

Retrospective Study Investigating the Seroprevalence of *Anaplasma phagocytophilum* in Manitoba, Canada: 2011–2014

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Human granulocytic anaplasmosis is currently not nationally notifiable in Canada. This for the most part accounts for gross under-estimation of true incidence and prevalence of HGA and would potentially culminate in clinical missed opportunities. To the best of our knowledge, this is the first report on the seroprevalence of *Anaplasma phagocytophilum* in a Canadian jurisdiction with known established black-legged tick populations.

Keywords. *Anaplasma phagocytophilum*; HGA; Manitoba; seroprevalence.

Human granulocytic anaplasmosis (HGA) is among the most common tickborne infectious diseases in the United States, and it became a nationally notifiable disease in 1999 [1]. Human granulocytic anaplasmosis is caused by the obligate intracellular bacterium *Anaplasma phagocytophilum* (Ap) through black-legged tick (*Ixodes ricinus* complex) bites. The latter ticks are also well known for transmission of Lyme disease among others [2–6].

There has been a steep rise in number of incident cases of HGA in several US states with established black-legged tick populations, with incidence as high as 58/100 000 in the general population and particularly in some counties in Minnesota and Wisconsin. The latter rise has been seen since HGA became nationally notifiable in 1999 [1, 7–9]. Human granulocytic anaplasmosis is currently not nationally notifiable in Canada, which has led to gross underestimation of its incidence and prevalence. The province of Manitoba and the state of Minnesota are neighbors. Human granulocytic anaplasmosis has been notifiable in Minnesota since 1995, and residents

there have been experiencing increasing rates of the disease. However, no systematic study has been done in Manitoba to determine incident HGA.

The presence of Ap in black-legged ticks in Manitoba has been shown recently [10]. In the current retrospective study (encompassing the period of 2011–2014), we examined the seroprevalence of Ap among healthy transplant donors and among patients who had been screened for Lyme disease during the tick activity season in Manitoba, which is from May through the end of October.

METHODS

In this retrospective study, a total of 446 residual serum specimens that had previously sent to Cadham Provincial Laboratory (CPL), Winnipeg, Manitoba, Canada, from 2011 to 2014 were used. The sera were collected for routine Lyme disease diagnosis based on presumed or definite tick exposure in the context of suggestive signs and symptoms.

From the pool described above, we selected 77 consecutively received residual serum samples initially collected and sent to CPL for organ donor screening (age- and sex-matched with the group described above), and these were used as a control group (where available). Manitoba (especially the southern part of the province) is considered a Lyme disease-endemic region with established *I. scapularis* tick populations with varying *Borrelia burgdorferi* positivity rates as well as definite clinical cases reported mostly from May through the end of October (local data from department of health).

The patients' age and gender as well as specimen collection month were also documented. Next, specimens were deidentified so no link could be established with the patients' information, and aliquoted sera were stored for Ap serological testing at a later date. The specimens were classified based on (1) Lyme disease screening test result (C6 peptide enzyme-linked immunosorbent assay [ELISA]; Immunetics, Boston, MA) and, where applicable, (2) Lyme immunoblot (LIB) results (following Centers for Disease Control and Prevention [CDC] criteria performed at the National Microbiology Laboratory [NML] in Winnipeg, Canada). Based on the aforementioned criteria, the randomly selected sera were categorized into 4 cohorts (cohorts 2–6) as described in Table 2.

Specimens were screened for Ap immunoglobulin (Ig) G. Sera positive for Ap IgG were further tested for Ap IgM. Both of the aforementioned tests were done by indirect fluorescent assay (IFA) using Ap IgG and Ap IgM IFA kits (Focus Diagnostics, Cypress, CA) according to manufacturer's instructions at CPL.

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RESULTS

Of 369 sera screened for Ap IgG, 86 (23.3%) tested positive and approximately one third (34.8%) of these Ap IgG seropositive subjects also tested positive for Ap IgM (Table 1). Of the 77 transplant donors, only 3 (3.8%) tested positive for Ap IgG, showing a statistically significant difference between the latter 2 groups (cohort 1 vs 7) (Table 2). This shows that any suspicion of exposure to ticks, even when it is not definitive, significantly increases the possibility of infection with Ap at an undetermined time. In our study, specimens with nonspecific or high background fluorescence on IFA were omitted to avoid potential false-positive results [11].

