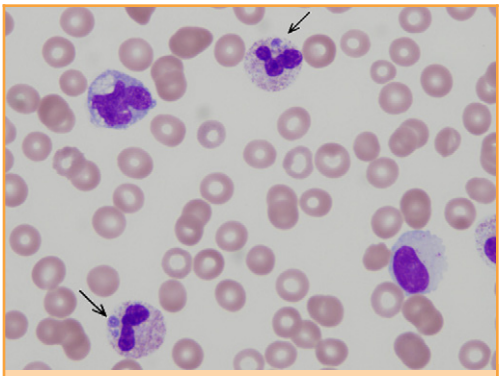

FIGURE 1. Morulae (arrows) in neutrophils of a 65-year-old man with a 2-day history of fever and diarrhea (Wright stain, original magnification ×100).

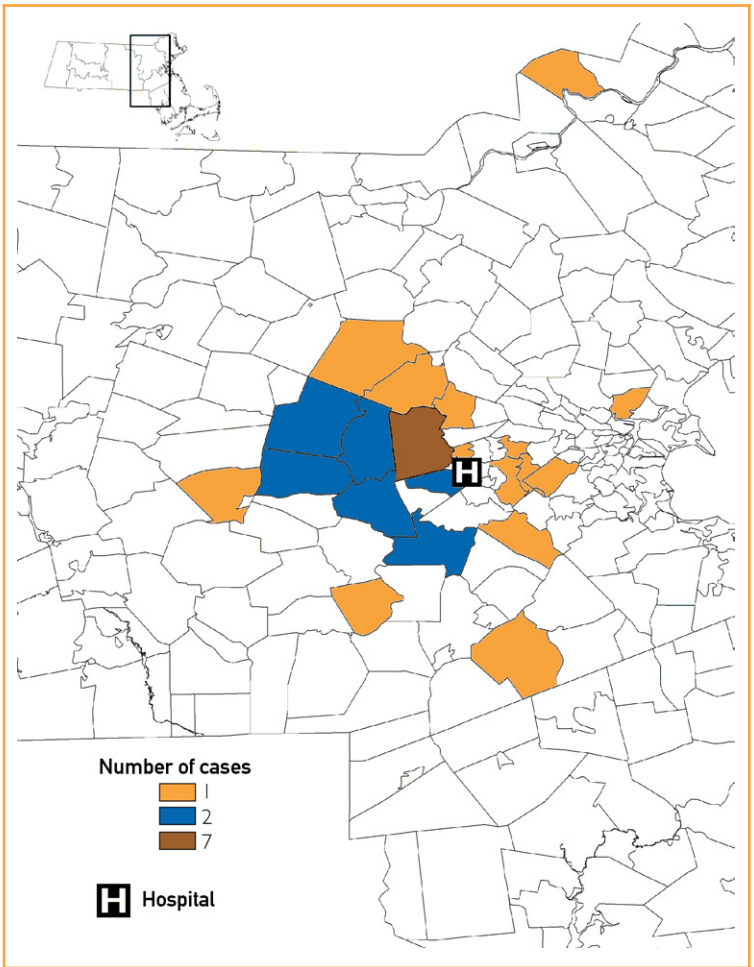

FIGURE 2. Concentration of human granulocytic anaplasmosis infections by zip code area surrounding Newton-Wellesley Hospital during the 2009 transmission season.

smear examination confirmed *A phagocytophilum* infection.

Patient residential postal zip code data were used to identify the possible area of disease transmission. The 33 patients resided in 21 different zip codes within the hospital catchment area (Figure 2). A single zip code in Weston, MA (02439) was the residence area of 7 of the 33 patients diagnosed with HGA (21% of all cases). Only 3% (11,079 of 350,854) of patients treated at Newton-Wellesley Hospital during the study period resided in this zip code; therefore, persons from this zip code are overrepresented among HGA cases at this hospital. The difference between the expected and observed number of patients with HGA in this zip code region was statistically significant ($\chi^2=271$; $P<.001$). These results suggest that this zip code locale may have increased transmission of HGA compared with surrounding areas.

