

Clinical, epidemiologic, and environmental surveillance for ehrlichiosis and anaplasmosis in an endemic area of northern California

Curtis L. Fritz, Lawrence R. Bronson, Charles R. Smith, Leta Crawford-Miksza, Elaine Yeh, and David Schnurr

Division of Communicable Disease Control, California Department of Health Services, Sacramento, CA 95899-7413, U.S.A.

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ABSTRACT: Two forms of tick-borne leukocytotropic rickettsioses have been recognized in California since the mid-1990s: human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis* and human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum*. Between 1997 and 1999, two cases of HME and four cases of HGA were diagnosed in residents of southern Humboldt County, California. Environmental followup at case-patients' residences revealed dense populations of *Ixodes pacificus* ticks, particularly in grassy roadside areas. PCR evidence of *A. phagocytophilum* was detected in approximately 2.0% of *I. pacificus*; *E. chaffeensis* was not detected in any of 625 ticks tested. Serologic antibody to *A. phagocytophilum* was detected in two of 54 participants in a community epidemiologic study; one of these also had antibody to *E. chaffeensis*. Over 85% of study participants reported finding a tick on themselves in the preceding 12 mo. Residents of southern Humboldt County are at significant risk of tick bites and should take appropriate prevention measures to avoid infection with rickettsia and other tick-transmitted pathogens. *Journal of Vector Ecology* 30 (1): 4-10. 2005.

Keyword Index: *Anaplasma phagocytophilum*, anaplasmosis, *Ehrlichia chaffeensis*, *Ehrlichia*, *Ixodes pacificus*, ehrlichiosis, tick-borne diseases.

INTRODUCTION

In the past two decades, several tick-borne, leukocytotropic rickettsia have been identified as potential causes of human infection and disease. Human monocytic ehrlichiosis (HME), an infection of mononuclear white blood cells by *Ehrlichia chaffeensis*, was first recognized in 1986 (Anderson et al. 1991, Dumler and Bakken 1995, Fishbein et al. 1994). Cases of human granulocytic anaplasmosis (HGA), first recognized in 1994 (Bakken et al. 1994, Chen et al. 1994), are caused by a rickettsia recently reclassified as *Anaplasma phagocytophilum* comb. nov. (formerly *E. phagocytophila*) (Dumler et al. 2001) that has a tropism for granulocytic white blood cells (Dumler et al. 1995). The canine pathogens *E. ewingii* and *E. canis* have been associated with rare cases of human infection (Buller et al. 1999, Perez et al. 1996). HME and HGA are clinically indistinguishable and characterized by headache, high fever, chills, and myalgias. HME and HGA can be readily treated in the early stage with antibiotics. Left untreated, however, severe illness can ensue, including secondary infections due to immunosuppression, renal dysfunction, respiratory failure, and occasionally death (Fritz and Glaser 1998).

Between 1986 and 1997, the Centers for Disease Control and Prevention (CDC) identified 1,223 cases of HME and HGA in the United States (McQuiston et al. 1999). However,

this case count likely significantly underestimated the true incidence for several reasons. First, the Council of State and Territorial Epidemiologists did not add HME and HGA to the list of diseases and conditions designated as nationally notifiable until 1999. Second, as these diseases are only recently described, knowledge and experience among the medical community are still developing. Third, only a few regional academic and public health laboratories have the necessary diagnostic capabilities (Fritz and Glaser 1998). As such, formally reported cases of HME and HGA likely represent only the more severe infections within a spectrum of symptomatic manifestations; patients with mild illness may not seek medical attention or, when they do, be properly diagnosed. Prior to 1997, one case of HME (Marin County, 1994) (Vugia et al. 1996) and two cases of HGA (Santa Cruz County, 1995) (Gewirtz et al. 1996) had been identified in California.

Since their first recognition as human pathogens in California, the California Department of Health Services (CDHS) has conducted surveillance and case investigations for HME and HGA in known or suspected endemic areas. This report summarizes the results of clinical, ecological, and epidemiologic surveillance for these tick-borne rickettsioses in one northern California county.

