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Incidence of human granulocytic anaplasmosis in returning travellers with fever

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Abstract

Background: Although tick-borne pathogens have been reported as an important cause of imported fever, the incidence of *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis (HGA), in travellers is unknown.

Methods: We conducted a prospective cohort study to investigate the aetiologies of fever in returning travellers (November 2017—July 2019). Polymerase chain reaction for *msp2* gene amplification and indirect immunofluorescence assay for *A. phagocitophilum* were performed in all returning travellers with undifferentiated non-malarial fever.

Results: Among 141 travellers included, 8 patients were diagnosed with probable or confirmed HGA. The overall incidence rate of HGA was 19.9 cases/1000 person—week of travel. The main destination of travel was Asia, accounting for 62.5% patients with HGA. Co-infections were found in 37.5% of patients with HGA.

Conclusions: Diagnosis of HGA and empirical treatment with doxycycline should be considered in travellers with fever.

Key words: *Anaplasma phagocytophilum*, fever, travel, ticks, doxycycline, emerging infections, vector-borne diseases

Introduction

Coronavirus disease 2019 (COVID-19) pandemic has brought out the difficulties of facing the diagnosis and treatment of emerging infections, especially those presenting with imported fever.[1] Febrile illness is estimated to occur in about 2–3% of returning travellers and accounts for up to 25% of patients seeking medical care in travel clinics.[2,3] After ruling out common diseases such as malaria or arboviral infections, a large number of undifferentiated fever cases remain undiagnosed.[4] Recently, some studies performed in endemic areas showed that pathogens transmitted by ticks or mites such as *Orientia tsutsugamushi* and several species of *Rickettsia spp*. must be considered as causative agents in non-malarial febrile illnesses.[5]

Anaplasma phagocytophilum is an obligate, intracellular Gram-negative bacteria transmitted by hard ticks from the family Ixodidae. It is the aetiological agent of Human Granulocytic Anaplasmosis (HGA), usually presenting as an undifferentiated febrile illness, with headache, myalgias/arthralgia and

cytopenias.[6] HGA can range from an asymptomatic self-limiting infection to a multi-organ failure fatal disease, requiring hospitalization in one-half of cases.[6,7]

HGA has mainly been described in America and Europe but, like other rickettsial infections, it seems to be severely underreported.[6] Besides factors such as climate change, the increase of international travels has been suggested as an important cause of the emergence of tick-borne infections.[7–9] Although HGA has been occasionally reported in febrile travellers,[10] incidence of HGA among returning travellers is unknown.

Methods

We performed a prospective cohort study in patients presenting with fever within 28 days after an international trip at the Hospital Clinic of Barcelona (HCB), Spain, from November 2017 to July 2019. Inclusion criteria were temperature ≥37.5°C or feverish sensation accompanied by 2 of the 3 following symptoms: arthralgia, myalgia or chills. Patients younger than 18 years old and those requiring admission in a high-level isolation unit were excluded. In order to rule out malaria, a blood smear was systematically performed in all febrile travellers or migrants returning from malaria endemic areas. Patients presenting with ≥3 stools per day, prominent cough, expectoration or dyspnea, urinary tract infection symptoms or signs consistent with skin or soft tissue infections were considered as differentiated fevers and were not eligible to participate. By contrary, febrile patients, presenting with no clear source of infection in whom malaria was ruled out, were defined as undifferentiated non-malarial fever (UNMF) cases and were eligible to participate.

In addition to the microbiological tests performed routinely (Table 1), blood samples at first visit and 28 days (range: 21–72) later were obtained in study participants. Polymerase chain reaction (PCR) for *msp2* gene amplification in acute-phase whole blood or plasma samples and indirect immunofluorescence assay (IFA) for *A. phagocitophylum* (Focus Diagnostics, Cypress, CA, USA) in paired sera were performed.[6,11–13] In patients diagnosed with HGA (either by IFA or PCR), PCRs and serologies for spotted fever group *Rickettsia* spp., typhus group *Ricketssia* spp., *O. tsutsugamushi*, *Coxiella burnetii*, *Bartonella quintana*, *Bartonella henselae* and serology for *Borrelia burgdorferi* were systematically performed in order to rule out possible cross-reactions and/or co-infections. Based on microbiological and clinical criteria, according to guidelines,[6,12] and after expert discussion, patients were classified as confirmed HGA, probable HGA or cases without active HGA (Table 1). Cases with stable titres of *A. phagocytophilum* antibodies in acute and convalescent sera and titres > 4-fold above the cut-off value were classified as probable cases when they presented with other confirmed infections. Possible co-infections were classified according to the scheme proposed by Phommarone *et al.*[14]

