Eur J Clin Microbiol Infect Dis (2001) 20:173–178

Article

First Long-Term Study of the Seroresponse to the Agent of Human Granulocytic Ehrlichiosis Among Residents of a Tick-Endemic Area of Sweden

B. Wittesjö, A. Björsdorff, I. Eliasson, J. Berglund

Abstract The seroprevalence of granulocytic ehrlichiosis has been documented in several studies, but little data exists on incidence rates. Using sera stored from an earlier study on Lyme borreliosis, 290 residents of Aspö Island could be followed prospectively during two tick seasons (1992–1994). Immunoglobulin G antibodies to granulocytic ehrlichiosis were detected by an immunofluorescence assay using *Ehrlichia equi* as antigen. Seroprevalence rates increased significantly over time, and at the 1994 follow-up, 28% of the residents were seropositive. Negative-to-positive seroconversion (incidence) rates were 3.9% and 11.1%, respectively, during the two seasons. A highly significant correlation was found between a positive serologic response for granulocytic ehrlichiosis and *Borrelia burgdorferi*. No such correlations were found for clinical Lyme borreliosis, self-reported arthralgia or number of recorded tick bites. It was concluded that granulocytic ehrlichiosis is highly endemic in this part of Sweden, with a seroconversion rate as high as 11% over a single tick season. Further studies are necessary to correlate these findings with clinical signs of human granulocytic ehrlichiosis.

Introduction

Granulocytic ehrlichiosis is a zoonotic infection caused by *Rickettsia*-like coccobacilli transmitted by ticks [1]. It is a well-recognised problem in veterinary medicine, occurring widely in tick-infested areas of Sweden [2, 3].

B. Wittesjö (✉)
Department of Communicable Disease Control,
Blekinge Hospital, 371 85 Karlskrona, Sweden
e-mail: bengt.wittesjo@telia.com
Tel.: +46-455-57952
Fax.: +46-455-57952

A. Björsdorff, I. Eliasson
Department of Clinical Microbiology, Kalmar County Hospital,
391 85 Kalmar, Sweden

A. Björsdorff
Department of Infectious Diseases and Medical Microbiology,
Lund University, 223 62 Lund, Sweden

J. Berglund
Blekinge Institute for Research & Development,
Erik Dahlbergsvägen 30, 374 37 Karlshamn, Sweden

J. Berglund
Department of Community Medicine, Lund University,
Malmö University Hospital, 205 02 Malmö, Sweden

The first human cases, caused by *Ehrlichia* organisms that primarily infected circulating neutrophil granulocytes, were described in the USA in 1994 [4]. Most cases of human granulocytic ehrlichiosis (HGE) characterised so far are caused by an *Ehrlichia* agent (the "HGE agent") that belongs to the same genogroup as *Ehrlichia equi* and *Ehrlichia phagocytophila*, both well-known pathogens of domestic animals. The HGE agent is also present in Sweden, where it causes granulocytic ehrlichiosis in horses, dogs and cats [5, 6]. *Ehrlichia ewingii*, an agent previously reported as a cause of granulocytic ehrlichiosis in dogs, has recently been shown to cause HGE as well [7].

The first four clinical cases of HGE in Europe were described in a prospective study performed in Slovenia in 1997 [8], and recently several HGE cases diagnosed in Sweden and Norway were verified by polymerase chain reaction [9].

The clinical presentation of granulocytic ehrlichiosis is typically a febrile illness with headache and myalgia, i.e. a flu-like condition, lasting for 1 to several weeks. Granulocytic ehrlichiae are transferred by ticks of the

genus *Ixodes*, in North America *Ixodes scapularis* [10, 11] and in Europe *Ixodes ricinus* [12–16]. Thus, granulocytic *Ehrlichia* share common vectors with *Borrelia burgdorferi* sensu lato; furthermore, as cases accumulate, the case distribution of HGE has been found to be similar to that of Lyme borreliosis [17, 18]. In fact, coinfection with *Borrelia* and *Ehrlichia* may act synergistically to favour the emergence of clinical Lyme borreliosis, based on the finding that granulocytic *Ehrlichia* impairs the immune status of the infected host [19]. This observation has been made in patients coinfected with *Babesia microti* and *Borrelia burgdorferi* [20].

