A Clinical, Diagnostic, and Ecologic Perspective on Human Anaplasmosis in the Upper Midwest

Anna M. Schotthoefer; Jennifer K. Meece; Thomas R. Fritsche, MD

ABSTRACT

Introduction: Human anaplasmosis caused by the bacterial pathogen *Anaplasma phagocytophilum* was first discovered in the Upper Midwest in 1990. Since that time the number of cases in the region has steadily increased, such that today, the pathogen rivals that of Lyme disease in causing human tick-borne–related illness.

Objective: We provide an overview of the biology, clinical characteristics, and epidemiology of the disease in the Upper Midwest and discuss currently available diagnostic methods.

Findings: Rapid differentiation of anaplasmosis from other acute febrile illnesses and targeted treatment are important for preventing severe disease and potentially fatal outcomes in infected individuals. Beyond blood smear analysis and serology, the development of real-time polymerase chain reaction (PCR) assays for clinical use holds promise in improving our ability to make rapid diagnoses and to differentiate *A phagocytophilum* infections from those produced by closely related *Ehrlichia* pathogens, which are also present in the region.

Conclusion: Continuing expansion of the range of the black-legged tick *(Ixodes scapularis)*, the principal vector of the disease, into areas heavily populated or visited by humans in the region likely will result in this pathogen becoming an even greater burden on human health. Efforts are needed to better characterize the current geographic distribution of human *Anaplasma* and *Ehrlichia* cases to identify emerging foci and to better understand the enzootic cycles that maintain the pathogens in the region. Improved diagnostics may assist with such efforts.

INTRODUCTION

Human anaplasmosis (HA; synonymously known as human granulocytic ehrlichiosis [HGE] and human granulocytic anaplasmosis [HGA]) is a zoonotic, tick-borne disease caused by the intracellular bacterium *Anaplasma phagocytophilum*, a member of the order *Rickettsiales*. The pathogen was first described in association with a febrile illness in 12 patients from Minnesota and Wisconsin in 1990-1993[1] (Table 1). Investigations that followed revealed that about 10% to 15% of patients reporting with a nonspecific febrile illness during the summer months to clinics in these states had evidence of infection with the agent,[2-4] and active surveillance studies reported annual incidence rates ranging between 9.3 and 16.1 per 100,000 individuals for the region.[5,6] The agent subsequently has been reported in several northeastern US states and in northern California, as well as in other regions of the world, including Europe and China.[7,8] However, the Upper Midwest (defined here as Minnesota and Wisconsin) has continued to be a primary focus of the disease with more than 56% of all US cases reported to the Centers for Disease Control and Prevention (CDC) in 2011 occurring in this region.[9] Moreover, since its discovery, the number of cases reported annually has steadily increased in the region, with an estimated number of 690 and 770 cases reported in Wisconsin and Minnesota, respectively, in 2011. These numbers represent at least a 10-fold increase in the number of cases reported in these states since it became a nationally reportable disease in 1998.[9]

A number of factors help to explain the rise in the numbers of HA cases in the Upper Midwest, including increased physician awareness and changes in reporting practices. Of perhaps greater significance, however, is the geographic expansion of the primary vector for the agent, *Ixodes scapularis* (the black-legged tick, also commonly referred to locally as the deer tick or bear tick). When HA was first discovered, the tick was primarily restricted to east-central Minnesota and northwestern Wisconsin (Figure 1A). Over the last decade, the tick has spread across Wisconsin and now is being found near more heavily populated areas of the state outside of Milwaukee and Green Bay, as well as areas

• • •

Author Affiliations: Marshfield Clinic Research Foundation, Marshfield, Wis (Schotthoefer, Meece); Marshfield Laboratories, Marshfield, Wis (Fritsche); University of Wisconsin-La Crosse (Fritsche).

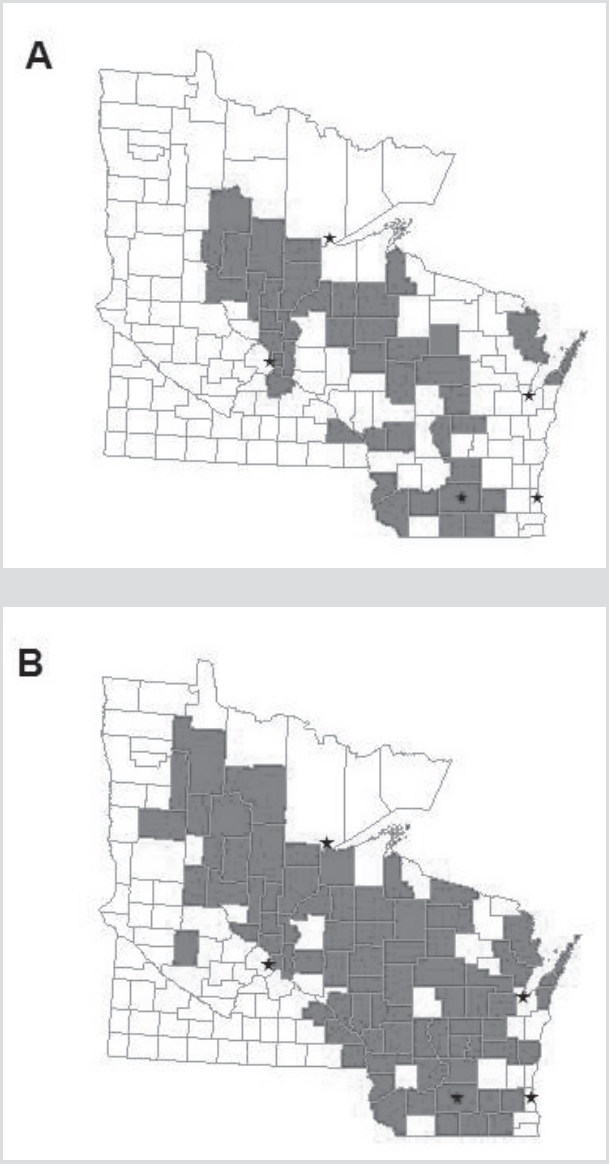
Corresponding Author: Anna M. Schotthoefer, PhD, Marshfield Clinic Research Foundation, 1000 N Oak Ave, Marshfield, WI 54449; phone 715.389.3520; fax 715.389.3319; e-mail Schotthoefer.Anna@mcrf.mfldclin.edu.