MATERIALS AND METHODS

Clinical surveillance

In 1997, CDHS collaborated with the local health agency in Humboldt County on a two-year program to enhance physician awareness of and surveillance for HME and HGA. Humboldt County is located in northwestern California and extends along the Pacific Coast from approximately 200 miles north of San Francisco to 100 miles south of the Oregon border (Figure 1). Approximately 120,000 persons permanently reside in Humboldt County's 3,500 square-miles, most (59%) of whom live in the greater Eureka area. The remaining residents live in one of five incorporated cities (400 to 10,000 population) or in unincorporated areas of the county. Humboldt County was selected for this study because the high density of ticks, rural lifestyle of most residents, and known endemicity for Lyme disease suggested that the potential for other tick-borne diseases was significant. In 1997 and 1998, approximately 200 physicians practicing in Humboldt County were mailed a packet of informational materials on HME and HGA and encouraged to submit appropriate specimens from patients presenting with compatible symptoms to the CDHS Viral and Rickettsial Disease Laboratory (VRDL) for testing, free of charge.

Suspect patients were defined as persons with an unexplained acute febrile illness encompassing all of the following signs/symptoms: fever > 38 C, headache, and leukopenia or thrombocytopenia. Serum specimens from suspect cases were tested for IgM and IgG antibody to *E. chaffeensis* in DH82 cells provided by the CDC, by indirect immunofluorescence assay (IFA), using methods previously described (Comer et al. 1999, Dawson et al. 1990). Methods of testing serum specimens for antibody to *A. phagocytophilum* were as previously described (Fritz et al. 1997) with the modifications that commercial slides were obtained from IDEXX Laboratories (Westbrook, Maine), incubation time was 30 min, and slides were mounted in polyvinyl alcohol in 20% glycerol. Buffy coat fractions were tested by polymerase chain reaction (PCR) for presence of 16S ribosomal DNA of *E. chaffeensis* (Dawson et al. 1994) and *A. phagocytophilum* (Barlough et al. 1996). Stained buffy coat smears were requested and reviewed for presence of intraleukocytic morulae.

A confirmed case of HME or HGA was defined as a patient with clinically compatible illness plus a) a four-fold rise in titre between acute and convalescent serum specimens, b) amplification of *E. chaffeensis* or *A. phagocytophilum* genetic material by PCR, or c) observation of morulae on blood or buffy coat smear and a positive titre. Patients with a single elevated titre were considered probable cases.

Ecologic surveillance

Following confirmation of a clinical case, efforts were made to conduct tick collection at the case-patient's residence and other areas where the patient reported engaging in outdoor activities in the weeks preceding onset of illness. Ticks were collected with a standard meter-square flannel flag and identified to species and sex. Ticks were separated into pools of four to six ticks each, extracted by boiling in sodium

hydroxide and guanidinium lysis, and tested for the presence of *E. chaffeensis* and *A. phagocytophilum* 16S rRNA genes by PCR. Quality of extracted DNA and the presence of inhibitors were assessed by PCR for tick mitochondrial rDNA (Black and Piesman 1994).

Community seroprevalence and risk assessment

An estimate of the background frequency of exposure to *E. chaffeensis* and *A. phagocytophilum* in the general population of Humboldt County was assessed through a community seroprevalence study. Based on results of the clinical surveillance, the target study population included all permanent residents of southern Humboldt County. The study area was defined from Redcrest southward to the Humboldt-Mendocino County border, and from the Pacific coast eastward to the Humboldt-Trinity County border, encompassing a population of approximately 10,000 residents. Individuals were deemed ineligible to participate if they did not consider southern Humboldt County their place of permanent residence, or if they had lived in the area less than one year.

Publicity for the study was initiated approximately three weeks prior to the study's scheduled commencement. Announcements were placed weekly in local print and broadcast media and posted at senior centers, community centers, and other highly trafficked public venues. One week before commencement, the study was discussed during an hour-long call-in talk show broadcast on local radio and at a seminar for physicians and allied staff at the primary health care facility serving the study area.

Opportunities for residents to enroll in the study were scheduled over a four-day period in November 2000. A single meeting was held in each of five local communities, for a total of 25 recruitment hours. Prospective participants who attended were provided information about the study and the diseases under investigation. Following informed consent, participants were asked to complete a brief self-administered questionnaire that solicited information on age, sex, race, residence type, travel and activities, experience with ticks and tick bites, and recent illnesses. A separate questionnaire was completed by each individual participant.