Several seroepidemiologic studies of tick-exposed populations in different parts of Europe have shown a seroprevalence of granulocytic ehrlichiosis of approximately 10% [21–24]. In a population-based seroepidemiologic study on the west coast of Sweden (Koster Islands), 11% were positive for *Ehrlichia equi* antibodies when tested by an immunofluorescence assay [25]. During 1996 we conducted a prospective clinical study (unpublished) to determine if tick-associated febrile illness could be attributed to HGE. No confirmed cases were found, but 11% of the patients showed serological signs of exposure to granulocytic *Ehrlichia* and falling titres after 6–12 months, indicative of recent infection.

To our knowledge, reports from longitudinal serologic studies of HGE with follow-up periods of more than one tick season are not yet available. We used sera stored from an earlier investigation of Lyme borreliosis on Aspö Island [26] in an attempt to study retrospectively the serologic evidence of HGE over time in a highly tick-exposed population.

The aims of the present investigation were to study the following: (i) the seroconversion rate (incidence) for granulocytic *Ehrlichia* during two tick seasons (1992–1993 and 1993–1994); (ii) the seroprevalence of granulocytic *Ehrlichia* during 3 years (1992, 1993 and 1994) and the influence of antibiotic treatment on serologic results; (iii) immunoglobulin G seropositivity for granulocytic *Ehrlichia* as related to the number of years spent in the area; (iv) the number of persons coinfected with granulocytic *Ehrlichia* and *Borrelia burgdorferi* sensu lato and clinical manifestations of the latter; and (v) the correlation between seropositivity for granulocytic *Ehrlichia* and the number of self-reported tick bites.

Materials and Methods

Study Area and Population. Aspö is an island with an area of 8 km^2 on the coast of the Baltic Sea in southeastern Sweden. In 1991, 540 individuals in 253 families inhabited the island. *Ixodes ricinus* ticks are numerous, and about 20% of the investigated population were found to be infected by *Borrelia burgdorferi* sensu lato in 1991 [27].

Study Design. As described by Berglund et al. [26], all inhabitants were invited to participate in a study that included blood sampling for serological tests and a questionnaire on age, years spent on the island, profession, tick exposure and history of previous Lyme borreliosis or symptoms commonly associated with the disease. Of the 540 residents, 480 agreed to participate and 301 (56%) were monitored during the entire study period.

Blood samples were drawn in May 1991 and thereafter once a year for 3 consecutive years. The participants were also asked to keep a record of tick bites observed and to complete annual questionnaires on symptoms or manifestations of Lyme borreliosis and any antibiotic treatment received during the period. The diagnosis of Lyme borreliosis required a clinically diagnosed erythema migrans, or one or more clinical manifestations (Lyme arthritis, neuroborreliosis, acrodermatitis chronica atrophicans and borrelial lymphocytoma) plus serological confirmation.

Using the same cohort for our present study, we were able to analyse the material with regard to serological evidence of granulocytic ehrlichiosis during two consecutive tick seasons, 1992–1993 and 1993–1994. Sera drawn in May 1991 in the original study could not be used due to the scanty quantity.

Serologic Testing. All individual serum samples from the original *Borrelia* study were divided in two test tubes (except for samples drawn in 1991) and stored at –70 °C. One of the test tubes drawn from each serum sample was not thawed until 1999, when serologic testing for *Ehrlichia* was performed at the Department of Clinical Microbiology, Kalmar County Hospital. IgG antibody titres to granulocytic *Ehrlichia* were determined by an indirect immunofluorescence assay using a commercially available *Ehrlichia equi* antigen (Protatek International, USA). The indirect immunofluorescence assay method used was adapted from the method described by Ristic et al. [28]. A fluorescein thiocyanate-conjugated goat anti-human IgG antibody (product no. 209–095–088; Jackson ImmunoResearch Laboratories, USA), diluted 1:100 in phosphate-buffered saline, was used as secondary antibody. Positive and negative serum controls were included for each immunofluorescence assay run and consisted of convalescent-phase sera from two verified cases of HGE (kindly provided by Dr. R. Massung, Centers for Disease Control and Prevention, Atlanta, USA) and of serum from a healthy staff member with no clinical history of HGE infection.