The sera with positive Ap IgG screen results were titrated to the end point: as the Ap IgG titers increased, Ap IgG positivity decreased, which may suggest that the majority of cases were due to past infections; however, low titers cannot reliably

differentiate between current and past infection because it has been reported in the literature that Ap IgG can last for more than 3 years [12, 13]. Further in support of the latter finding, we found that both Ap IgG titers as well as Ap IgM positivity increased (to 100%). These two findings suggest recent acute Ap infection most likely during the preceding 45–60 days because IgM can persist that long postinfection [2].

As also evident from Table 2, C6 ELISA positivity is significantly associated with Ap IgG positivity, which could be due to more objective evidence of exposure to the same tick that transmits both Lyme disease and HGA. At CPL, C6 ELISA is used for screening of sera with Lyme serology request, and when the result is equivocal or positive (antibody index ≥ 1.1), the serum is forwarded to NML for LIB confirmation following CDC criteria. According to our data (not shown), sera with a C6 ELISA antibody index of ≥ 4.0 have higher likelihood of positivity on LIB confirmation; therefore, that cutoff was used in our study for further stratification (low vs high C6 cohorts) (Table 2). The latter 2 cohorts had negative LIB results (both IgM and IgG). No significant difference for Ap IgG geometric mean titer ([GMT] with positive screen results) was found (using Mann-Whitney *U* test; $P = .07$) between the C6-negative cohort and low C6-positive cohort; however, the difference between C6-negative cohort versus other 3 cohorts (cohorts 4–6; Table 2) was statistically significant (Mann-Whitney *U* test; negative C6 cohort vs cohorts 4, 5, and 6 [$P = .0038$, $P < .0001$, and $P < .0001$, respectively]), suggesting that higher C6 ELISA antibody index values are predictive of Ap IgG positivity, whereas low C6 antibody index values are possibly due to non-specific reactivity. As Table 2 shows, the highest odds ratio (8.9; 95% confidence interval, 2.4–32.3) as well as Ap IgG GMT seen in cohort 5 suggest recent Lyme-HGA coinfection or sequential

Table 1. Titers of IgG and IgM Antibodies to Ap Obtained by IFA in Patients Who Had Previously Been Screened for Lyme Antibody

Ap IgG Titer	No. (%) of Patients (n = 369)	Ap IgM Screen Positive No. (%)
Positive (titer of ≥ 64)	86 (23.30)	NA*
64	38 (10.29)	4/38 (10.52)
128	18 (4.87)	4/18 (22.22)
256	7 (1.89)	2/7 (28.57)
512	7 (1.89)	5/7 (71.42)
1024	6 (1.62)	5/6 (83.33)
2048	10 (2.71)	10/10 (100)

Abbreviations: AP, *Anaplasma phagocytophilum*; IFA, indirect fluorescent assay; Ig, immunoglobulin; NA, not applicable.

*Ap IgM screen test was only performed on those sera with positive Ap IgG screen result.

Table 2. Titers of IgG and IgM Antibodies to Ap Obtained by IFA in Transplant Donors and Patients Who Had Previously Been Screened for Lyme Antibody Stratified by C6 Peptide ELISA and Lyme Immunoblot Results

Cohort	Cohort Description	Ap Screen IgG Positive No (%)	Ap IgG GMT	Ap Screen IgM Positive No (%)	Ap IgM GMT	Analysis Groups	OR (95% CI)	P Value ^a
1	Transplant Donors	3/77 (3.89)	NA ^b	0/3	0	1 vs 7	5.9 (1.8–19.4)	.0003
2	C6 Negative	29/182 (15.93)	83.24	1/29 (3.44)	80	2 vs 1	4.0 (1.2–13.8)	.017
3	Low C6 Positive	8/28 (28.57)	128	1/8 (12.5)	20	3 vs 1	7.3 (1.8–29.6)	.007
4	High C6 Positive	12/39 (30.76)	203.18	4/12 (56.56)	56.56	4 vs 1	7.8 (2.1–29.6)	.001
5	LIB IgM ⁺ /IgG ⁻	16/46 (34.78)	378.06	13/16 (46.93)	46.93	5 vs 1	8.9 (2.4–32.3)	.0003
6	LIB IgG ⁺	21/74 (28.37)	301.93	11/21 (52.38)	40	6 vs 1	7.2 (2.08–25.4)	.0005
7	Tick-Exposed (C6 positive and negative)	86/369 (23.30)	178.12	30/86 (34.88)	44.89	NA	NA	NA
8	C6 Positive	57/187 (30.48)	262.30	29/57 (50.87)	44.01	8 vs 2 ^c	NA	$P < .0001$

Abbreviations: AP, *Anaplasma phagocytophilum*; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titer; IFA, indirect fluorescent assay; Ig, immunoglobulin; LIB, Lyme immunoblot; NA, not applicable; OR, odds ratio.