A 5 ml sample of blood was collected by venipuncture from each participant. Blood specimens were labeled with a unique identifying number, allowed to clot, and refrigerated prior to shipping. A second series of serum samples to be used as a comparison group was collected from residents of northern Humboldt County. These serum samples were obtained from units of blood donated at a community blood center in Eureka in June 2001. All blood specimens were delivered to the VRDL for testing. Immunoglobulin G antibodies to *E. chaffeensis* and *A. phagocytophilum* were assessed by IFA. Reciprocal titres of greater than or equal to 64 were considered positive. Titers of positive specimens were determined up to a dilution of 1:256.

Table 1. Demographic, clinical, and laboratory features of human cases of human monocytic ehrlichiosis (HME) and human granulocytic anaplasmosis (HGA) diagnosed in Humboldt County, California, 1997-1999.

Case No.	HME/HGA	Age (yrs)	Sex	Onset (mo-yr)	Symptoms	Acute serum IgG titre		Conv. serum IgG titre		PCR	Morulae
						<i>Ehrlichia chaffeensis</i>	<i>Anaplasma phagocytophilum</i>	<i>Ehrlichia chaffeensis</i>	<i>Anaplasma phagocytophilum</i>		
1	HME	43	M	5-97	fever, myalgia, chills	<1:64	<1:64	1:512	1:64	ND	NA
2	HGA	35	M	8-97	fever, headache, back pain	<1:64	1:128	NA	NA	ND	NA
3	HME	46	M	3-98	fever, headache, malaise	<1:64	<1:64	1:256	<1:64	Neg	NA
4	HGA	55	F	6-98	fever, headache, sweats	<1:64	<1:64	<1:64	1:256	Pos	NA
5	HGA	44	M	6-98	fever, headache, malaise, arthralgias	1:128	1:2048	1:64	1:2048	Pos	NA
6	HGA	49	M	5-99	fever, headache, malaise	<1:64	<1:64	<1:64	1:256	Pos	Pos

NA: Serum/smear not collected.

ND: Diagnostic procedure not performed.

RESULTS

Clinical case surveillance

Between May 1997 and May 1999, specimens from 29 patients in Humboldt County were submitted to the VRDL for HME/HGA diagnostics. Two cases of HME and four cases of HGA were identified (Table 1). Two of these cases (Nos. 4 and 5) have been previously described (Foley et al. 1999). Two cases of HME (Nos. 1 and 3) were confirmed by a four-fold rise in *E. chaffeensis* IgG titer. Two cases of HGA (Nos. 4 and 5) were confirmed by PCR identification of *A. phagocytophilum*, and one (No. 6) by PCR, a four-fold rise in IgG titer to *A. phagocytophilum*, and observation of morulae in leukocytes. One probable case of HGA (No. 2) was identified by a single elevated IgM titer to *A. phagocytophilum*.

All six case-patients were residents of southern Humboldt County (Figure 1). Two cases were identified in 1997, three in 1998, and one in 1999. During this same period, no autochthonous HME/HGA cases were reported elsewhere in California.

Five (83%) case-patients were male and the median age was 45 years-old (range, 35 to 55 years). Months of illness

onset were March (1), May (2), June (2), and August (1). All six case-patients recalled a recent tick bite prior to onset of illness. Frequently reported symptoms included headache, fever, malaise, myalgia, and arthralgia. Mild leukopenia (WBC range, 1900 to 8100 /:L) and thrombocytopenia (platelet range, 67,000 to 139,000 /:L) were observed in most patients. Five patients were treated with doxycycline. One patient (Case No. 3) was already recovering at the time the diagnosis was confirmed, five wk after onset, and did not receive antimicrobials. All patients recovered without complication.

Ecologic surveillance

Tick surveillance was conducted at residences and areas of activity reported by three ehrlichiosis case-patients (Cases No. 1, 4, and 5). Dense brush and a steep ravine precluded tick flagging at Case No. 3's residence. Case No. 2 refused permission to access the residential property and Case No. 6 was lost to followup.