Overall incidence of HGA was presented as an incidence rate (IR) of confirmed and probable cases. Period at risk for HGA was considered as the time from the beginning of the trip until the onset of fever in participants diagnosed with HGA presenting with fever during the trip and as the overall duration of travel in the others. For the IR calculation, long trips (≥6 months), expatriates and long term residents were excluded from the analysis.

Categorical variables are presented in percentages and quantitative variables are presented as median and interquartile ranges (IQR). Fisher's exact test was used to compare categorical variables between groups, while Mann–Whitney U test was used for quantitative variables. The statistical analysis was carried out using Stata 15 (StataCorp.2017).

The study was conducted following the principles of the Declaration of Helsinki. The ethical committee of the HCB approved the study (HCB/2017/0612). Informed written consent was given by all participants.

Results

Incidence of HGA among febrile returning travellers

During the study period, 141 patients with available paired sera out of a total of 179 patients with UNMF were included in the study. Of them, a total of 8 patients were diagnosed with probable or confirmed HGA, showing an overall IR of HGA of 19.9 cases/1000 person—week of travel (95%CI: 9.6–41.5). Figure 1 illustrates the microbiological results of participants with HGA.

Two participants fulfilled the criteria for confirmed HGA. One had a seroconversion with no alternative diagnosis. The other confirmed case was diagnosed both by seroconversion and amplification of *msp2* gene, with subsequent *A. phagocitophilum* sequencing. Based on confirmed cases, minimum IR of HGA is estimated to be 5.7 cases/1000 person—week of travel (95%CI: 1.4–22.6).

Six participants fulfilled the criteria for probable HGA. Two of them presented seroconversion for *A. phagocitophylum* and an alternative diagnosis. The other 4 participants presented with high positive IFA titers (≥64) in acute and convalescent sera or <4-fold elevation of IFA titers for *A. phagocitophylum*. Then, IR of probable HGA was estimated to be 14.2 cases/1000 person with UNMF—week of travel (95%CI: 6.0–34.0). Data on incidence of probable and confirmed HGA are summarized in Table 1.

Among the 141 patients with UNMF included, positive IFA for *A. phagocytophilum* was detected in an additional 9 participants (17 patients in total), showing a global seroprevalence of *A. phagocytophilum* IgG antibodies of 12.1% (95%CI: 7.2–18.6).

Epidemiological and clinical characteristics of travellers diagnosed with HGA

Table 2 describes the epidemiological and clinical features of the 8 travellers with HGA. The main destination of travel was Asia, accounting for 5 cases (62.5%). Indeed, the two confirmed cases of HGA came from Thailand and Malaysia. All of them visited rural areas. Three (37.5%) patients presented a clear risk of tick exposure: two of them had contact with animals and only one referred a tick bite, but none of them presented with an eschar on physical exam. The maximal incubation period (measured from the last day of the trip) was 6 days. All patients presented fever within the first 24 hours of symptoms' onset. Other common symptoms were arthralgia, myalgia, malaise, headache and rash. Common laboratory abnormalities were cytopenias (75.0%) and

Table 1. Incidence and definition criteria of confirmed and probable HGA

	Confirmed HGA	Probable HGA[a]
Case definition	Compatible clinical presentation AND: (i) positive PCR assay and *A. phagocytophilum* sequencing OR (ii) seroconversion or ≥ 4-fold increase of IFA titers with no alternative confirmed diagnosis.[b]	Compatible clinical presentation AND: (i) seroconversion or ≥ 4-fold increase of IFA titers with an alternative confirmed diagnosis[a] OR (ii) high positive IFA titers (≥64) in acute and convalescent sera or < 4-fold elevation of IFA titers with no alternative diagnosis.[b]
IR[c]	5.7 cases/1000 person with UNMF—week of travel	14.2 cases/1000 person with UNMF—week of travel
95% CI	1.4–22.6 cases/1000 person with UNMF—week of travel	6.0–34.0 cases/1000 person with UNMF—week of travel