^aAp IgM screening was only done for those sera with positive IgG screen results. GMTs were only calculated for those sera with positive screen results. The positivity cutoff for C6 peptide ELISA was 1.1, and the enhanced cutoff for low vs high was 4.

^bFisher's exact test (including OR with 95% CI) for Ap IgG result only. Results were considered statistically significant at $P < .05$.

^cGiven the small number of screen-positive sera in this cohort, GMT was not calculated to avoid bias. Three sera in this cohort had titers of 64 (2) and 128, with negative IgM results suggesting past exposures to Ap.

^dComparison of Ap IgG GMTs between cohorts 2 and 8 using Mann-Whitney *U* test showed statistically significant difference ($P < .0001$).

infection. Future studies using patients' medical records should pay particular attention to this cohort, with special attention paid to patients with laboratory abnormalities including leukopenia, thrombocytopenia, and elevated liver enzymes.

DISCUSSION

Consistent with the literature [7, 12], we showed that Ap IgG positivity as well as GMT both increased (although nonsignificantly, despite the visual trend) with age, which suggested the following: (1) more lifetime chance of exposure to Ap; (2) occupational exposure (median age of adults, 48 years; male/female ratio, 1.6:1), and (3) higher incidence of severe HGA in adults with higher age that subsequently brings them to medical attention. Figure 1 shows the potential usefulness of Ap IgM in diagnosis of acute Ap infection as sera with higher titers of Ap IgM were significantly more likely to have higher Ap IgG titers. In addition, sera with Ap IgM titers of 40 and 80 combined had Ap IgG GMT of 927 (higher than the single cutoff of ≥ 640 , suggesting acute Ap infection in endemic areas [2, 3, 12]). In further support of the latter, those sera with Ap IgG GMT >512 were all from adults with a median age of 56 years, which is very close to the median age reported for incident HGA in the literature, especially in those with severe disease and usually with underlying medical conditions [2, 3, 12, 14, 15]. Because the median age of confirmed Lyme disease diagnosis in adults in Manitoba is 54 (CPL Lyme testing data), the latter 2 findings suggest a higher probability of coinfection in this group of individuals; therefore, these patients may benefit from Ap screening when clinically and epidemiologically warranted. Furthermore, 94% of sera from the above group had positive Ap IgM with GMT of 60, and 81% of them were collected in the months of July, August, and September. The latter results make more sense when taking into account that Ap IgM can last 45–60 days postinfection and

that the peak of black-legged tick nymphs activity in Manitoba is in May and June.

Finally, the Ap IgG positivity did not reach statistical significance by comparing pediatric (<18 years of age) with adult patients (20% vs 23.7%, respectively), suggesting a bimodal epidemiology. The median age in the former was 15 years (interquartile range [IQR], 12.5–16), whereas the median age in the latter was 48 years (IQR, 34–60). Only 11% of the pediatric patients with positive Ap IgG result (GMT of 118) also had Ap IgM positive result; this suggests exposure to Ap in previous years because Ap IgG can last more than 3 years, and the median age of confirmed Lyme diagnosis among pediatric patients in Manitoba is 12 years (CPL Lyme testing data).

CONCLUSIONS

According to CPL Lyme testing data, the annual number of Ap IgG-positive cases in Manitoba, regardless of titer, is much less than what was found in this study, suggesting that HGA is probably underdiagnosed in this province; the latter conclusion was further strengthened by finding of sera with Ap IgG titers >512 , which suggests acute Ap infection. Reasonable public health approaches in this setting would include to enhance awareness among practitioners in this province regarding HGA, conduct similar studies in other Canadian jurisdictions with established black-legged tick populations, and finally make HGA nationally notifiable.