Case No. 1 lived in a mobile home on a fenced one-acre lot in redwood-alder-oak mix habitat. A 40-foot deep, heavily brushed ravine on the north precluded tick surveillance efforts immediately adjacent to the case-patient's property. Flagging

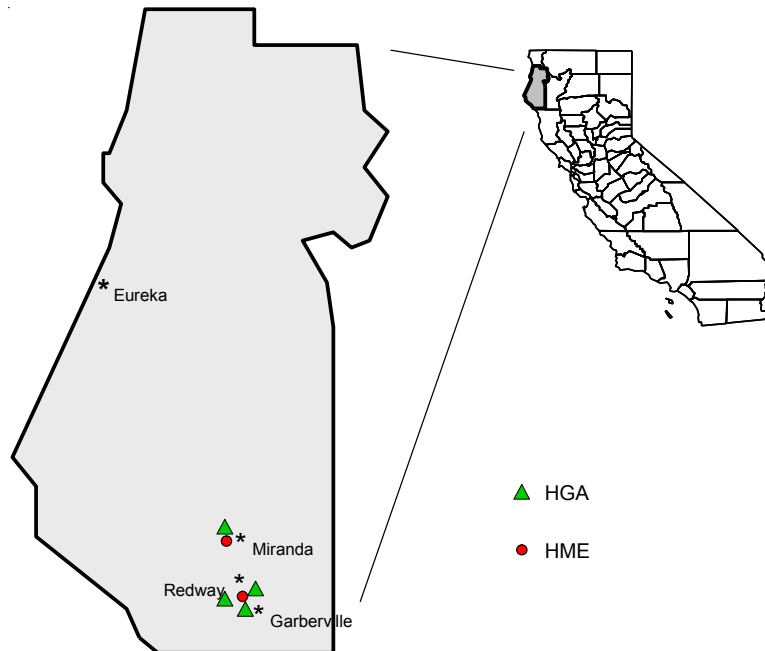


Figure 1. Cases of human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME) diagnosed in Humboldt County, California, 1997-1999.

on undeveloped state park land across the ravine, approximately one-quarter mile from the case-patient's property, yielded 42 *Ixodes pacificus* and 4 *Dermacentor occidentalis* ticks in April 1998. Follow-up efforts at the same location in March 1999 yielded an additional 50 *I. pacificus* and 6 *D. occidentalis*.

Case No. 4 lived on approximately four acres of densely wooded terrain, consisting of Douglas fir and madrone, on the north slope of a riparian canyon. Tick surveillance was conducted at the case's residence, alongside the driveway, and along an adjacent road in March and April 1999. A total of 123 *I. pacificus* and 4 *D. occidentalis* were collected, the majority from the driveway and road. Additional flagging conducted at the same time approximately eight km from the case-patient's property yielded an additional 107 *I. pacificus*.

Case No. 5 lived in a dense redwood grove. As the case-patient's property was destroyed by fire subsequent to his illness, tick surveillance efforts were relocated to an embankment along the major road near his residence and a roadside picnic area approximately 400 m north. Flagging in March 1999 yielded 119 *I. pacificus*. Flagging at several sites proximal to the case-patient's former residence in April 1999 yielded an additional 32 *I. pacificus* and 8 *D. occidentalis*. In addition to the case-associated surveillance, 59 *I. pacificus* and 61 *D. occidentalis* ticks were collected from other sites in southern Humboldt County during the same time period.

Of the 542 *I. pacificus* (108 pools), 82 *D. occidentalis* (20 pools), and one *D. variabilis* (1 pool) collected, *A. phagocytophilum* was detected in one of ten pools of female *I. pacificus* (minimum infection proportion = 0.0196) collected near Case No. 5's residence, and in one of six pools of female *I. pacificus* (minimum infection proportion =

0.0312) collected along the roadside near Case No. 4's residence. No pool was positive for *E. chaffeensis*.

Community study

A total of 54 residents of southern Humboldt County enrolled in the epidemiologic study. Participants were residents of ten communities in the study area. Thirty-three (61%) participants were female and the median age was 51 years (range, 17 to 80 years).

Participants had lived at their current residences a median of 10 years (range, 1 to 60 years). Thirty-nine (74%) participants lived in single-family homes on at least one-quarter acre of land; of these, 33 reported living on property of more than four acres. Twenty-seven participants lived on land that was composed of more than 50% woods. Of these, 16 reported seeing deer on their property at least once per mo.

Forty-six (85%) participants reported finding a tick on themselves at least once during the preceding year; nine participants reported having found ten or more ticks on themselves. Ticks were reported as having been found most frequently during the spring (34 of 46 respondents) and least frequently during the winter (14 of 46 respondents).