End-point titres were set at the reciprocal of the last dilution giving an unequivocal specific fluorescence. Cut-off values for the immunofluorescence assay were established on the basis of the values for sera collected in 1996 from a control group of 100 healthy blood donors from northern Sweden, where *Ixodes* ticks are not encountered. A titre of <80 correlated with the presumptive absence of antibodies in sera from healthy persons. In sera diluted less than 1:80, nonspecific reactions are likely to occur. Accordingly, a titre of <80 was interpreted as negative and an antibody titre of 80 or higher was considered positive.

Statistical Analysis. The chi-square method, with help of Epi Info 6.0 (Centers for Disease Control and Prevention, USA), a large sample test of proportions and the central limit theorem, was used for statistical analyses.

Ethics. The Research Ethics Committee of Lund University approved the study.

Results

Of the 480 residents initially included and the 301 monitored for 3 years in the *Borrelia* study, 290 could be evaluated in the present study (Figure 1). The

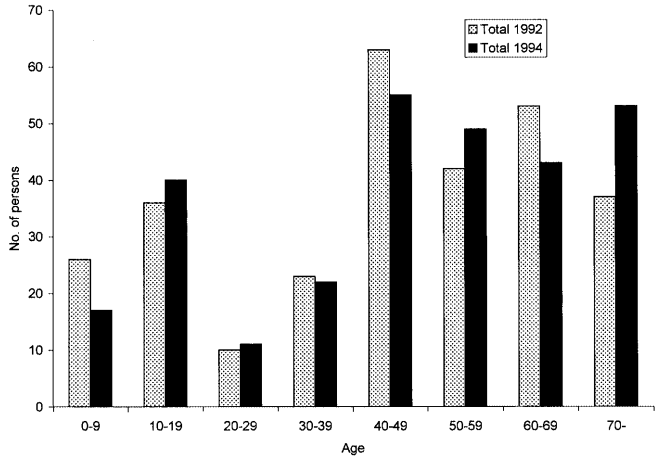

Figure 1 Number of persons per age group among the cohort of 290 residents of Aspö Island from 1992 to 1994

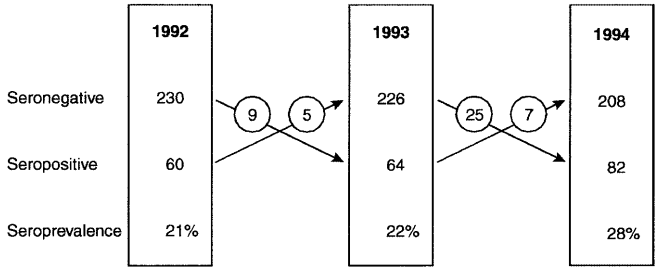

Figure 2 Dynamics of seroprevalence of IgG antibodies to *Ehrlichia equi* in 290 residents of Aspö Island from 1992 to 1994

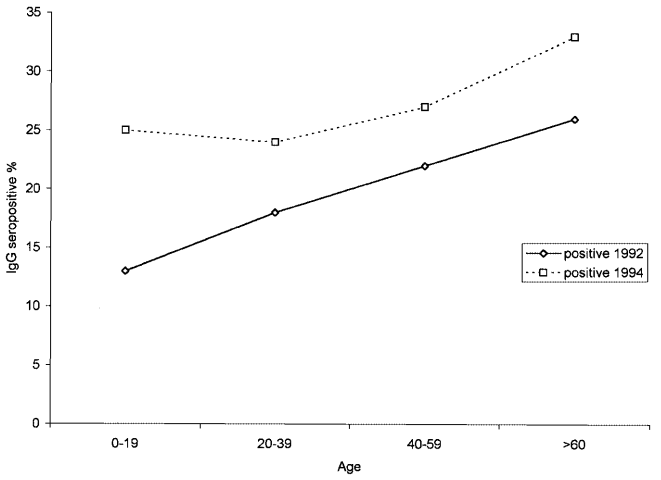

Figure 3 IgG seropositivity for *Ehrlichia equi* as related to age among 290 residents of Aspö Island who were followed-up for 2 years

