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# Update on Human Granulocytic Anaplasmosis

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**ABSTRACT:** Granulocytic anaplasmosis (GA) is a disease of humans, domestic animals, and wildlife caused by *Anaplasma phagocytophilum*, formerly *Ehrlichia phagocytophila*, *E. equi*, and the unnamed agents of “human granulocytic ehrlichiosis” (HGE). This pathogen is inoculated into host skin by the bite of *Ixodes* spp. ticks, including *I. pacificus* in California and *I. scapularis* in the eastern U.S. After inoculation, *A. phagocytophilum* disseminates to the blood and is phagocytosed into host neutrophils. The clinical characteristics of GA in people vary from no symptoms to fever, headache, neurological symptoms, and occasionally death. Horses with GA may experience high fever, depression, reduced ability to eat, limb edema, jaundice, and ataxia. GA is an emerging disease in the eastern U.S. but only a handful of human cases have been reported in the western U.S., despite relatively common reports of disease in horses and dogs. Wildlife and dog-sentinel studies have clarified that infection is common in the coast range mountains and Sierra Nevada foothills, with ongoing research focusing on ecological determinants that can modify the prevalence of infection in any particular area. One known reservoir is the dusky-footed woodrat. However, important, poorly understood ecological determinants serve to modify the probability of rodent as well as human infections, including climate, vegetation, and possibly the presence of other reservoir-competent rodents and nidicolous woodrat-specialist ticks such as *I. spinipalpis*.

**KEY WORDS:** *Anaplasma phagocytophilum*, disease, dusky-footed woodrat, *Ixodes pacificus*, *Ixodes spinipalpis*, *Neotoma fuscipes*, tick-borne disease

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## THE ORGANISM AND DISEASE

### Phylogeny and Biological Characteristics

Granulocytic anaplasmosis (GA) is a disease of humans, domestic animals, and wildlife caused by the newly renamed pathogen, *Anaplasma phagocytophilum* (Dumler *et al.* 2001). This new species combines *Ehrlichia phagocytophila*, *E. equi*, and the unnamed agents of “human granulocytic ehrlichiosis” (HGE), which cross-react serologically (Walker and Dumler 1995) and are indistinguishable morphologically (Popov *et al.* 1998). There is microvariability in the 16S rRNA gene of strains from the upper midwestern, eastern, and western U.S. among different host species (Chen *et al.* 1994, Reubel *et al.* 1998), but analysis of the 16S, 444 bp-Ank, and groESL heat shock protein genetic regions from equine and human-origin isolates from northern California suggested that the HGE agent and *E. equi* in the U.S. are synonymous, with multiple strains that may vary geographically but do not appear to be host-differentiated (Chae *et al.* 2000).

*A. phagocytophilum* shares common biological characteristics and immunopathological mechanisms in hosts with other ehrlichial and rickettsial bacteria. This organism is inoculated into host skin by tick bite, in context with tick saliva, which contains an array of anti-inflammatory and immunomodulatory chemicals during feeding to prevent host blood clotting, platelet aggregation, NK cell activity, and T<sub>H</sub>1 cytokine responses (Wikel 1999). After tick inoculation and local host immune responses, *A. phagocytophilum* disseminates to the blood, where this obligate intra-cellular pathogen must persist within host neutrophils. The bacterium enters the neutrophil after attaching via an outer surface protein to a host cell receptor and being phagocytosed into a small host membrane-bound site called a

phagosome. *A. phagocytophilum*-infected neutrophils secrete chemokines that recruit macrophages, which then secrete IFN- $\gamma$  and IL-10 and amplify local and systemic inflammation (Dumler *et al.* 2000, Klein *et al.* 2000). Within the phagosome, *A. phagocytophilum* can inhibit fusion of this protected environment with the lysosome, overcoming initial host immune attacks against the bacteria. Ultimately, however, when host cells are primed with IFN- $\gamma$ , they are able to kill the intracellular *A. phagocytophilum* (Webster *et al.* 1998, Mott *et al.* 1999).

### Clinical Outcomes of Infection

The clinical characteristics of GA in people are variable including pyrexia, headache, myalgia, nausea, and ataxia (Goodman *et al.* 1996). More severe cases may result in organ failure, susceptibility to opportunistic infections, neuritis, or respiratory complications, with a case fatality rate up to 5% (Walker and Dumler 1995, Foley 2000). GA in horses is characterized by high fever, depression, reduced ability to eat, limb edema, petechiation, icterus, and ataxia (Madigan 1993). Counts of blood cells may document anemia, low platelet counts, and low white blood cell counts. Because much of the pathogenesis of GA is immune-mediated, there may be downstream immune-mediated sequelae, including possible polyarthritis and vasculitis.