On the basis of the national case definition, the 33 patients diagnosed by TAPP in our study fulfill criteria for HGA case confirmation. Of these 33 PCR-positive cases, 32 (97%) were reported to the Massachusetts Department of Public Health (MDPH), and 20 of the 32 cases (63%) were designated as confirmed cases because clinically compatible information was reported by physicians caring for the patient or by patient report. The discrepancy between eligibility for case confirmation (32 cases) and actual confirmation (20 cases) was due to a lack of clinical data available to the MDPH and represents an underestimate of true incident infections.

DISCUSSION

We describe a cohort of 33 patients diagnosed with HGA at a Boston suburban hospital during the 2009 transmission season. Overall, signs and symptoms of infection and clinical outcomes were consistent with previously published descriptions.[19] There were no sensitive or specific solitary laboratory findings associated with *A phagocytophilum* infection in this series, and clinical findings were also nonspecific. This serves as a reminder that HGA is an undifferentiated febrile illness, and in the appropriate season and geographic area, fever accompanied by few or no localizing signs or symptoms should prompt suspicion for tick-borne disease. When questioned, the majority of patients with HGA in this series reported recent outdoor activity. It is unclear if patients were asked about exposure because clinicians suspected tick-borne disease based on clinical presentation or if clinicians were more likely to request testing for tick-borne disease in patients who reported outdoor exposure. Additionally, as noted in earlier reports, many infected patients did not recall a recent tick bite.[4,20]

Mayo Clin Proc. ■ March 2012;87(3):233-239 ■ doi:10.1016/j.mayocp.2011.09.008
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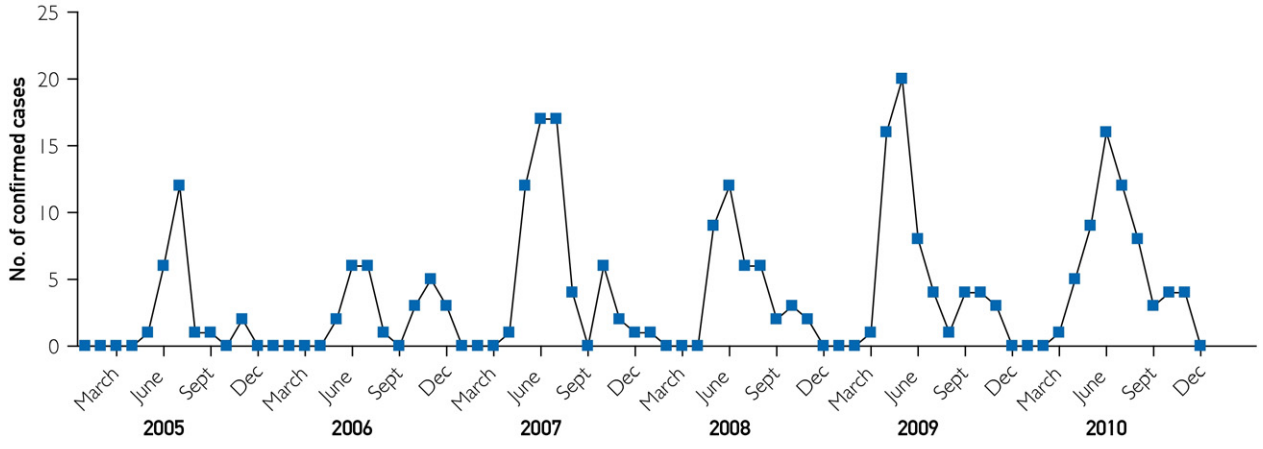
Awareness by clinicians and laboratory personnel has led to an increase in diagnosis of HGA in our hospital. In 2 cases included in this series, a diagnosis was made because laboratory personnel detected inclusion bodies in neutrophils consistent with *A phagocytophilum* during routine evaluation of peripheral smears, independent of clinician request for a peripheral blood smear examination for tick-borne infection. These findings demonstrate the importance of technician familiarity with the appearance of positive smears.