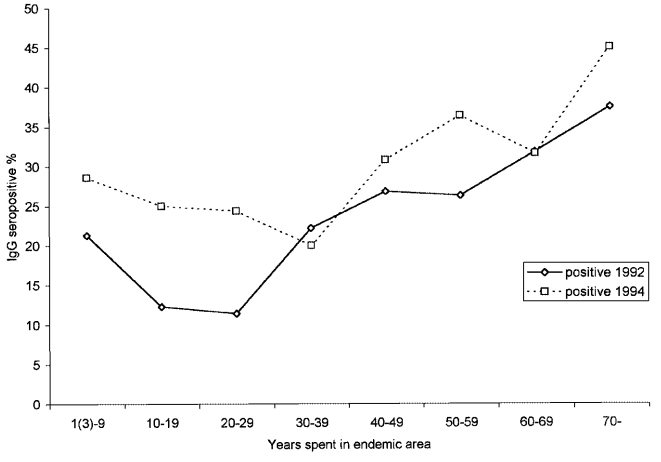

Figure 4 IgG seropositivity for *Ehrlichia equi* as related to years spent on Aspö Island among 290 individuals followed-up for 2 years

reasons for dropout were as follows: nonattendance at one or more blood samplings ($n=92$), departure from the island ($n=71$), death ($n=16$) and scanty quantities of serum remaining from the original study ($n=11$). Of the 290 residents included, 141 were men and 149 women. The mean age was 45 years (range 2–91 years) at the start of the study (1992).

IgG Antibodies to Ehrlichia equi. Of the 290 residents studied, 60 (21%) were IgG seropositive in the sera collected in 1992. Seropositivity rates increased significantly ($P<0.05$) over time, and at the 1994 follow-up 82 (28%) of the residents were seropositive (Figure 2). When comparing separate age groups (Figure 3), the increase in the seropositivity rate was significant ($P<0.05$) only in the lowest age interval, 0–19 years. This was confirmed also when comparing age groups in 10-year intervals.

The seropositivity rate also increased with the number of years spent on the island (Figure 4). Most individuals who were or who became seropositive remained positive during the study period. Twelve residents lost their seroreactivity, of whom only one received antibiotic treatment for Lyme borreliosis. Thirty-four individuals seroconverted to positive during the two seasons studied, of whom six received antibiotic treatment for confirmed or suspected Lyme borreliosis. Antibiotic treatment for any cause or specifically for Lyme borreliosis during the two seasons did not affect the outcome of HGE seroreactivity.

One of the healthy control blood donors was seropositive (titre 80). The remaining 99 blood donors tested negative.

Correlation Between Serologic Titres and Manifestations of Lyme Borreliosis and Ehrlichia equi Titres. A positive IgG serologic response to *Borrelia burgdorferi* sensu lato was found more frequently in individuals who also had a positive serologic response to *Ehrlichia equi*. No correlation was found between self-reported chronic arthralgia and negative-to-positive seroconversion for *Ehrlichia equi*. Likewise, no correlations were

Table 1 Correlation between serological, clinical and self-reported findings in 290 individuals over 2 years

Exposition (horizontal)	Correlate (vertical)	Status	1992–1993		*P* value	1993–1994		*P* value
			No. pos.	No. neg.		No. pos.	No. neg.	
E. equi IgG antibodies (1993 & 1994)	*B. burgdorferi* IgG antibodies (1993 & 1994)	pos.	33	31	$P<0.001$	43	39	$P<0.001$
		neg.	61	165		53	155	
E. equi seroconversion	lyme borreliosis[a]	pos.	0	9	n.s	2	23	n.s.
		neg.	14	267		11	254	
E. equi seroconversion	arthralgia[b]	pos.	2	7	n.s	2	23	n.s.
		neg.	15	266		7	258	
Tick bite	*E. equi* seroconversion	pos.	8	239	n.s	23	239	n.s.
		neg.	1	42		2	26	

[a] Diagnosed and treated by a physician
[b] Self-reported
pos., positive; neg., negative; n.s., not significant

found between negative-to-positive seroconversion or positive serologic response to *Ehrlichia equi* and any manifestation of Lyme borreliosis (Table 1).