CI: confidence interval. IFA: indirect immunofluorescence assay.
[a] *Definition criteria for confirmed and probable HGA adapted from international guidelines.*[6]
[b] *PCRs and serologies against dengue virus, chikungunya virus and Zika virus were routinely performed in all patients presenting with fever ≤ 14 days after the trip. PCRs and serologies for spotted fever group Rickettsia spp., typhus group Ricketssia spp., O. tsutsugamushi, C. burnetii, B. quintana, B. henselae and serology for B. burgdorferi were systematically performed in patients diagnosed with HGA. Tests looking for other infections such as leptospirosis or HIV were performed based on clinical suspicion and risk exposures.*
[c] *Long trips (≥6 months), expatriates and long-term residents excluded.*

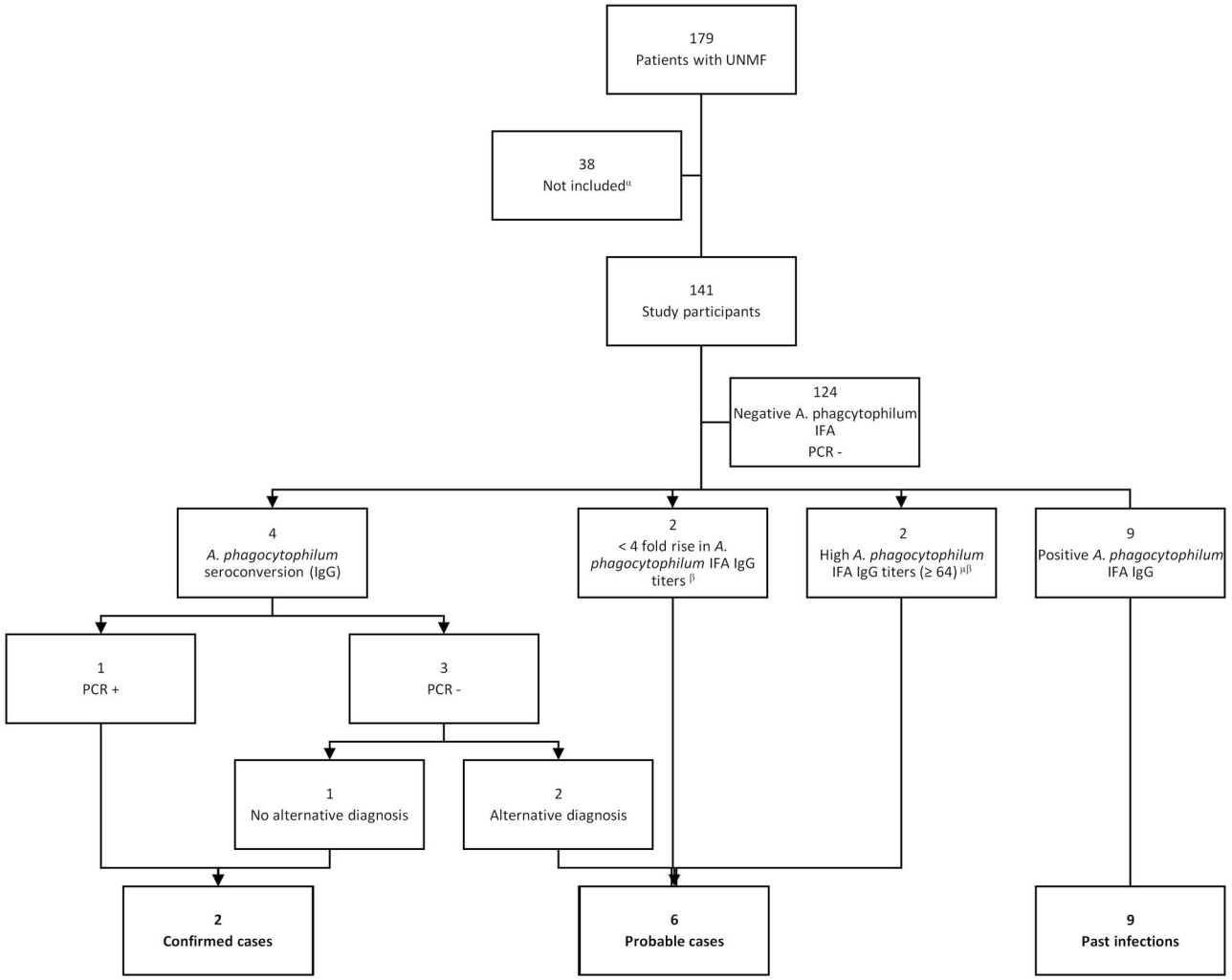

Figure 1. Flowchart of study participants with HGA.
IFA: indirect immunofluorescence assay. IgG: immunoglobulin G. [α] 38 patients with no convalescent serum sample were excluded from the study [β] Without an alternative diagnosis [μ] In acute and convalescent sera. [π] Not fulfilling criteria of previous categories.

