The prevalence of Anaplasma phagocytophilum in host-seeking Ixodes ricinus ticks in Norway

O. Rosef [1] , J. Radzijevskaja [2] , A. Paulauskas [2] and C. Haslekås [1]

[1] Telemark University College, Bø i Telemark, Norway and [2] Vytautas Magnus University, Kaunas, Lithuania
Corresponding author and reprint requests: O. Rosef, Department of Environmental and Health studies, Telemark University College, Hallvard Eikas Plass, 3800 Bø, Norway. E-mail: olav.rosef@hit.no

Introduction

In recent years, cases of human granulocytic anaplasmosis (HGA), as well as presence of A. phagocytophilum in ticks and various vertebrate hosts, have been reported throughout Europe. In Norway, tick-borne fever caused by A. phagocytophilum has been recorded in moose, red deer, roe deer and sheep [1], as well as in humans [2]. The prevalence of A. phagocytophilum in I. ricinus ticks is important for risk assessment of HGA. In this study, we present the prevalence of A. phagocytophilum in host-seeking I. ricinus ticks collected during a 2-year period from 18 locations situated from northwest to southeast Norway.

Material and Methods

Sample collection

A total of 1634 Ixodes ricinus ticks representing adult females, males and nymphs from 18 sites were collected from wooded habitats by the standard flagging method during spring and summer 2006 and 2007. The ticks were picked from the towel and placed into 1.5 mL tubes filled with 70% ethanol and stored until DNA extraction.

DNA extraction

For DNA extraction, a modified procedure following that of Stańczak et al. [3] was used. DNA was isolated individually from each tick and the lysates were stored at −20°C until use.

Analyses

For I. ricinus identification, the lysates were analysed with species-specific primers (IxriF 5`GGA AAT CCC GTC GCA CG 3` and IxriR 5`CAA ACG CGC CAA CGA AC3`) resulting in a 150bp segment of the 5.8S rRNA gene [4]. We used primer sequences specific for the A. phagocytophilum msp2 gene identified as ApMSP2r 5'- ATGGAAGGTA GTGTTGGTTATGGTATT and ApMSP2r 5'-TTGGTCTTGAAGCGCTCGTA to generate a 77-bp fragment by using TaqMan probe ApMSP2p-FAM 5'- TGGTGCCAGGGTTGAGCTTGAGATTG as described by Courtney et al. [5]. Real-time PCR was performed by using TaqMan Master Mix in a quantitative thermal cycler (iCycler™, Bio-Rad Laboratories, Inc. Hercules, California, USA). Positive and negative controls were used in all runs.

Parameter values were analysed statistically by means of Pearson's χ^2 test by using the statistical package STATISTICA for WINDOWS 5.5.

Results and Discussion

Out of the 1634 ticks analysed, 93 harboured A. phagocytophilum (5.7%). The prevalence varied between the sites from zero, to 1.4%, to 19.4% (Table 1). The highest prevalence of infection was detected on the Hitra and Jomfruland islands, situated in northwestern and southern Norway, respectively. The overall infection rate in 2006 and 2007 was 4.0% and 6.5%, respectively ($\chi^2 = 3.53$, $p = 0.06$). The prevalence of A. phagocytophilum infection in I. ricinus in Europe varies in different areas and between development stages of the tick [1]. We found that adult ticks collected in 2007 were more infected than nymphs (7.7% and 3.5%) ($\chi^2 = 5.97$, $p = 0.01$) in contrast to 2006, where 2.1% adults and 2.7% nymphs where infected ($\chi^2 = 2.39$, $p = 0.12$).

Table 1. The prevalence of A. phagocytophilum in questing I. ricinus ticks

			Female			**Male**			**Nymph**			**Total**		
				Prevalence			**Prevalence**			**Prevalence**			**Prevalence**	
No.	**Locations***	**Years**	**N**	**n**	**%**	**N**	**n**	**%**	**N**	**n**	**%**	**N**	**n**	**%**
1.	Hitra, Fjelløyvær N7059209E504490	2007	23	4	17.4	24	3	12.5	30	2	16.7	77	12	15.6
		2006	2	1	50	6	0	0	56	2	3.6	64	3	4.7
2	Hitra, Strøm N7048360E498426	2007	40	9	22.5	35	9	25.7	33	3	9.1	108	21	19.4
		2006	5	1	20	3	1	33.3	89	8	8.9	97	10	10.3
3.	Surnadal N6987483E476360	2007	30	1	3.3	22	1	4.5	21	0	0	73	2	2.7
4.	Stranda N6908365E394344	2007	13	0	0	16	1	6.25	21	0	0	50	1	2