Tick Bites. Approximately 1,600 and 1,900 tick bites were registered by the participants during the two seasons 1992–1993 and 1993–1994, respectively, corresponding to an average of six tick bites per resident per year. Only 16 of 290 (5.5%) residents did not register any tick bite during the study period. No correlation was found between negative-to-positive seroconversion and number of registered tick bites (0 vs. any number of tick bites; Table 1), even when comparing zero versus >10 tick bites. The same applied for seropositivity for *Ehrlichia equi*.

Discussion

In this highly tick-exposed population, we found a significant ($P<0.05$) increase in the seroprevalence of granulocytic *Ehrlichia*, from 21% in 1992 to 28% in 1994. These are the highest rates reported so far, considerably greater than those from other studies among tick-exposed populations [21–25, 29]. In all studies the diagnostic method was similar, i.e. an immunofluorescence antibody test against *Ehrlichia equi*. When comparing age groups, the increase in the seroprevalence rate was significant only in the lower age interval, probably due to a higher degree of tick exposure.

For the first time seroconversion rates for granulocytic ehrlichiosis have been established. Among this highly tick-exposed population, 3.9 and 11.1% developed serological evidence of exposure to granulocytic *Ehrlichia* during 1992–1993 and 1993–1994, respectively. We do not, however, know how many of these individuals actually developed any symptoms consistent with HGE. Fever as a nonspecific symptom was not recorded, and haematological analyses, other than serologic assays, were not performed in this study, which was originally designed for Lyme borreliosis. Other studies with active case collection for HGE have yielded an incidence of 14–16 and 24–58 cases per 100,000 population in different parts of the USA, respectively [30]. The demonstration of proven infection in the absence of specific therapy suggests that HGE may frequently be subclinical or may occur with few and mild clinical manifestations [29, 31].

In our study the seroprevalence of granulocytic ehrlichiosis increased with age and years spent on the island. Similar results were found in a seroprevalence study of HGE among permanent residents of northwestern Wisconsin [29] as well as in the original investigation concerning *Borrelia burgdorferi* [26].

A positive-to-negative seroconversion rate of 8.3 and 10.9% was found in the periods 1992–1993 and 1993–1994, respectively. These findings could indicate recent exposure to granulocytic *Ehrlichia*, but again, we cannot correlate this with signs of HGE. Forty-nine (16.9%) individuals showed a positive serologic response to *Ehrlichia equi* during the entire study period. This may indicate a "vaccination effect" due to multiple exposures to the antigen. In this limited material, antibiotic treatment was not found to have any significant influence on HGE seroreactivity.

As expected, a highly significant correlation was found between seropositivity for granulocytic *Ehrlichia* and *Borrelia burgdorferi*, both agents being transmitted by the same vector. Concurrent HGE and Lyme disease has been documented [32], and the risk of a more severe disease has been suggested in case of coinfection [19], though this has still not been proven clinically. We found no correlation between negative-to-positive seroconversion for granulocytic *Ehrlichia* and either clinically verified Lyme borreliosis treated by a physician or self-reported arthralgia.

A positive correlation between the number of tick bites and seropositivity for granulocytic *Ehrlichia* would be

expected, as has been shown in a previous seroepidemiologic study on Lyme borreliosis and tickborne encephalitis on Lisö Island in Sweden [33]. No such correlation was found in our study regarding granulocytic ehrlichiosis. However, compared to the subjects in the Lisö study, our population was exposed to ticks much more frequently: an average of six tick bites per resident per year were reported, with only 5.5% of the residents reporting that they had never been bitten by a tick. In the seroprevalence study of HGE in Wisconsin, 45.7% reported a tick bite within the last 12 months, but no association between the results of the immunofluorescence assay and history of tick bites was found [29]. The study from the west coast of Sweden [25] also showed that tick bites were not more frequent in *Ehrlichia equi*-seropositive than in -seronegative persons; the degree of tick exposure, however, was not stated. The only known transmission route is through the bite of an *Ixodes* tick. One might speculate that a bite at the nymphal stage is infectious but not recognised and recorded as a tick bite.