### Laboratory Findings in GA and Diagnosis

Although most cases of GA occur in areas where tick biting is common, not all human patients report a tick bite and not all dog or horse owners recognize that their animal has been infected. The symptoms and laboratory diagnostic results of GA are non-specific and may include elevated liver enzymes, thrombocytopenia, and neutro-

penia. In some cases, the symptoms (e.g., headache and fever) are severe, and yet there are only mild or no laboratory abnormalities.

Probably the most rapid method for diagnosis of GA is to find the characteristic membrane-bound inclusions (morulae) in neutrophils on blood smears, but the proportion of neutrophils with visible morulae may be as low as 1-2%. The Centers for Disease Control (CDC) criteria for a confirmed diagnosis require that visualization of morulae be accompanied by positive titers ( $\geq 64$ ), probably because some cellular inclusions may appear similar to morulae. Although a single titer  $\geq 64$  may be part of a presumptive diagnosis, this does not document active infection. However, a 4-fold rise in specific titer over 4 weeks is considered confirmatory for GA. Titers become positive approximately 2 weeks after onset of clinical signs and remain elevated after exposure for months to years, so there is a brief window early in the infection when the patient may be ill but seronegative.

Culture of *A. phagocytophilum* is time-consuming, specialized, and somewhat expensive. Polymerase chain reaction (PCR) for specific DNA is extremely valuable for confirming a suspected diagnosis but generally not available rapidly enough in practice for use in primary diagnosis and to guide treatment. There are multiple published protocols for PCR of *A. phagocytophilum*. PCR may be most useful early in acute infection before the patient seroconverts, late in infection to rule-out chronic infection, or when serological titers to several agents are similar.

## ECOLOGY

### Emergence

Human granulocytic anaplasmosis (HGA) has emerged dramatically in the eastern and midwestern U.S. over the past 25 years. Since first reported in Wisconsin in 1994, over 400 cases of HGA have been diagnosed in the upper midwestern and northeastern United States (CDC 2000). In contrast, only 8 human cases have been documented in California as of April 2002, despite being reportable to the California Department of Health Services (CDHS), availability of diagnostic laboratory support, and dissemination of information to physicians by CDHS personnel and others. Yet, GA is common in dogs and horses (Madigan and Gribble 1987, Foley *et al.* 2001). However, following targeting of the north coast of California by state and local health officials, 4 cases of HGE were documented in one county within 12 months where no case had previously been confirmed (Foley *et al.* 1999a). Thus, cases within the state are no doubt somewhat under-reported.

### Tick Vectors

Worldwide, *Anaplasma phagocytophilum* is vectored by at least 4 tick species and hosted by dozens or hundreds of mammals, reptiles, and birds. *Ixodes scapularis* is the vector in the eastern U.S., *I. pacificus* in California, *I. ricinus* in Europe, and *I. persulcatus* in Asia (MacLeod and Gordon 1933, Pancholi *et al.* 1995, Richter *et al.* 1996, Telford *et al.* 1996, Des Vignes and Fish 1997, Petrovec *et al.* 1997). Locally important or enzootic vectors may include *I. hexagonus* in England

and *I. spinipalpis* in the western U.S.

The spatial distribution of human and equine cases of GA in the western U.S. corresponds with the range of *Ixodes pacificus*, the western black-legged tick, a common tick throughout much of low mountain California (Furman and Loomis 1984, Madigan and Gribble 1987, Richter *et al.* 1996). *I. pacificus* has been recorded from 55 of 58 counties in California, and adult *I. pacificus* containing *A. phagocytophilum* DNA have been detected in Alameda, El Dorado, Humboldt, Napa, Orange, Sacramento, Santa Cruz, Sonoma, and Yolo Counties (Barlough *et al.* 1997, Kramer *et al.* 1999; Nicholson *et al.* 1999; Lane *et al.* 2001), although tick surveillance has been limited. The vector competence of *I. pacificus* for *A. phagocytophilum* has been evaluated. Infected *I. pacificus* individuals can be found in nature (Richter *et al.* 1996; Barlough *et al.* 1997, Kramer *et al.* 1999, Lane *et al.* 2001); horses can be experimentally infected using field-caught, naturally-infected ticks (Reubel *et al.* 1998); and the agent can be transmitted from experimentally-infected horses to naïve horses by feeding of *I. pacificus* (Richter *et al.* 1996).