CME available. See page 115 for more information.

Table 1. Timeline of Major Events in the Discovery and Reporting of *A phagocytophilum* in the Upper Midwest

Year(s)	Event	Reference(s)
1990-1993	*A phagocytophilum* first detected in febrile patients in Wisconsin and Minnesota; designated as human granulocytic ehrlichiosis (HGE)	Chen, et al[1]
1995	HGE becomes a reportable disease in Minnesota	Bakken, et al[5]
1998	HGE officially becomes a nationally notifiable disease, including in Wisconsin	Council of State and Territorial Epidemiologists[9]
2001	HGE, *E equi* and *E phagocytophila* are synonymized as *A phagocytophilum*; designated as human granulocytic anaplasmosis (HGA) or human anaplasmosis (HA)	Dumler, et al[10]
2008	Case definition changes to accommodate reporting of infections with specific species of *Anaplasma* and *Ehrlichia*	Natonal Notifiable Diseases Surveillance System[11]

Figure 1. Expansion of *Ixodes scapularis* Populations in the Upper Midwest



Gray areas are counties where the tick was found during surveillance studies conducted through 1994 (A) and 2009 (B), based on previous reports.[12] Stars mark the locations of major metropolitan areas in the region: Milwaukee, Madison, and Green Bay, Wis; and Minneapolis-St. Paul and Duluth, Minn.

in northern Wisconsin that are visited each year by thousands of tourists who engage in summer recreational activities[12] (Figure 1B). In Minnesota, the tick also has spread into areas north and west of its original range, including into areas bordered by Canada.[13] Expansion of the tick into these areas is of concern not only because of the potential for increasing transmission of HA to humans, but also because the tick transmits other important diseases endemic to the region, including Lyme disease, babesiosis, and the Powassan encephalitis virus (Table 2). Because of the biological competency of *I scapularis* to carry and transmit a variety of etiologic agents, concurrent infections with HA and these other pathogens may be expected to become more frequent. Patients with co-infections are known to experience more severe illness and their effective treatment may require multiple therapies[14] (Table 2).

Increased contact between humans and ticks also creates opportunities for the emergence of new pathogens. In 2009 an agent novel to North America, designated as *Ehrlichia* species Wisconsin, was discovered in 1 Minnesota and 3 Wisconsin patients by a consortium consisting of the Mayo Clinic Laboratories, Wisconsin Division of Public Health (WDPH), and the CDC.[15] Although the importance of this new pathogen has yet to be elucidated, it emphasizes the need for continued disease surveillance in the region and the development of diagnostic tests that are sensitive and specific to each pathogen and that can be readily adopted by clinical laboratories. In addition, continuing efforts to educate health care providers and the public about these diseases will be important for their rapid diagnosis and treatment, as well as for their prevention.

In this review, we summarize the current state of knowledge regarding the biology, clinical characteristics and epidemiology of HA in the Upper Midwest and discuss the diagnostic methods employed in detection of the disease; areas for future research are also outlined. For more general reviews of anaplasmosis and erhlichioisis, readers are referred to Dumler et al[16] and Thomas et al.[17]

FINDINGS

Biology and Taxonomy

Anaplasma phagocytophilum, like all other members in the order *Rickettsiales* is an obligate intracellular bacterium.[10] It was

Table 2. Summary of Tick-borne Pathogens Occurring in the Upper Midwest

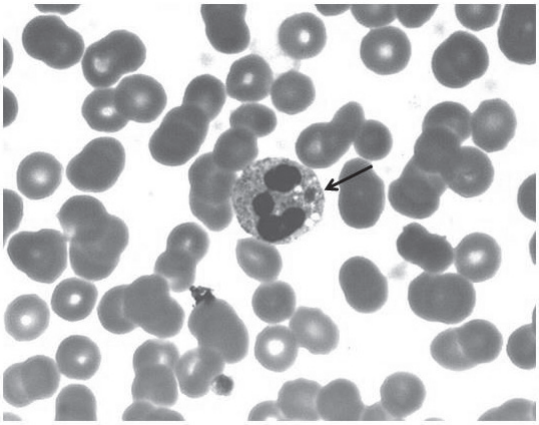
Pathogen	Name(s) of Disease	Primary Tick Vector	Diagnostic Tests[a]	Recommended Treatment[b]
Endemic and commonly transmitted to humans in the Upper Midwest				
Anaplasma phagocytophilum	Human anaplasmosis (HA), human granulocytic anaplasmosis (HGA), Human granulocytic ehrlichiosis (HGE)[c]	*Ixodes scapularis*	1. Blood smear tests for visualization of morulae in neutrophils. 2. Detection of antibodies in serum. 3. Real-time PCR assays for detection of DNA in peripheral blood.	**First line:** Doxycycline or tetracycline for 5-14 days for all age groups, including children. **Alternative:** Rifampin for pregnant patients or patients with a history of allergy to tetracycline.
Borrelia burgdorferi	Lyme Disease	*Ixodes scapularis*	1. Two-tier serologic testing for detection of antibodies in serum: enzyme immunoassay (EIA), followed by the immunoblot (Western blot) test. 2. Detection of antibodies in synovial fluid. 3. Real-time polymerase chain reaction (PCR) assay for detection of DNA in cerebrospinal fluid. 4. Growth of organism in cell culture from skin biopsy.	**For patients with early Lyme disease (eg, with erythema migrans):** Doxycycline for 10-21 days, or amoxicillin, or cefuroxime axetil for 14-21 days. **For patients with Lyme meningitis or early neurologic Lyme disease:** Ceftriaxone for 10-28 days. **For patients with Lyme arthritis:** Doxycycline, amoxicillin, or cefuroxime axetil for 28 days. **For patients with late Lyme disease (eg, recurrent Lyme arthritis and/or late neurologic disease):** Parenteral or intravenous treatment with ceftriaxone, cefotaxime, or penicillin G for 14-28 days.[18]
Endemic and emerging or rarely transmitted to humans in the Upper Midwest				
Babesia microti	Babesiosis	*Ixodes scapularis*	1. Blood smear tests for visualization of parasites in red blood cells. 2. Detection of antibodies in serum. 3. Real-time PCR assays for detection of DNA in peripheral blood.	Combination of atovaquone plus azithromycin, or clindamycin plus quinine for 7-10 days.
Ehrlichia species Wisconsin		Evidence suggests *Ixodes scapularis*	Real-time PCR assays for detection of DNA in peripheral blood.	**First line:** Doxycycline or tetracycline for 5-14 days for all age groups, including children. **Alternative:** Rifampin for pregnant patients or patients with a history of allergy to tetracycline.
Powassan virus		Evidence suggests *Ixodes scapularis*	Detection of antibodies in serum	Supportive therapy [WDPH; http://www.dhs.wisconsin.gov/]. Accessed May 14, 2014.
Not known to be endemic, but may be currently invading the Upper Midwest				
Ehrlichia chaffeensis	Ehrlichiosis, Human monocytic erhlichiosis (HME)	*Amblyomma americanum*	1. Blood smear tests for visualization of morulae in monocytes. 2. Detection of antibodies in serum. 3. Real-time PCR assays for detection of DNA in peripheral blood.	**First line:** Doxycycline or tetracycline for 5-14 days for all age groups, including children. **Alternative:** Rifampin for pregnant patients or patients with a history of allergy to tetracycline.