Among the 46 participants who found at least one tick on themselves, prevention measures reported to be performed "often" or "always" included wearing long-sleeved shirt (76%) and long pants (76%), checking body (54%) and clothes (43%) for ticks, and wearing light-colored clothing (30%). Repellants were reported applied at least "often" to clothing or skin by one and no respondents, respectively.

Thirty participants reported experiencing flu-like symptoms (fever, fatigue, headache, myalgia) at least once in

the preceding 12 mo. Three participants reported that they believed they had HME/HGA, currently or previously; two of these reported having received a physician's diagnosis of HME/HGA. Ten participants believed they had Lyme disease, currently or previously; eight of these reported having received a physician's diagnosis of Lyme disease.

Antibody to *A. phagocytophilum* was detected in serum specimens from two (3.7%) participants (titres 1:64 and >1:256). One *A. phagocytophilum*-positive specimen also had detectable antibody to *E. chaffeensis* (1:64); antibody to *E. chaffeensis* was not detected in any of the other serum specimens. IgG antibody (1:64) to *A. phagocytophilum* was detected in two (2.9%) serum specimens from the blood donors. Antibody to *E. chaffeensis* was not detected in any of the blood donor specimens.

DISCUSSION

In the three years preceding this study (1994-1996), three autochthonous cases of HME/HGA had been identified in all of California. During the two years the enhanced surveillance and physician awareness program was conducted, six cases were identified in southern Humboldt County. The estimated incidence rate of approximately 30 cases per 100,000 person-years is comparable to reports of active surveillance for HGA in endemic counties in Connecticut (14 to 16 per 100,000 person-years) (Bakken et al. 1996) and Wisconsin (24 to 51 per 100,000 person-years) (Ijdo et al. 2000).

All cases were identified by demonstration of specific serum antibody or detection of *Ehrlichia/Anaplasma* genetic material by PCR. As blood and buffy coat smears were rarely prepared by the submitting physicians, morulae were observed in leukocytes of only one patient. Although morulae are definitive evidence for acute HME/HGA, the proportion of infected leukocytes is highly variable. Morulae have been observed in <1% and up to 41% of granulocytes in HGA patients. By comparison, only 1-2% of mononuclear leukocytes are infected in HME patients (Aguero-Rosenfeld et al. 1996, Centers for Disease Control 1995, Fritz and Glaser 1998). Nevertheless, buffy coat smears should be prepared and examined by an experienced microbiologist for all suspect HME/HGA cases as identification of morulae can provide immediate confirmation.

All six case-patients recalled a specific tick bite that preceded their illnesses, with at least one tick being identified at the county public health laboratory as the western black-legged tick, *I. pacificus*. Field and experimental data support *I. pacificus* as the primary vector for *A. phagocytophilum* in California (Barlough et al. 1997, Kramer et al. 1999, Richter, Jr. et al. 1996). *Ixodes pacificus* is prevalent throughout the Pacific states and is most abundant in the humid, temperate forests of northern coastal California (Dennis et al. 1998). In the present study, over 500 adult *I. pacificus* were collected from vegetation during approximately 24 person-hours of flagging (~one tick every three min). Ticks were collected most frequently from unmaintained grass areas and fringe habitat adjacent to roads, driveways, walkways, and other areas of human traffic. The proportion of *I. pacificus* infected

with *A. phagocytophilum* in the present study (0.020) was less than what was previously estimated in Alameda (0.047), Santa Cruz (0.062), and Sonoma (0.067) Counties (Holden et al. 2003, Kramer et al. 1999). However, the greater abundance of *I. pacificus* ticks in Humboldt County may translate to more frequent opportunities to be bitten by an infected tick, despite the comparatively low infection proportion.

Little information is available on the vector of *E. chaffeensis* in California. The principal tick vector in the midwestern and southern United States is the Lone Star tick, *Amblyomma americanum* (Anderson et al. 1993). However, the described range of this tick does not extend to the Pacific states. *Ehrlichia chaffeensis* has also been detected in the American dog tick, *D. variabilis* (Roland et al. 1998). *Ehrlichia chaffeensis* was detected more frequently in *D. variabilis* than *I. pacificus* collected in Santa Cruz County, California (Holden et al. 2003, Kramer et al. 1999). As only one *D. variabilis* was collected in the present study from Humboldt County, it is impossible to estimate the infection prevalence of *E. chaffeensis*.