The prevalence of *A. phagocytophilum* in ticks ranges from 0-51% in the eastern U.S. (Magnarelli *et al.* 1995, Pancholi *et al.* 1995, Daniels *et al.* 1997, Varde *et al.* 1998), but typically is much lower in the western U.S., from 0.8-11% (Barlough *et al.* 1997, Kramer *et al.* 1999, Nicholson *et al.* 1999, Lane *et al.* 2001). The prevalence in *I. pacificus* nymphs is also low, with 0/465 nymphs positive even where the adult prevalence was 11% (Lane *et al.* 2001) and only 1/47 pools positive from Sonoma County (Barlough *et al.* 1997). Yet, nymphs may cause most human, deer, and mountain lion cases, because these peak typically in June and July, corresponding to nymphal tick abundance (Lane 1990; Foley *et al.* 1998, 1999b, 2001; CDC 2000). In contrast, cases in horses and dogs seem to peak in the winter, corresponding to peak activity of *I. pacificus* adults (Madigan and Gribble 1987, Vredevoe *et al.* 1999, Foley *et al.* 2001), although dog cases also occur in late spring, at the time of the emergence of nymphs (Foley *et al.* 2001). *A. phagocytophilum* is not transovarially transmitted among ticks (Lewis 1979, Munderloh and Kurtti 1995).

### Mammalian Hosts and Reservoirs

Knowledge of natural reservoirs of *A. phagocytophilum* in California is limited. Immature *I. pacificus* commonly feed on lizards, birds, and small mammals, and occasionally on deer and carnivores (Furman and Loomis 1984). Adult *I. pacificus* occur frequently on dogs, coyotes, bears, bobcats, horses, deer, cattle, humans, and other large animals (Furman and Loomis 1984, Westrom *et al.* 1985, Lane 1994). The PCR-prevalence of *A. phagocytophilum* in cervids ranges from 9-64% across Connecticut, Wisconsin, and Maryland (Bakken *et al.* 1996, Belongia *et al.* 1997, Massung *et al.* 1998, Magnarelli *et al.* 1999a). In California, the *A. phagocytophilum* PCR-prevalence was 5% in Columbia black-tailed deer (*Odocoileus hemionus columbianus*) and 31% in tule elk (*Cervus elaphus nannodes*) (Foley *et al.* 1998). DNA sequencing from the blood of deer and elk confirmed that California cervid isolates were

homologous with horse isolates from northern California (Foley *et al.* 1998). Infected cervids have been observed from Sonoma, Marin, and Mendocino Counties. Because experimental studies have not been performed in deer, it is not known how they contribute to the maintenance of infection in nature, although their role as reservoirs would be limited to the number of nymphs they support.

Infections with *A. phagocytophilum* are common in several rodent species. Only a few incidents of infection with *A. phagocytophilum* have been reported in house mice (*Mus musculus*), chipmunks (*Tamias* spp.), and voles (*Microtus pennsylvanicus*), all in the eastern U.S. (Tyzzer 1938, Telford *et al.* 1996, Walls *et al.* 1997). The primary reservoir for *A. phagocytophilum* in the eastern U.S. is the white-footed mouse (*Peromyscus leucopus*), with a seroprevalence from 1-50% (Telford *et al.* 1996, Walls *et al.* 1997, Nicholson *et al.* 1998, Stafford *et al.* 1999). However, even though the PCR-prevalence ranged from 10.5-36%, infections in these rodents were transient (Walls *et al.* 1997, Nicholson *et al.* 1998). Reservoir competence was documented by experimental transmission of *A. phagocytophilum* by feeding larval *I. scapularis* on infected *P. leucopus*, allowing these larvae to molt, and transmitting the infection to naïve *P. leucopus* individuals via nymphs (Telford *et al.* 1996). There is not much evidence of anaplasmosis in other *Peromyscus* spp., with 11% seropositive in *P. boyleyi*, 6% in *P. maniculatus*, and 8% in *P. gossypinus* (Nicholson *et al.* 1998, 1999). PCR-positivity in *P. maniculatus* ranged from 0-21% (Nicholson *et al.* 1998, 1999; Zeidner *et al.* 2000).

Woodrats are likely reservoirs for *A. phagocytophilum* in California. Serologic evidence of exposure was found in *Neotoma fuscipes* (17.4-26.0%), *N. lepida* (25%), *N. albigula* (1%), and *N. mexicana* (4.7%) (Nicholson *et al.* 1998, 1999), and *A. phagocytophilum* DNA was recovered from seropositive woodrats (Nicholson *et al.* 1999). Titers in woodrats averaged 194 (geometric mean titer) compared with 410 in all seropositive *Peromyscus* (Nicholson 1998). Persistent infection (1-8 months) with *A. phagocytophilum* was documented in wild-caught *N. fuscipes* with no clinical signs (Nicholson *et al.* 1999, Foley *et al.* 2002). The agent in woodrat blood was detectable by PCR and was transmissible via needle inoculation to susceptible woodrats. *Neotoma fuscipes* is particularly common in oak woodlands, which is also optimal habitat for *I. pacificus*.