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References

- Centers for Disease Control and Prevention (CDC). Anaplasmosis: statistics and epidemiology. Available at: <http://www.cdc.gov/anaplasmosis/stats/>. Accessed 30 June 2016.
- Dumler JS, Walker DH. *Ehrlichia chaffeensis* (human monocytotropic ehrlichiosis), *Anaplasma phagocytophilum* (human granulocytotropic anaplasmosis), and other anaplasmataceae. In: Bennett JE, Dolin R, and Blaser MJ (eds). Principles and Practice of Infectious Diseases. Philadelphia, PA: Elsevier Saunders; 2015: pp 2227–33.
- Reller ME, Dumler JS. *Ehrlichia*, *Anaplasma*, and related intracellular bacteria. In: Jorgensen JH, Carroll KC, Tenover FC, et al (eds). Manual of Clinical Microbiology. Washington DC: ASM Press; 2015: pp 1135–49.
- Rikihisa Y. *Anaplasma phagocytophilum* and ehrlichia chaffeensis: subversive manipulators of host cells. Nat Rev Microbiol 2010; 8:328–39.
- Rikihisa Y. Mechanisms of obligatory intracellular infection with *Anaplasma phagocytophilum*. Clin Microbiol Rev 2011; 24:469–89.
- Katavolos P, Armstrong PM, Dawson JE, Telford SR 3rd. Duration of tick attachment required for transmission of granulocytic ehrlichiosis. J Infect Dis 1998; 177:1422–5.
- Bakken JS, Goellner P, Van Etten M, et al. Seroprevalence of human granulocytic ehrlichiosis among permanent residents of northwestern Wisconsin. Clin Infect Dis 1998; 27:1491–6.
- Dumler JS, Bakken JS. Human granulocytic ehrlichiosis in Wisconsin and Minnesota: a frequent infection with the potential for persistence. J Infect Dis 1996; 173:1027–30.
- Ijdo JW, Meek JJ, Cartter ML, et al. The emergence of another tickborne infection in the 12-town area around Lyme, Connecticut: human granulocytic ehrlichiosis. J Infect Dis 2000; 181:1388–93.

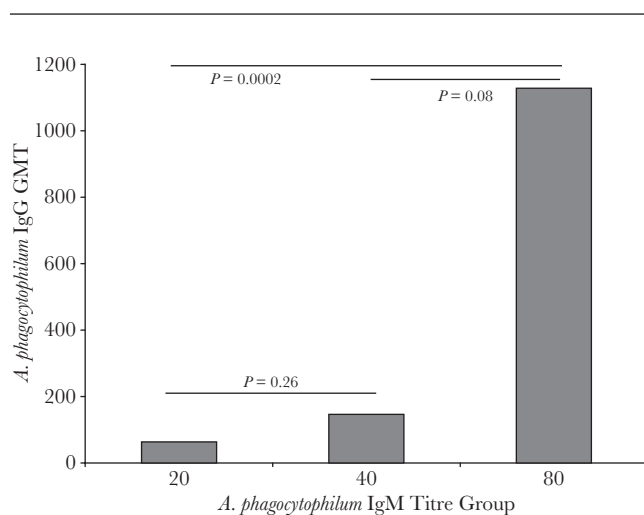


Figure 1. The relationship between *Anaplasma phagocytophilum* (Ap) immunoglobulin (IgM) titer groups and Ap IgG geometric mean titer (GMT). Results were considered statistically significant at $P < .05$ using Mann-Whitney U test.

10. Dibernardo A, Cote T, Ogden NH, Lindsay LR. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* ticks collected in Canada. *Parasit Vectors* **2014**; 7:183.
11. Wong SJ, Thomas JA. Cytoplasmic, nuclear, and platelet autoantibodies in human granulocytic ehrlichiosis patients. *J Clin Microbiol* **1998**; 36:1959–63.
12. Bakken JS, Dumler JS. Human granulocytic anaplasmosis. *Infect Dis Clin North Am* **2015**; 29:341–55.
13. Aguero-Rosenfeld ME, Kalantarpour F, Baluch M, et al. Serology of culture-confirmed cases of human granulocytic ehrlichiosis. *J Clin Microbiol* **2000**; 38:635–8.
14. Bakken JS, Krueh J, Wilson-Nordskog C, et al. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *JAMA* **1996**; 275:199–205.
15. Ramsey AH, Belongia EA, Gale CM, Davis JP. Outcomes of treated human granulocytic ehrlichiosis cases. *Emerg Infect Dis* **2002**; 8:398–401.