5.	Hellesylt N6888479E390922	2007	5	0	0	3	0	0	1	0	0	9	0	0
6.	Utvik N6856929E371115	2007	22	0	0	26	1	3.8	22	0	0	70	1	1.4
7.	Hermansverk N6783930E385519	2007	33	0	0	21	0	0	11	0	0	65	0	0
8.	Mundheim N6673817E329790	2007	8	1	12.5	5	1	20	17	0	0	30	2	6.7
9.	Etne N6614567E325293	2007	11	1	9.1	15	1	6.7	21	2	9.5	47	4	8.5
10.	Lista N6442343E377447	2007	43	2	4.7	32	1	3.1	54	0	0	129	3	2.3
11.	Søgne N6441736E428101	2007	6	0	0	5	2	40	14	2	14.3	25	4	16
12.	Odderøya N6440362E447818	2007	31	0	0	20	1	2.2	21	0	0	72	1	1.4
13.	Tjore N6463382E473032	2007	19	0	0	22	0	0	21	0	0	63	0	0
		2006	4	0	0	4	0	0	16	0	0	24	0	0
14.	Tromøy N6479560E489828	2007	13	1	7.7	19	2	10.5	5	0	0	37	3	8.1
		2006	24	0	0	11	0	0	6	0	0	41	0	0
15.	Hinnebu N6493848E469418	2007	27	0	0	18	0	0	5	0	0	50	0	0
		2006	42	0	0	32	0	0	32	0	0	106	0	0
16.	Tvedestrand N6499879E503053	2007	23	1	4.3	20	1	5	27	1	3.7	70	3	4.3
		2006	10	0	0	17	0	0	19	0	0	46	0	0
17.	Risør N6510507E510485	2006	19	0	0	18	0	0	22	0	0	59	0	0
18.	Jomfruland N6524446E533677	2007	50	8	16	32	3	9.4	49	4	8.2	131	15	11.5
		2006	8	1	12.5	8	1	12.5	75	6	8	91	8	8.7
Total												1634	93	5.7
in 2006												528	21	4.0
in 2007												1106	72	6.5

N, number of tested ticks; n, number of infected ticks.

*The coordinates of the sites are given in UTM 32 (Euref 89).

In Hitra (Fjelløyvær island) the prevalence of A. phagocytophilum increased significantly from 2006 (4.7%) to 2007 (15.6%) ($\chi^2 = 4.37$, $p = 0.03$). In other sites differences in prevalence of infection were observed but were not statistically significant: in Hitra (Strøm) infection rate increased from 10.3% to 19.4% ($\chi^2 = 3.32$, $p = 0.06$); in Jomfruland from 8.7% to 11.5% ($\chi^2 = 0.41$, $p = 0.52$); and from zero to 8.1% at Tromøy ($\chi^2 = 3.46$, $p = 0.063$) and to 4.3% at Tvedestrand ($\chi^2 = 2.02$, $p = 0.15$).

The life cycle of A. phagocytophilum in Europe has involved I. ricinus ticks and wild animals as natural reservoirs [1]. Transovarial transmission of A. phagocytophilum in ticks appears to be inefficient, mammals are considered to play a major role in the maintenance and distribution of these bacteria in nature. Recent studies indicate that migrating birds may be important in the

dispersal of A. phagocytophilum-infected I. ricinus in Europe [1], and the role of migratory birds as reservoirs has been hypothesised. In the present study, the sites with the highest prevalence of infection are situated in the coastal areas where many migratory and sea birds cluster, which could play a role in the transmission of infection. In Jomfruland, larvae and nymphs collected on passerine birds in 2006 and 2007 were infected with A. phagocytophilum (Paulauskas et al., in press).

Small rodents are known to be the main host for the immature stages of Ixodes ricinus ticks, while red deer and roe deer play an important role for the mature tick stage. We found the highest prevalence of A. phagocytophilum in Hitra, which has a very high density of roe deer and red deer. They may play an important role in the infection route of A. phagocytophilum.

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