[a] Only the tests commonly offered by clinical laboratories are listed; other diagnostic tests may exist.
[b] Antibiotic therapies as recommended by the CDC, http://www.cdc.gov/ticks/index.html, and cited references.
[c] The name "ehrlichiosis" still is frequently used in the Upper Midwest in reference to infections with *A phagocytophilum*. It should be recognized that there are distinct *Ehrlichia* species agents that can cause similar diseases as *A phagocytophilum*, but that do not frequently occur in the Upper Midwest. Discontinued use of the name ehrlichiosis and adoption of anaplasmosis for infections caused by *A phagocytophilum* is recommended.

described first as a pathogen of veterinary importance and originally was designated as 2 separate *Ehrlichia* species: *E phagocytophila* in ruminants and *E equi* in horses.[8] When it was first detected in humans, it was referred to as the human granulocytic ehrlichiosis (HGE) agent.[1] In 2001, phylogenetic analyses based on molecular data supported the reclassification of *E phagocytophila, E equi,* and HGE into the single species, *A phagocytophilum*[10] (Table 1).

The pathogen naturally cycles between wild mammalian hosts and tick vectors. Within its mammalian hosts, including humans, the pathogen infects neutrophils and replicates within intracytoplasmic vacuoles to form inclusions known as morulae[10] (Figure

Figure 2. Human Peripheral Blood Granulocyte Infected with a Morula of *A phagocytophilum*



2). In the eastern United States, the nominal wild reservoir for infections is the white-footed mouse, *Peromyscus leucopus,*[19,20] although other small mammals, including short-tailed shrews, *Blarina brevicauda,* and eastern chipmunks, *Tamias striatus*, also significantly contribute to enzootic maintenance.[20] Other mammalian species, including white-tailed deer, *Odocoileus virginianus,* and birds also may harbor infections.[21,22]

Recent investigations suggest that the pathogen exists as biologically and ecologically distinct subpopulations that are adapted to specific reservoir hosts and tick species with varying capacities to infect and cause disease in humans and domestic animals.[23,24] In particular, the 16S rRNA gene variant referred to as Ap-ha is the only known *A phagocytophilum* variant that has been isolated to date from humans in the eastern United States and it appears to be maintained in the *P leucopus–I scapularis* enzootic cycle in that region.[25,26] Distinct variants not found in humans have been recovered from deer, other ruminants, and horses, suggesting only a subset of *A phagocytophilum* variants cause disease in humans.[25,27]

The potential role that different mammalian species have as reservoirs in the Upper Midwest remains largely unexplored; however, the Ap-variants 1, WI-1, and WI-2 have been detected in deer in the region.[28-31] Small rodents, including *P leucopus, T striatus,* and *Clethrionomys gapperi* have also been found to harbor *A phagocytophilum* infections, although the identities of the variants in these hosts have not been determined.[31]

Infections in dogs and horses also appear to be common in the region and may be on the rise.[32,33] For instance, more than 50% of dogs tested in some counties in Minnesota and Wisconsin were seropositive during a national surveillance study conducted in 2006-2007, and overall prevalence of samples with antibodies to *A phagocytophilum* was 6.7% in the Midwest, a higher prevalence than that reported for any other region.[34] Dogs appear to be infected with the same genetic variants of *A phagocytophilum* as humans[24,27] although their role, as well as that of wild canids, in maintaining HA infections is unknown.

In the Upper Midwest *I scapularis* is the principle vector for the pathogen. The tick species is widely distributed across the eastern USA and surveys conducted in Wisconsin and Minnesota have reported *A phagocytophilum* prevalences of 9% to 12% in adult ticks.[29,35-37] Ticks typically acquire infections as larvae or nymphs, following feeding on a reservoir-competent host. Ticks may retain infections as they molt between stages, but infected females are unlikely to transmit infections to offspring transovarially,[10] though transovarial transmission was demonstrated for a nonpathogenic variant of *A phagocytophilum* found circulating between *Dermacentor albipictus* and white-tailed deer in Minnesota.[30] Unlike transmission of *B burgdorferi,* which may require[36-48] hours or more of tick attachment, laboratory studies on mice indicated transmission of *A phagocytophilum* may occur within 24 hours of tick attachment,[38,39] suggesting that faster detection and removal of attached ticks is necessary to prevent transmission. Most human cases of HA occur in June and July, corresponding to the activity of nymphal *I scapularis*, suggesting this is the most likely stage to transmit infections to humans.