mild elevation of C-reactive protein (62.5%). The aforementioned clinical and demographic characteristics did not differ between travellers with HGA and those without HGA (Table 2).

Although only 2 (25.0%) patients received an adequate antibiotic treatment (doxycycline 100 mg bid for 7–14 days), fever disappeared in less than 5 days in 6 patients (75.0%).

Table 2. Comparison of epidemiological and clinical characteristics of travellers with and without HGA

	Confirmed HGA ($n=2$)	Probable HGA ($n=6$)	Overall HGA ($n=8$)	Non-HGA undifferentiated fevers ($n=133$)	*P*-value[a]
Male sex, *n*/*N* (%)	1/2 (50.0)	3/6 (50.0)	4/8 (50.0)	71/133 (53.4)	>0.999[b]
Age (years), Md (IQR)	40.5 (39–42)[c]	26 (26–53)	32.5 (26–47.5)	34 (28–43)	0.954[d]
Travel region, *n*/*N* (%)					0.938[b]
• Asia/Southeast Asia[e]	2/2 (100)	3/6 (50.0)	5/8 (62.5)	61/132 (46.2)	
• Africa[f]	0/2	2/6 (33.3)	2/8 (25.0)	33/132 (25.0)	
• America[g]	0/2	1/6 (16.7)	1/8 (12.5)	29/132 (22.0)	
• Europe	0/2	0/6	0/8	9/132 (6.8)	
Duration of travel (days), Md (IQR)	16.5 (16–17)	15.5 (13–16)	16 (14–16.5)	17 (12–26)	0.688[d]
Risk factors, *n*/*N* (%)					
• Rural areas	2/2 (100)	6/6 (100)	8/8 (100)	118/133 (88.7)	0.600[b]
• Animal contact	1/2 (50.0)	1/6 (16.7)	2/8 (25.0)	58/133 (43.6)	0.467[b]
• Tick bite	0/2	1/6 (16.7)	1/8 (12.5)	18/133 (13.5)	>0.999[b]
Days of fever, Md (IQR)	3.5 (3–4)[c]	6 (2–6)	4 (2–6)	5 (3–7)	0.864[d]
Symptoms and signs, *n*/*N* (%)					
• Fever	2/2 (100)	6/6 (100)	8/8 (100)	133/133 (100)	-
• Malaise/fatigue	1/2 (50.0)	5/6 (83.3)	6/8 (75.0)	105/133 (78.9)	0.678[b]
• Headache	1/2 (50.0)	5/6 (83.3)	6/8 (75.0)	99/133 (74.4)	>0.999[b]
• Arthralgia/myalgia	2/2 (100)	3/6 (50.0)	5/8 (62.5)	97/133 (72.9)	0.685[b]
• Rash	2/2 (100)	2/6 (33.3)	4/8 (50.0)	54/133 (40.6)	0.717[b]
• Eschar	0/2	0/6	0/9	11/133 (8.3)	>0.999[b]
Blood tests, *n*/*N* (%)					
• Any cytopenia	1/2 (50.0)	5/6 (83.3)	6/8 (75.0)	82/131 (62.6)	0.710[b]
• Leukopenia	1/2 (50.0)	3/6 (50.0)	3/8 (37.5)	40/131 (30.5)	0.703[b]
• Neutropenia	1/2 (50.0)	3/6 (50.0)	4/8 (50.0)	49/131 (37.4)	0.480[b]
• Lymphopenia	1/2 (50.0)	4/6 (66.7)	5/8 (62.5)	55/131 (42.0)	0.255[b]
• Thrombocytopenia	1/2 (50.0)	0/6	1/8 (12.5)	18/130 (13.9)	> 0.999[b]
• Anemia	0/2	1/6 (16.7)	1/8 (12.5)	11/129 (8.53)	0.529[b]
• Elevated CRP	1/2 (50.0)	4/6 (66.7)	5/8 (62.5)	81/133 (60.9)	>0.999[b]
• Elevated transaminases	1/2 (50.0)	2/6 (33.3)	3/8 (37.5)	68/133 (51.1)	0.493[b]
Hospital admission, *n*/*N* (%)	0/2	1/6 (16.7)	1/8 (12.5)	26/133 (19.5)	>0.999[b]
Cure at 30 days, *n*/*N* (%)	2/2 (100)	6/6 (100)	8/8 (100)	101/133 (75.9)	0.198[b]