We conclude that granulocytic ehrlichiosis is highly endemic in this part of Sweden, and for the first time a long-term study has shown a seroconversion rate as high as 11% over one season. In order to correlate these findings with clinical signs of HGE, new prospective studies are needed.

Acknowledgment We thank Åke Björnham for excellent help with statistical analysis and Landstinget Blekinge for economical support.

References

1. Gribble DH: Equine ehrlichiosis. Journal of the American Veterinary Medical Association (1969) 155:462–469
2. Björsdorff A, Christenson D, Johnsson A, Sjöström AC, Madigan JE: Granulocytic ehrlichiosis in the horse – the first verified cases in Sweden. (In Swedish, summary in English). Svensk Veterinärtidning (1990) 42:357–360
3. Egenvall A, Hedhammar A, Olson P, Björsdorff A: Fästingburna infektioner hos hund i Sverige. Tickborne infections of dogs in Sweden. (In Swedish, summary in English). Svensk Veterinärtidning (1994) 46:321–327, 329
4. Bakken JS, Dumler SJ, Chen SM, Eckman MR, Van Etta LL, Walker DH: Human granulocytic ehrlichiosis in the upper midwest United States, a new species emerging? JAMA (1994) 272:212–218
5. Johansson KE, Pettersson B, Uhlen M, Gunnarsson A, Malmqvist M, Olsson E: Identification of the causative agent of granulocytic ehrlichiosis in Swedish dogs and horses by direct solid phase sequencing of PCR products from the 16S rRNA gene. Research in Veterinary Science (1995) 58:109–112
6. Björsdorff A, Svendenius L, Owens JH, Massung RF: Feline granulocytic ehrlichiosis – a report of a new clinical entity and characterisation of the infectious agent. Journal of Small Animal Practice (1999) 40:20–24
7. Buller RS, Arens M, Hmiel SP, Paddock CD, Summer JW, Rikhisa Y, Unver A, Gaudreault-Keener M, Manian FA, Liddell AM, Schmulewitz N, Storch GA: *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. New England Journal of Medicine (1999) 341:148–155
8. Lotric-Furlan S, Petrovec M, Zupanc TA, Nicholson WL, Sumner JW, Childs JE, Strle F: Human granulocytic ehrlichiosis in Europe: clinical and laboratory findings for four patients from Slovenia. Clinical Infectious Diseases (1998) 27:424–428
9. Björsdorff A, Berglund J, Kristiansen BE, Söderström C, Eliasson I: Varying clinical picture of human granulocytic ehrlichiosis. Twelve Scandinavian cases of the new tick-borne zoonosis are presented. (In Swedish, summary in English). Läkartidningen (1999) 96:4200–4204
10. Varde S, Beckley J, Schwartz I: Prevalence of tick-borne pathogens in *Ixodes scapularis* in a rural New Jersey county. Emerging Infectious Diseases (1998) 4:97–99
11. Daniels TJ, Falco RC, Schwartz I, Varde S, Robbins RG: Deer ticks (*Ixodes scapularis*) and the agent of Lyme disease and human granulocytic ehrlichiosis in a New York City park. Emerging Infectious Diseases (1997) 3:353–355
12. Schouls LM, Van De Pol I, Rijpkema SG, Schot CS: Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. Journal of Clinical Microbiology (1999) 37:2215–2222
13. Pusteria N, Leutenegger CM, Huder JB, Weber R, Braun U, Lutz H: Evidence of the human granulocytic ehrlichiosis agent in *Ixodes ricinus* ticks in Switzerland. Journal of Clinical Microbiology (1999) 37:1332–1334
14. Guy E, Tasker S, Joynson DH: Detection of the agent of human granulocytic ehrlichiosis (HGE) in UK ticks using polymerase chain reaction. Epidemiology and Infection (1998) 121:681–683
15. Petrovec M, Sumner JW, Nicholson WL, Childs JE, Strle F, Barlic J, Lotric-Furlan S, Avsic Zupanc T: Identity of ehrlichial DNA sequences derived from *Ixodes ricinus* ticks with those obtained from patients with human granulocytic ehrlichiosis in Slovenia. Journal of Clinical Microbiology (1999) 37:209–210
16. von Stedingk LV, Gürtelschmid M, Hanson HS, Gustafson R, Dotevall L, Engvall EO, Granström M: The human granulocytic ehrlichiosis (HGE) agent in Swedish ticks. Clinical Microbiology and Infection (1997) 3:573–574
17. Cinco M, Padovan D, Murgia R, Heldtander M, Engvall EO: Detection of HGE agent-like *Ehrlichia* in *Ixodes ricinus* ticks in northern Italy by PCR. Wiener Klinische Wochenschrift (1998) 110:898–900
18. Daniels TJ, Boccia TM, Varde S, Marcus J, Le J, Bucher DJ, Falco RC, Schwartz I: Geographic risk for Lyme disease and human granulocytic ehrlichiosis in southern New York state. Applied and Environmental Microbiology (1998) 64:4663–4669
19. Dumler JS: Is human granulocytic ehrlichiosis a new Lyme disease? Review and comparison of clinical, laboratory, epidemiological, and some biological features. Clinical Infectious Diseases (1997) 25, Supplement 1:43–47
20. Krause PJ, Telford SR III, Spielman A, Sikand V, Ryan R, Christianson D, Burke G, Brassard P, Pollack R, Peck J, Persing DH: Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. JAMA (1996) 275:1657–1660
21. Bakken JS, Krueth J, Tilden RL, Dumler JS, Kristiansen BE: Serological evidence of human ehrlichiosis in Norway. European Journal of Clinical Microbiology & Infectious Diseases (1996) 15:829–832
22. Fingerle V, Goodman JL, Johnson RC, Kurtti TJ, Munderloh UG, Wilske B: Human granulocytic ehrlichiosis in southern Germany: increased seroprevalence in high-risk groups. Journal of Clinical Microbiology (1997) 35:3244–3247
23. Brouqui P, Dumler JS, Lienard R, Brossard M, Raoult D: Human granulocytic ehrlichiosis in Europe. Lancet (1995) 346:782–783
24. Björsdorff A, Brouqui P, Eliasson I, Massung RF, Wittesjö B, Berglund J: Serological evidence of *Ehrlichia* infection in Swedish Lyme borreliosis patients. Scandinavian Journal of Infectious Diseases (1999) 31:51–55