Clinical Characteristics and Epidemiology

HA is characterized by nonspecific symptoms including fever, headache, myalgia and fatigue that may be accompanied by other symptoms, including cough, abdominal pain, and nausea. Clinical features commonly associated with the disease are leukopenia, thrombocytopenia, and elevated serum hepatic aminotransferases.[5,6] Patients usually develop symptoms 5 to 21 days after receiving a bite from an infected tick[16] and most frequently seek medical attention during the acute phase of illness (< 1 week following onset of symptoms).[4,40,41] Although the case fatality rate in humans is less than 1%,[42] up to 50% of patients may be hospitalized and infections have been associated with severe organ failure and opportunistic infections that appear secondarily.[5,6,42]Risk factors associated with severity of outcomes include patient age, delayed diagnosis and treatment, and underlying medical comorbidities.[42] In particular, patients 60 years of age and older, or reporting an immunosuppressive condition, are at greatest risk of life-threatening complications and death.[42] Clinical infections in general are more common in older people, with the highest rates reported for patients 50 years of age or older; infections also appear to be slightly more common among men than women.[6]

The nonspecific clinical symptoms seen in infected patients can pose difficulties in diagnosing HA. Moreover, the clinical and laboratory manifestations of HA are similar to other tick-borne diseases, preventing differentiation of these diseases on symptomatology alone, although differences do exist in the frequencies

in which patients present with specific symptoms. For instance, patients infected with the closely related pathogen *Ehrlichia chaffeensis,* the causative agent of human monocytic ehrlichiosis (HME), are more likely to report rashes and gastrointestinal illness than patients infected with HA.[16] The hospitalization and case fatality rates for HME also are higher than for HA, as patients with HME are more likely to experience life-threatening complications related to renal failure, respiratory distress, disseminated intravascular coagulopathy, or meningitis/encephalitis.[5,42] Opportunistic infections, such as yeast pneumonitis caused by *Candida* and *Cryptococcus* species, and rhabdomyolysis have been reported more frequently as complications in HA infections.[14,42,43] Currently, however, HME and HA cases occur in fairly distinct geographic regions,[42] such that patients with ehrlichiosis-like symptoms in the Upper Midwest who have not travelled outside of the region would most likely be infected with *A phagocytophilum*. Conversely, the geographic distributions of HA and Lyme disease cases overlap significantly in the USA, such that identifying clinical differences in their presentations may be more useful. In a case-control study involving patients in Wisconsin, patients diagnosed with HA infections were more likely to report fever, chills, and dyspnea than Lyme disease patients, although these symptoms did overlap between the 2 groups of patients. Laboratory findings such as leukopenia, thrombocytopenia, and elevated alanine aminotransferase levels, on the other hand, were significantly more associated with patients infected only with HA.[44] Importantly, the rash of erythema migrans also is highly specific for patients infected with the Lyme disease spirochaete and not HA.[44] These general findings recently were supported in a comparison of Lyme disease and HA patients infected in the northeastern United States.[45]

Because *A phagocytophilum* is transmitted by the same tick species that transmits the agents of Lyme disease, babesiosis, and Powassan encephalitis, patients may be concurrently infected with more than 1 pathogen.[14] In Wisconsin in the 1990s, an active surveillance study detected apparent co-infections in 2 of 62 (3.2%) patients presenting with a summer febrile illness,[4] and a prospective study found 4% of 283 patients suspected of having a tick-borne disease with evidence of co-infection with HA and Lyme disease.[44] Further evidence that patients in the Upper Midwest may be concurrently or sequentially infected with more than 1 pathogen was obtained in a cross-sectional study during the same time period. Twelve of 115 (10.4%) patients that had a laboratory-confirmed diagnosis of acute HA or Lyme disease also had serologic evidence of infection with another pathogen; 3 patients (2.6%) in this study had evidence of triple infections with HA, Lyme disease, and babesiosis.[46] More recently, an HA and Lyme disease co-infection rate of 9% was detected in patients in northwestern Wisconsin where HA is an emerging disease.[37] An increased incidence of human co-infections in endemic areas of the Upper Midwest in recent years has not been investigated, but might be expected with increasing prevalence of infections in reservoir host and tick populations. Because patients co-infected with *A phagocytophilum* and Lyme disease may be less likely to report fever, chills, and fatigue than patients infected with HA alone and may still develop an erythema migrans rash[37,44] testing for both pathogens in patients suspected of a tick-borne illness may be indicated.

HA is treated effectively with doxycycline (Table 2). A 10 to 14 day regimen of 100 mg for adults or 2.2 mg/kg for children 8 years of age or older (weighing < 100 lbs) every 12 hours is recommended.[17,18,47] For children less than 8 years of age, doxycycline is still the recommended treatment, as the risk for dental staining has been shown to be minimal,[47] although treatment may be limited to 4 to 5 days or about 3 days after resolution of fever.[18] Response to therapy is rapid with clinical improvement in 24 to 72 hours. Rifampin has been used successfully when doxycycline cannot be used, such as in pregnant women.[18,47] Doxycycline is the recommended treatment for Lyme disease as well, therefore, a treatment course of 10 to 14 days should be sufficient in patients suspected to be infected with both HA and Lyme disease.[18,47] Because HA infections can rapidly progress to a life-threatening disease, treatment should not be delayed for confirmatory laboratory diagnosis. However, HA does appear to be a self-limiting infection in the majority of human patients. Ramsey et al[48] conducted a case-control study to evaluate the likelihood of persistent or recurrent symptoms in patients diagnosed with HA in Wisconsin in 1996-1998. Patients were more likely to report repeated fevers, chills, sweats, and fatigue 1 year after diagnosis, although there was no evidence that cases experienced more bodily pain or were in poorer physical condition than controls. Of the 85 enrolled cases in the study, one retained an elevated HA serologic titer (1:256) and reported recurrent fatigue, vomiting, and headaches.