CPR: C-reactive protein. Md: Median. n: number of cases. *N*: total number of patients diagnosed with HGA.
[a]Comparison between overall HGA and non-HGA undifferentiated fever groups.
[b]Fisher's exact test.
[c]Range.
[d]Wilcoxon rank-sum test.
[e]Countries visited in Asia included Thailand, Vietnam, Singapore, Malaysia, Indonesia and India.
[f]Countries visited in Africa included Madagascar and Angola.
[g]The visited country in the America was Costa Rica.

Only one patient required hospital admission. One month after inclusion, all patients but one were completely asymptomatic. The only patient that was still symptomatic was diagnosed with myelodysplastic syndrome; symptoms after 1 month were not considered any more as related to *A. phagocytophilum* infection.

Co-infections

Three out of 8 HGA participants (37.5%) had confirmed, probable or possible co-infections with Chikungunya, Dengue and *C. burnetii*. Supplementary Table 1 shows detailed microbiological information of HGA co-infections.

Discussion

The main finding of our study is an overall IR of HGA of 19.9 cases/1000 person with UNMF—week of travel, that means that patients presenting with fever after an international travel have an increased risk of HGA of almost 2% for every week of travel. Although there is a scarcity on the available published information about the incidence of HGA in travellers, our study shows that the incidence of HGA among returning travellers with fever is not negligible. In addition, the incidence of vector-borne infections is expected to increase due to recent lockdowns and breakdowns in control strategies in endemic areas due to COVID-19 pandemic.[15] As with some other neglected diseases, the non-specific presentation of HGA, the low awareness of this infection by physicians and the lack of access to diagnostic tools in several centers can explain why HGA is currently underreported.[6–8,16] Moreover, since two patients had stable antibody levels against *A. phagocytophilum*, we cannot completely rule out that these patients acquired the infection in Spain before travelling. This is not the case for the remaining patients. Time since the last day of the trip to the onset of fever was below 5 days

in all but one case, and time since the beginning of the trip to the onset of fever was consistent with the usual median HGA incubation period of 11 days (range 5–21).[6] The only patient who presented with fever 6 days after the last day of the trip denied any exposure or risk factor for HGA since the arrival, making very unlikely the acquisition of HGA in Spain.

Most cases of HGA have been previously reported in North America and Europe.[7,8] Nevertheless, in our study, *A. phagocytophilum* infection was commonly found in travellers from Asia, where *A. phagocytophilum* is increasingly been reported.[7,17–21] Moreover, it has been communicated at least once in Africa[22] and it has been found in ticks in South America.[23,24] Despite the relatively small sample size and the unicenter nature of the study, data showed in this study add further evidence that distribution of HGA is widespread.

In line with previous reports, possible co-infections were detected in more than one third of cases in our cohort.[19–21] Physicians must be aware of possible co-infections not only with tick-borne microorganisms but also with mosquito-transmitted arboviruses.

Although most cases presented a self-limited evolution, effective antibiotic treatment against intracellular bacteria such as doxycycline should be considered in travellers with undifferentiated fever presenting with severe disease, after excluding malaria and, especially, if they had visited rural areas and/or presented with cytopenias.[5,20,21,24]

In conclusion, HGA should be included in the differential diagnosis of returning travellers with fever and no clear source of infection. Antibiotic treatment such as doxycycline should be considered in travellers with undifferentiated fever presenting with severe disease, after discarding malaria and, especially, if they had visited rural areas and/or presented with cytopenias. Physicians must be aware of possible co-infections with other tick-borne microorganisms and mosquito-transmitted arboviruses.

Supplementary data

Supplementary data are available at *JTM* online.

Conflict of interests

The authors declare no conflict of interests.

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