Serodiagnosis is the most sensitive diagnostic method for HGA but requires paired serum samples and is therefore not practical when rapid diagnosis is needed. Detection of morulae on patient peripheral smears is not a sensitive method for diagnosis, as our results demonstrate, since morulae are most reliably visible in the acute phase of infection.[21,22] However, our results demonstrate that when a trained technician detects characteristic inclusion bodies in a peripheral blood smear, a diagnosis of HGA may be achieved more rapidly than with PCR. Due to the high sensitivity provided by PCR, it is the diagnostic method of choice in acute illness.[19,21,23]

In total, 146 positive HGA PCR laboratory reports from across Massachusetts were received by the MDPH from patients tested during the study period. Accompanying clinical information was received for only 84 (58%) of the total PCR-positive cases statewide. Due to either insufficient clinical information or a clinical description inconsistent with HGA, only 61 of the 84 cases (73%) fulfilled both the clinical and laboratory criteria for a confirmed case.[24] As noted previously in this report, only 20 of the 32 cases (63%) reported from our hospital in 2009 became confirmed MDPH cases, solely due to lack of clinical information transmitted to the MDPH. Often, patient history and clinical data are not reported by physicians, and resources for direct follow-up with patients are scarce. Our discussions with MDPH authorities and the results of this study demonstrate that current reporting of HGA cases is underestimated using epidemiological case-finding methods. It is crucial for practitioners to report clinical data to public health authorities, who otherwise have only laboratory testing results that alone are insufficient for defining HGA cases.

Limitations of our study include the retrospective nature of data collection. Active follow-up to determine patient outcomes was not conducted, and confirmatory testing including serologic studies was not conducted on patients in this study. The



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2005	0	0	0	0	1	6	12	1	1	0	2	0
2006	0	0	0	0	2	6	6	1	0	3	5	3
2007	0	0	0	1	12	17	17	4	0	6	2	1
2008	1	0	0	0	9	12	6	6	2	3	2	0
2009	0	0	0	1	16	20	8	4	1	4	4	3
2010	0	0	1	5	9	16	12	8	3	4	4	0

FIGURE 3. Epidemiologically confirmed cases of human granulocytic anaplasmosis in Massachusetts, 2005-2010 (as of August 20, 2011). Unpublished data from the Commonwealth of Massachusetts, Massachusetts Department of Public Health, with permission.

HGA PCR testing was carried out in one laboratory using one assay. Polymerase chain reaction assays are not standardized across laboratories, and sensitivity and specificity are likely to vary. The exact location of patient exposure to ticks was not determined in our study, and we assumed that exposure most likely occurred in the zip code of residence. The frequency with which clinicians are testing for, detecting, and reporting infection with *A phagocytophilum* in other areas of Massachusetts is unknown. Therefore, it is not possible to determine if the nature and epidemiology of the HGA cases reported herein are unique to this region.

Cases of confirmed HGA have increased yearly in Massachusetts since 2005, as shown in Figure 3. An increase in confirmed cases may reflect an increase in incidence of infections, an increase in diagnosis or reporting of infections, or a combination of these factors. This trend suggests an overall increase in HGA infections during this time period. Notably, several cases were reported outside the window of the traditional tick-borne disease transmission season, especially during the winter months. An additional limitation is our study period, which could have excluded HGA cases in the very early and late months of 2009. These results should encourage clinicians to have a high suspicion for tick-borne disease in the spring and summer and to suspect tick-borne disease in the appropriate clinical setting year-round. In the medical records reviewed for this case series, clinicians frequently documented suspicion for Lyme disease based on history and clinical presentation, and non-Lyme tick-borne diseases were infrequently included in the differential diagnosis. In these cases, use of a TAPP resulted in appropriate diagnosis and treatment. Clinicians, especially, should be alert to the fact that there are at least 2 other potentially life-threatening infections borne by the same ticks that transmit Lyme disease, and these infections merit consideration when febrile illness occurs in the appropriate season in an endemic area.

CONCLUSION

Diagnosis of HGA requires a high suspicion for infection. We suggest that many cases of HGA are underdiagnosed, and use of a TAPP for evaluating suspected tick-borne disease may be prudent where tick-borne diseases are endemic. An accurate epidemiological assessment of the HGA burden is dependent on confirmation of clinical findings with public health authorities.

ACKNOWLEDGMENTS

We are grateful for the support of Carlin H. Bachrach, BS, Susan Manning, BS, and the hematology laboratory technicians at Newton-Wellesley Hospital, in providing technical assistance. Dr Bobbi Pritt provided valuable technical assistance. We also thank Dr Michael Lew for helpful comments on the manuscript.

Presented in part at the Infectious Diseases Society of America meeting; October 22, 2011; Boston, MA.

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