The combined seroprevalence to *E. chaffeensis* and *A. phagocytophilum* estimated in the community study (0.037) was comparable to that reported previously in northern California (0.050) (Fritz et al. 1997) but was lower than estimates reported for endemic areas elsewhere in the United States (Wisconsin, 0.149) (Bakken et al. 1998) and Europe (Sweden, 0.169) (Dumler et al. 1997). The apparent discrepancy between the relatively high clinical incidence and seroprevalence estimates is difficult to interpret, as incidence and seroprevalence studies of HME/HGA have not previously been conducted in the same population. Furthermore, the seroprevalence estimate (0.037) suffers from poor precision (95% CI = 0.00, 0.407) due to the small number of participants.

The seroprevalences to *E. chaffeensis* and *A. phagocytophilum* for the study group in southern Humboldt County were not significantly different from those estimated for the comparison group of Eureka blood donors. Eureka blood donors were believed to represent a geographically proximal but less rural population. However, as information on demographics, residence, activities, and tick bite prevention practices was not available for blood donors, it is unknown whether they shared risk factors with the study population (e.g., outdoor activities, exposure to ticks). Additional ecological surveillance is needed throughout Humboldt County to better define the distribution, density, and dynamics of rickettsial infection in ticks.

A previous study of tick-transmitted diseases conducted in Sonoma County estimated seroprevalence to *E. chaffeensis* at 4.6% (Fritz et al. 1997). In the present study, one participant (1.9%) had detectable antibody to *E. chaffeensis* (1:64), as well as to *A. phagocytophilum* (1:256). Dual infection with *E. chaffeensis* and *A. phagocytophilum* has not been previously reported in humans, but serologic cross-reactivity between *E. chaffeensis* and *A. phagocytophilum* antigens is a recognized phenomenon which has been reported in over half of specimens from HGA patients (Comer et al. 1999). Serologic cross-reactivity may confound confirmation of

HME cases in non-endemic areas if other diagnostics to differentiate HME from HGA (i.e., observation of morulae, PCR) are not available or performed.

Serum antibodies are detectable within two wk of onset in 70 to 90 percent of clinical HME/HGA patients and peak around six wk after onset (Aguero-Rosenfeld et al. 1996, Aguero-Rosenfeld et al. 2000, Dawson et al. 1990). The duration of circulating antibodies to these rickettsia is unknown. While follow-up studies have detected positive titers in some HGA patients up to 24 mo after onset (Lotric-Furlan et al. 2001), titers in HME patients decline 6-12 weeks after onset (Dawson et al. 1990). One participant in the community study was also one of the six clinical cases. At the time of illness, the patient's convalescent titer to *E. chaffeensis* was 1:512; at the time of the community study (3+ years post-infection) the patient's titer was <1:64. If IgG antibodies to these rickettsiae are relatively short-lived, seroprevalence figures alone may understate the risk of exposure to these agents. Also, nearly half of HGA cases nationwide occur in June and July (Comer et al. 1999). If serum antibodies to *A. phagocytophilum* and *E. chaffeensis* are relatively transient, serologic studies conducted during the late autumn, such as the present study, may detect fewer exposures than if conducted in early summer.

The inferential significance of the community study is compromised by the comparatively small number of participants. The few number of seroreactive participants precluded identification of risk factors for exposure to *E. chaffeensis* and *A. phagocytophilum*. Also, the small number of self-selected participants may not be representative of all southern Humboldt County residents. Persons who regularly encountered ticks or had a recent illness may have been more likely to participate, thus positively biasing the seroprevalence estimate and epidemiologic exposure results. Conversely, participants from the Eureka blood center likely derived from a healthier base population than those self-selected from a general population.

Although the seroprevalences to *E. chaffeensis* and *A. phagocytophilum* suggested a low risk of exposure to these organisms, information gathered in the questionnaire indicated that exposures to ticks was common. Tick bites were reported by 85% of participants, but adherence to standard tick bite prevention recommendations was generally low. Physicians and other health care providers in Humboldt County should be aware that because of their clientele's frequent contact with ticks, HME, HGA, and other tick-transmitted diseases should be considered in any patient who presents with compatible symptoms. Subsequent to completion of this study, a case of HGA was diagnosed in June 2002 in a 54-year-old female resident of southern Humboldt County. This continued evidence of autochthonous transmission emphasizes the ongoing risk to residents in, and visitors to, this endemic area of California.

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