25. Dumler JS, Dotevall L, Gustafson R, Granström M: A population-based seroepidemiologic study of human granulocytic ehrlichiosis and Lyme borreliosis on the west coast of Sweden. Journal of Infectious Diseases (1997) 175:720–722
26. Berglund J, Eitrem R, Norrby R: Long-term study of Lyme borreliosis in a highly endemic area in Sweden. Scandinavian Journal of Infectious Diseases (1996) 28:473–478
27. Berglund J, Eitrem R: Tick-borne borreliosis in the archipelago of southern Sweden. Scandinavian Journal of Infectious Diseases (1993) 25:67–72
28. Ristic M, Huxsoll DL, Weisiger RM, Hildebrandt PK, Nyindo MBA: Serological diagnosis of tropical canine pancytopenia by indirect immunofluorescence. Infection and Immunology (1972) 6:226–231
29. Bakken JS, Goeliner P, Van Etten M, Boyle DZ, Swonger OL, Mattson S, Krueth J, Tilden RL, Asanovich K, Walls J, Dumler JS: Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. Clinical Infectious Diseases (1998) 27:1491–1496
30. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS: Clinical and laboratory characteristics of human granulocytic ehrlichiosis. JAMA (1996) 275:199–205
31. Petrovec M, Furlan SL, Zupanc TA, Strle F, Brouqui P, Roux V, Dumler JS: Human disease in Europe caused by a granulocytic *Ehrlichia* species. Journal of Clinical Microbiology (1997) 35:1556–1559
32. Nadelman RB, Horowitz HW, Hsieh TC, Wu JM, Aguero-Rosenfeld ME, Schwartz I, Nowakowski J, Varde S, Wormser GP: Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis. New England Journal of Medicine (1997) 337:27–30
33. Gustafson R, Svenungsson B, Gardulf A, Stiernstedt G, Forsgren M: Prevalence of tick-borne encephalitis and Lyme borreliosis in a defined Swedish population. Scandinavian Journal of Infectious Diseases (1990) 22:297–306