Infection with *A. phagocytophilum* has been described also in carnivores, including coyotes (Pusterla *et al.* 2000), mountain lions (Foley *et al.* 1999b), black bears (Walls *et al.*, unpubl. data), dogs (Madewell and Gribble 1982, Greig *et al.* 1996, Foley *et al.* 2001), and raccoons, cats, skunks, and bobcats (J. Foley, unpubl. data). The seroprevalence in coyotes, 46%, is surprisingly high, and a PCR-positive coyote has been described (Pusterla *et al.* 2000). The titers in coyotes were relatively high as well, with two coyotes having titers  $\geq 1,280$ . Seropositive coyotes were reported from Los Angeles, Santa Clara, and Tehama Counties, and a PCR-positive coyote was detected in Santa Clara County (Pusterla *et al.* 2000). Mountain lions had relatively high serologic (17%) and PCR-prevalence (16%) of *A. phagocytophilum*, although

with low titers (Foley *et al.* 1999b). If infections in mountain lions are clinically similar to infections in cats, the disease may be mild (Lewis *et al.* 1975). The prevalence of *A. phagocytophilum* in mountain lions varied from 19% in north coastal California to 25% in the Sierra Nevada and 0% from Monterey. Both coyotes and mountain lions have extensive home ranges or migration patterns and are likely to encounter hundreds of individual ticks from several independent foci, from which they might acquire *A. phagocytophilum*.

Dogs in California develop relatively mild clinical signs of anaplasmosis and have been used as sentinels of human risk, because of the frequency with which they are bitten by *I. pacificus* and their close association with people (Foley *et al.* 2001). The California statewide seroprevalence of *A. phagocytophilum* in dogs was 8.7% (Foley *et al.* 2001). Dogs in the eastern U.S. also have been reported to experience infection with *A. phagocytophilum*. Clinical, serological, and molecular descriptions were provided for 17 dogs with anaplasmosis from Minnesota and Wisconsin (Greig *et al.* 1996) and 3 dogs from North Carolina and Virginia (Goldman *et al.* 1998). A serosurvey of tick-borne disease in Oklahoma documented 33% prevalence of antibodies to *A. phagocytophilum* in dogs, but 0/100 in horses (Rodgers *et al.* 1989). In Connecticut and New York, 9.4% of ill dogs had antibodies to *A. phagocytophilum* (Magnarelli *et al.* 1997). In California, canine GA has been reported from Marin County (northern coast range) and the Sierra Nevada foothills (Madewell and Gribble 1982). A large serosurveillance study of 1,082 healthy dogs across California using Geographic Information Systems technology indicated a Bayes' adjusted prevalence (BAP) 0.01 to 0.47 (Humboldt County) (Foley *et al.* 2001). Other north coast counties with moderately high seroprevalence were Mendocino, Sonoma, Lake, and Marin (from 0.15-0.20). Counties in the Sierra Nevada foothills had lower seroprevalence, ranging from 0.09-0.13. Santa Cruz County had a BAP of 0.10.

In contrast to dogs, horses (particularly older animals) experience relatively severe clinical disease (Madigan and Gribble 1987). Equine granulocytic anaplasmosis is common in California, with early reports from horses in northern coastal ranges (Sonoma and Mendocino Counties) and the foothills of the Sierra Nevada (Gribble 1969, Madigan and Gribble 1987, Madigan *et al.* 1990). Case reports were described for 5 clinically affected horses from the northeastern U.S. (Madigan *et al.* 1996). In horses from Connecticut and New York with clinical signs of anaplasmosis, the seroprevalence was 29% (Magnarelli *et al.* 1999b), and in well horses from Minnesota and Wisconsin, the seroprevalence was 17.6% from tick-endemic vs. 3.8% from non tick-endemic areas (Bullock *et al.* 2000). Infection with human origin *A. phagocytophilum* protected horses from challenge with *E. equi* (Barlough *et al.* 1995). Equine cases in California are reported regularly from counties in the northern coast ranges and Sierra Nevada foothills, with seroprevalence in horses from coast range and Sierra foothills of 10% (Madigan *et al.* 1990). A questionnaire-based study evaluated the spatial distribution of equine anaplasmosis (Vredevoe *et al.* 1999). Of 62 practices in northern

California, cases were diagnosed in the foothills of the Sierra Nevada and coast range mountains with spatial clustering based on nearest neighbor statistical analysis.

In summary, GA is a threat to humans, domestic animals, and wildlife in a range of habitats typical of *I. pacificus* ticks, although the spatial ecology is characterized by distinct patchiness. There appears to be a low disease incidence in humans but not other animals, suggesting key features of the ecology remain unidentified.

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