Current Diagnostics

Infections with *A phagocytophilum* can be diagnosed using several methods, each of which has advantages and limitations. The most widely available methods in diagnostic laboratories include direct visualization of morulae on stained blood smears, serology, and polymerase chain reaction (PCR) assays (Table 2). The best diagnostic approach will depend on the stage of infection at which the presenting patient is tested and the agents known to occur in the geographic region where the patient may have been exposed. Typically, the first 1 to 2 weeks following onset of symptoms coincides with a high number of circulating infected leukocytes. Therefore, detection by direct visualization of morulae on blood smears will have its highest sensitivity during this phase of infection. However, sensitivity of this method rapidly wanes as the infection progresses and fewer infected neutrophils are present in

peripheral blood. Sensitivity also is optimized by having blood smears reviewed by an experienced technologist and/or pathologist.[47] Despite these limitations, examination of peripheral blood smears may allow for a rapid diagnosis and for the specific differentiation between *A phagocytophilum,* which infects granulocytes, and *Ehrlichia* species, which will be found primarily in monocytes.[16,17]

Serologic tests frequently are used clinical tests, but their accuracy also depends on the timing of sample collection with respect to the course of infection;[17,41] cross-reactivity between closely related organisms may also prevent definitive diagnoses.[47] The ability to detect antibodies during the acute phase of illness, in particular, is low.[49] Seropositivity also may represent asymptomatic or previously resolved infections when IgG class antibodies are detected by laboratory tests and which may persist for over a year in some patients.[49] While IgM-targeted HA antibody tests are available from some reference laboratories, and may prove helpful during acute infection, detection of these antibodies is highly problematic due to false-positive reactions known to occur with this class of antibodies. Because of these difficulties, the current CDC case definitions for confirmed diagnoses of anaplasmosis and ehrlichiosis specify a 4-fold increase in IgG-specific antibody titers in the indirect immunofluorescence assay (IFA) between paired acute and convalescent serum samples collected within 2 to 4 weeks of each other.[11]

PCR-based diagnostic tests for anaplasmosis offer several advantages over traditional diagnostic methods. They have sensitivity and specificity rates that may approach 100%, tend to have a higher degree of sensitivity than serology and blood smear tests in the acute phase of illness when the majority of patients are tested, and they have the potential to detect co-infections simultaneously in multiplexed reactions.[50,51] They also provide confirmatory laboratory evidence of infection.[11] A variety of PCR assays for *A phagocytophilum* have been developed that vary in their performance to accurately detect the implicated pathogen in clinical specimens. Several assays that target different regions in the 16S rRNA gene have been developed, with differing performance characteristics. In particular, an evaluation by Massung and Slater[52] revealed differences in detection limits and specificity for various primer sets, with some primer sets lacking the specificity required for definitive clinical diagnosis. Assays targeting other genes appear to perform as well, or better, with tests targeting amplification of genes with multiple copies (eg, amplification of genes with multiple copies (eg, major surface protein 2 [msp2] and ankyrin-repeat protein [ankA]) and that) and that are, in general, more sensitive than assays targeting the 16S rRNA gene.[37,52,53]

A real-time PCR assay developed by Bell and Patel[51] that targets the *groEL* operon of the heat shock protein, shows promise for molecular diagnosis and differentiation of the closely related pathogens *A phagocytophilum*, *E chaffeenis, E ewingii,* and the newly described *Ehrlichia* species Wisconsin.[15] In fact, the use of this test allowed for the discovery of the *Ehrlichia* species Wisconsin in patients presenting with an *Ehrlichia*-like illness.[15]Further work evaluating the use of this test in detecting *A phagocytophilum* infections in different phases of infection revealed that its greatest sensitivity was in patients reporting illness for 4 or fewer days, although the method was able to detect infections in patients that had been sick for up to 30 days. Moreover, the assay was more sensitive than blood smear analysis.[41] These results were similar to those reported for other PCR assays designed to detect *A phagocytophilum*.[2,16] Thus, despite the many advantages PCR-based methods may offer for diagnosis, their limited abilities to detect pathogens in patient specimens beyond the acute phase of infection persists. Multiple lines of testing still may be required for a definitive diagnosis in some patients, including amplification of pathogen-specific DNA, a 4-fold increase in antibody titer, or visualization of morulae on a blood smear.[41,50]

Future Needs in Diagnostics, Surveillance and Control

As we continue to learn more about the biology of *A phagocytophilum* and the risks it poses to human and animal health in the Upper Midwest, we are faced with the challenges of identifying effective methods of control and prevention. Current public health measures to combat tick-borne diseases in the United States rely primarily on an individual's abilities to minimize exposure to tick vectors. Such personal protection measures include wearing long pants and sleeves when working outdoors or during recreational activities, tucking pants into socks, and applying tick repellents to skin or clothing before entering tick habitats to reduce the risk of tick exposure. Conducting thorough and frequent body checks and promptly removing any attached ticks after spending time outdoors reduces the likelihood of pathogen transmission. It remains unclear if there are specific behavioral risk factors associated with tick-borne diseases in the Upper Midwest. In 1 study, HA cases were more likely to report residence in a rural neighborhood and camping compared to controls;[44] however, other studies reported that individuals who worked outdoors or spent time in the woods were at no greater risk of infection.[3,4] Therefore, it is unknown if education campaigns focused on changing people's behaviors would help reduce the incidence of these diseases.

Developing spatial risk maps to identify environmental factors correlated with ticks or human-tick encounters and to alert health care providers and the public to geographic regions where the risks of acquiring infections are greatest represents an alternate strategy that may improve the public's ability to make decisions regarding the use of protective measures and a provider's ability to correctly diagnose a tick-borne illness.[54,55] It is also possible that such risk maps could be used to cost-effectively target appro-

priate community-wide efforts aimed at the control of ticks or reservoir hosts in the environment.[55] High quality patient or tick infection data are required to develop maps that most accurately predict risk.[56] A major disadvantage of this approach, however, is that underlying models often are based on outdated or single time points of data, thus limiting their utility for predicting current or future risks. The adoption of electronic medical records, electronic case reporting systems, and molecular diagnostic assays that allow for the distinction between active and resolved infections, in addition to the availability of high quality environmental data, technological advances for processing data, and the public's widespread access to smartphones and the Internet may remove this disadvantage and allow for the distribution of relatively current information about the risks of pathogen exposure in a particular region. Delivery of such "real-time" alerts may have the greatest impact on an individual's decision to try to avoid ticks, and future efforts should explore the integration of surveillance systems with publicly available media (eg, websites and phone applications) to accomplish this.

A better understanding of the circulating variants of *A phagocytophilum* in the Upper Midwest, their enzootic cycles, and their abilities to infect humans and domestic animals is another priority. Such information would strengthen models that predict areas of greatest infection risk, and may help explain the distribution of human cases. For instance, Massung et al[26] demonstrated that varying prevalence of the AP-ha and nonhuman infecting *A phagocytophilum* variants in mice, chipmunks, and ticks corresponded to differences in rates of human infections in Connecticut and Rhode Island. Moreover, having the ability to differentiate distinct *A phagocytophilum* variants may allow for development of better diagnostic tests, or possibly vaccines, in the future.[57,58]

CONCLUSION

Tick-borne diseases represent some of the most important emerging infectious diseases affecting human and domestic animal populations in the temperate regions of the world. In the Upper Midwest, infections with *A phagocytophilum* particularly appear to be on the rise, though less is known about this pathogen in this region than in other parts of its range in the United States. To mitigate the effects of infections on human health, continued health care provider awareness, development and adoption of reliable diagnostic technologies, and efforts to better understand the ecology of *A phagocytophilum* in human-modified environments in the region are needed. In particular, diagnostic methods that reliably differentiate *A phagocytophilum* from closely related *Ehrlichia* spp infections should be adopted by clinical laboratories and health care providers to improve rates of definitive diagnoses and accurate case reporting and surveillance. In addition, identifying the principal reservoir hosts for the strains of the pathogen that are most likely to infect humans in the Upper Midwest would provide knowledge useful for predicting and, perhaps, controlling, transmission to humans.

Acknowledgement: The authors thank Dr Brad Sullivan for providing comments on the manuscript.

Funding/Support: Financial support was provided by the Marshfield Clinic Research Foundation (Sp code: FRI10110).

Financial Disclosures: None declared.

Planners/Reviewers: The planners and reviewers for this journal CME activity have no relevant financial relationships to disclose.

REFERENCES

1. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol.*1994;32(3):589-595.

2. Dumler JS, Bakken JS. Human granulocytic ehrlichiosis in Wisconsin and Minnesota: a frequent infection with the potential for persistence. *J Infect Dis.* 1996;173(4):1027-1030.

3. Bakken JS, Goellner P, Van Etten M, et al. Seroprevalence of human granulocytic ehrlichioisis among permanent residents of northwestern Wisconsin. *Clin Infect Dis.* 1998;27(6):1491-1496.

4. Belongia EA, Reed KD, Mitchell PD, et al. Tickborne infections as a cause of nonspecific febrile illness in Wisconsin. *Clin Infect Dis.* 2001;32(10):1434-1439.

5. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS. Clinical and laboratory characteristics of human granulocytic ehrlichioisis. *JAMA.* 1996;275(3):199-205.

6. Belongia EA, Gale CM, Reed KD, et al. Population-based incidence of human granulocytic ehrlichiosis in northwestern Wisconsin, 1997-1999. *J Infect Dis.* 2001;184(11):1470-1474.

7. Zhang S, Hai R, Li W, et al. Seroprevalence of human granulocytotropic anaplasmosis in central and southeastern China. *Am J Trop Med Hyg.* 2009;81(2):293-295.

8. Woldehiwet Z. The natural history of *Anaplasma phagocytophilum. Vet Parasitol.* 2010;167(2-4):108-122.

9. Centers for Disease Control and Prevention. Summary of Notifiable Diseases--United States, 2011. *MMWR Morb Mortal Wkly Rep.* 2013;60(53):1-117.

10. Dumler JS, Barbet AF, Bekker CP, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia,* descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila. Int J Syst Evol Microbiol.* 2001;51(Pt 6):2145-2165.

11. Natonal Notifiable Diseases Surveillance System. Ehrlichiosis and Anaplasmosis, 2008 Case Definition. http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=667&DatePub=1/1/2008. Accessed April 28, 2014.

12. Lee X, Hardy K, Johnson DH, Paskewitz SM. Hunter-killed deer surveillance to assess changes in the prevalence and distribution of *Ixodes scapularis* (Acari: Ixodidae) in Wisconsin. *J Med Entomol.* 2013;50(3):632-639.

13. Neitzel D, Kemperman MM. Tick-borne diseases in Minnesota: an update. *Minn Med.* 2012; 95(8):41-44.

14. Swanson SJ, Neitzel D, Reed KD, Belongia EA. Coinfections acquired from *Ixodes* ticks. *Clin Microbiol Rev.* 2006;19(4):708-727.

15. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *N Engl J Med.* 2011;365(5):422-429.

16. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis.* 2007;45(Suppl 1):S45-51.

17. Thomas RJ, Dumler JS, Carlyon JA. Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis. *Expert Rev Anti Infect Ther.* 2009;7(6):709-722.

18. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2006;43(9):1089-1134.

19. Telford SR 3rd, Dawson JE, Katavolos P, Warner CK, Kolbert CP, Persing DH. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proc Natl Acad Sci USA.* 1996;93(12):6209-6214.

20. Keesing F, Hersh MH, Tibbetts M, et al. Reservoir competence of vertebrate hosts for *Anaplasma phagocytophilum. Emerg Infect Dis.* 2012;18(12):2013-2016.

21. Levin ML, Nicholson WL, Massung RF, Sumner JW, Fish D. Comparison of the reservoir competence of medium-sized mammals and *Peromyscus leucopus* for *Anaplasma phagocytophilum* in Connecticut. *Vector Borne Zoonotic Dis.* 2002;2(3):125-136.

22. Dugan VG, Yabsley MJ, Tate CM, et al. Evaluation of white-tailed deer *(Odocoileus virginianus)* as natural sentinels for *Anaplasma phagocytophilum*. *Vector Borne Zoonotic Dis.* 2006;6(2):192-207.

23. Foley JE, Nieto NC, Massung R, Barbet A, Madigan J, Brown RN. Distinct ecologically relevant strains of *Anaplasma phagocytophilum. Emerg Infect Dis.* 2009;15(5):842-843.

24. Scharf W, Schauer S, Freyburger F, et al. Distinct host species correlate with *Anaplasma phagocytophilum* ankA gene clusters. *J Clin Microbiol.* 2011;49(3):790-796.

25. Massung RF, Mauel MJ, Owens JH, et al. Genetic variants of *Ehrlichia phagocytophila,* Rhode Island and Connecticut. *Emerg Infect Dis.* 2002;8(5):467-472.

26. Massung RF, Priestley RA, Miller NJ, Mather TN, Levin ML: Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. *J Infect Dis.* 2003;188(11):1757-1763.

27. Morissette E, Massung RF, Foley JE, Alleman AR, Foley P, Barbet AF. Diversity of *Anaplasma phagocytophilum* strains, USA. *Emerg Infect Dis.* 2009;15(6):928-931.

28. Belongia EA, Reed KD, Mitchell PD, et al. Prevalence of granulocytic *Ehrlichia* infection among white-tailed deer in Wisconsin. *J Clin Microbiol.* 1997;35(6):1465-1468.

29. Michalski M, Rosenfield C, Erickson M, et al. *Anaplasma phagocytophilum* in central and western Wisconsin: a molecular survey. *Parasitol Res.* 2006;99(6):694-699.

30. Baldridge GD, Scoles GA, Burkhardt NY, Schloeder B, Kurtti TJ, Munderloh UG. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). *J Med Entomol.* 2009;46(3):625-632.

31. Johnson RC, Kodner C, Jarnefeld J, Eck DK, Xu Y. Agents of Human Anaplasmosis and Lyme Disease at Camp Ripley, Minnesota. *Vector Borne Zoonotic Dis.* 2011;11(12): 1529-1534.

32. Bullock PM, Ames TR, Robinson RA, Greig B, Mellencamp MA, Dumler JS. *Ehrlichia equi* infection of horses from Minnesota and Wisconsin: detection of seroconversion and acute disease investigation. *J Vet Intern Med.* 2000;14(3):252-257.

33. Nicholson WL, Allen KE, McQuiston JH, Breitschwerdt EB, Little SE. The increasing recognition of rickettsial pathogens in dogs and people. *Trends Parasitol.* 2010;26(4):205-212.

34. Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Vet Parasitol.* 2009;160(1-2):138-148.

35. Pancholi P, Kolbert CP, Mitchell PD, et al. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. *J Infect Dis.* 1995;172(4):1007-1012.

36. Steiner FE, Pinger RR, Vann CN, et al. Infection and co-infection rates of *Anaplasma phagocytophilum* variants, *Babesia* spp., *Borrelia burgdorferi,* and the rickettsial endosymbiont in *Ixodes scapularis* (Acari: Ixodidae) from sites in Indiana, Maine, Pennsylvania, and Wisconsin. *J Med Entomol.* 2008;45(2):289-297.

37. Lovrich SD, Jobe DA, Kowalski TJ, Policepatil SM, Callister SM. Expansion of the Midwestern focus for Human granulocytic anaplasmosis into the region surrounding La Crosse, Wisconsin. *J Clin Microbiol.* 2011;49(11): 3855-3859.

38. Katavolos P, Armstrong PM, Dawson JE, Telford SR 3rd. Duration of tick attachment required for transmission of granulocytic ehrlichiosis. *J Infect Dis.* 1998;177(5):1422-1425.

39. des Vignes F, Piesman J, Heffernan R, Schulze TL, Stafford KC 3rd, Fish D. Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs. *J Infect Dis.* 2001;183(5):773-778.

40. Bakken JS, Dumler JS. Human granulocytic ehrlichiosis. *Clin Infect Dis.* 2000;31(2):554-560.

41. Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a real-time PCR method with serology and blood smear analysis for diagnosis of human anaplasmosis: importance of infection time course for optimal test utilization. *J Clin Microbiol.* 2013;51(7):2147-2153.

42. Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuiston JH. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000-2007. *Am J Trop Med Hyg.* 2011;85(1):124-131.

43. Talsness SR, Shukla SK, Mazza JJ, Yale SH. Rhabdomyolysis-induced acute kidney injury secondary to *Anaplasma phagocytophilum* and concomitant statin use. *WMJ.* 2011;110(2):82-84.

44. Belongia EA, Reed KD, Mitchell PD, et al. Clinical and epidemiological features of early Lyme disease and human granulocytic ehrlichiosis in Wisconsin. *Clin Infect Dis.* 1999;29(6):1472-1477.

45. Wormser GP, Aguero-Rosenfeld ME, Cox ME, et al. Differences and similarities between culture-confirmed human granulocytic anaplasmosis and early lyme disease. *J Clin Microbiol.* 2013;51(3):954-958.

46. Mitchell PD, Reed KD, Hofkes JM. Immunoserologic evidence of coinfection with *Borrelia burgdorferi, Babesia microti,* and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *J Clin Microbiol.* 1996;34(3):724-727.

47. Chapman AS, Bakken JS, Folk SM, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: a practical guide for physicians and other health-care and public health professionals. *MMWR Recomm Rep.* 2006;55(RR-4):1-27.

48. Ramsey AH, Belongia EA, Gale CM, Davis JP. Outcomes of treated human granulocytic ehrlichiosis cases. *Emerg Infect Dis.* 2002;8(4):398-401.

49. Bakken JS, Haller I, Riddell D, Walls JJ, Dumler JS. The serological response of patients infected with the agent of human granulocytic ehrlichiosis. *Clin Infect Dis.* 2002;34(1):22-27.

50. Dumler JS, Brouqui P. Molecular diagnosis of human granulocytic anaplasmosis. *Expert Rev Mol Diagn*. 2004;4(4):559-569.

51. Bell CA, Patel R. A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum, Ehrlichia chaffeensis*, and *Ehrlichia ewingii. Diagn Microbiol Infect Dis.* 2005;53(4):301-306.

52. Massung RF, Slater KG. Comparison of PCR assays for detection of the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum. J Clin Microbiol.* 2003;41(2):717-722.

53. Walls JJ, Caturegli P, Bakken JS, Asanovich KM, Dumler JS. Improved sensitivity of PCR for diagnosis of human granulocytic ehrlichiosis using *epank1* genes of *Ehrlichia phagocytophila*-group ehrlichiae. *J Clin Microbiol.* 2000;38(1):354-356.

54. Piesman J, Eisen L. Prevention of tick-borne diseases. *Annu Rev Entomol.* 2008;53:323-343.

55. Eisen L, Eisen RJ. Using Geographic Information Systems and Decision Support Systems for the prediction, prevention, and control of vector-borne diseases. *Annu Rev Entomol.* 2001;56:41-61.

56. Eisen L, Eisen RJ. Need for improved methods to collect and present spatial epidemiologic data for vectorborne diseases. *Emerg Infect Dis.* 2007;13(12):1816-1820.

57. Dunning Hotopp JC, Lin M, Madupu R, et al. Comparative genomics of emerging human ehrlichiosis agents. *PLoS Genet.* 2006;2:e21.

58. de la Fuente J, Kocan KM, Blouin EF, et al. Functional genomics and evolution of tick-*Anaplasma* interactions and vaccine development. *Vet Parasitol.* 2010;167(2-4):175-186.

CME

To receive CME credit, complete this quiz and return it to the address listed below. See CME-designated article on pages 107-114.

Quiz: A Clinical, Diagnostic, and Ecologic Perspective on Human Anaplasmosis in the Upper Midwest

EDUCATIONAL OBJECTIVES

Upon completion of this activity, participants will be able to:

1. Understand the epidemiology of human anaplasmosis and other diseases in the Upper Midwest transmitted by the bite of *Ixodes scapularis*.
2. Describe the clinical characteristics of infections with *Anaplasma phagocytophilum.*
3. Describe the evaluation and treatment of patients with human anaplasmosis.

PUBLICATION DATE: June 16, 2014

EXPIRATION DATE: June 16, 2015

QUESTIONS

1. Human anaplasmosis (HA) is a zoonotic, tickborne disease caused by the intracellular bacterium *Anaplasma phagocytophilum* and is also known as human granulocytic anaplasmosis (HGA) and human granulocytic ehrlichiosis (HGE).
   ❑ True ❑ False
2. *A phagocytophilum* was first described in association with a febrile illness in patients from Sweden in the early 1900s.
   ❑ True ❑ False
3. About 10% to 15% of patients reporting with a nonspecific febrile illness during the summer months to clinics in Wisconsin and Minnesota had evidence of infection with *A phagocytophilum*.
   ❑ True ❑ False
4. *Ixodes scapularis* (the black-legged, deer, or bear tick) is the primary vector not only for HA, but also transmits other important diseases endemic to the region, including Lyme disease, babesiosis, and the Powassan encephalitis virus.
   ❑ True ❑ False
5. The range of *I scapularis* in Wisconsin and Minnesota has decreased significantly over the past decade.
   ❑ True ❑ False

• • •

You may earn CME credit by reading the designated article in this issue and successfully completing the quiz (75% correct). Return completed quiz to WMJ CME, 330 E. Lakeside St, Madison, WI 53715 or fax to 608.442.3802. You must include your name, address, telephone number and e-mail address. You will receive an e-mail from wmj@wismed.org with instructions to complete an online evaluation. Your certificate will be delivered electronically.

The Wisconsin Medical Society (Society) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

The Wisconsin Medical Society designates this journal-based CME activity for a maximum of 1.0 *AMA PRA Category 1 Credit*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

6. Analyses based on molecular data supported the reclassification of *Ehrlichia phagocytophila, Ehrlichia equi*, and the human granulocytic ehrlichiosis (HGE) agent into the single species, *A phagocytophilum*.
   ❑ True ❑ False
7. In human hosts, *A phagocytophilum* infects monocytes and replicates within intracytoplasmic vacuoles to form inclusions known as morula.
   ❑ True ❑ False
8. Wild reservoirs for infections with *A phagocytophilum* include the white-footed mouse, short-tailed shrew, eastern chipmunk, and white-tailed deer.
   ❑ True ❑ False
9. Surveys conducted in Wisconsin and Minnesota have reported a prevalence of *A phagocytophilum* in 9% to 12% of adult *I scapularis*.
   ❑ True ❑ False
10. The transmission of *A phagocytophilum* may occur within 24 hours of tick attachment; this is distinctly different from *Borrelia burgdorferi*, the pathogen in Lyme disease, which may require 36 to 48 hours or more of tick attachment for transmission to occur.
    ❑ True ❑ False
11. HA is characterized by nonspecific symptoms including fever, headache, myalgia, and fatigue; clinical features that are common also include leukopenia, thrombocytopenia, and elevated serum hepatic aminotransferases.
    ❑ True ❑ False
12. Symptoms of HA usually develop within 2-3 days after receiving a bite from an infected tick.
    ❑ True ❑ False
13. Since *A phagocytophilum* is transmitted by the same tick species that transmits the agents of Lyme disease, babesiosis, and Powassan encephalitis, patients may be concurrently infected with more than a single pathogen.
    ❑ True ❑ False
14. The rash of erythema migrans is seen often in patients infected with the Lyme disease spirochete, but a similar rash also is seen frequently in patients with HA.
    ❑ True ❑ False
15. The recommended first-line treatment for HA is amoxicillin.
    ❑ True ❑ False
16. Current diagnostic tests for infections with *A phagocytophilum* include direct visualization of morulae on stained blood smears, serology, and PCR assays.
    ❑ True ❑ False

advancing the art & science of medicine in the midwest

WMJ

WMJ (ISSN 1098-1861) is published through a collaboration between The Medical College of Wisconsin and The University of Wisconsin School of Medicine and Public Health. The mission of *WMJ* is to provide an opportunity to publish original research, case reports, review articles, and essays about current medical and public health issues.

© 2014 Board of Regents of the University of Wisconsin System and The Medical College of Wisconsin, Inc.

Visit www.wmjonline.org to learn more.