Prevalence of Anaplasma phagocytophilum and Babesia divergens in Ixodes ricinus ticks from Lithuania and Norway

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Abstract

We detected Anaplasma phagocytophilum and Babesia divergens in Ixodes ricinus ticks collected from different locations in Lithuania and Norway by using the Taq Man based real-time PCR method. The msp2 gene of A. phagocytophilum and the 18S rRNA gene of B. divergens have been chosen as amplification targets. The overall infection rate of A. phagocytophilum in Norwegian ticks was 4.5% (10/224) and in Lithuanian ticks 3% (4/140). The prevalence varied in locations between 0% and 9% in Lithuania and in Norway. Three out of 140 (2%) ticks were infected with B. divergens in Lithuania and two out of 224 (0.9%) in Norway. The prevalence of B. divergens infection varied from 0% to 3% and from 0% to 4% in different sites in Lithuania and Norway, respectively.

Keywords: Anaplasma phagocytophilum; Babesia divergens; Ticks; Real-time PCR

Introduction

During the last decades, the distribution and abundance of Ixodes ricinus ticks has increased in northern Europe due to higher temperatures, climatic changes, and human impact on the environment (Lindgren et al., 2000; Gray, 2002). This trend could lead to an increased incidence of tick-transmitted diseases as well as increased risk areas. In recent years, cases of human granulocytic anaplasmosis (HGA) and babesiosis as well as the presence of Anaplasma phagocytophilum and Babesia divergens in ticks and various vertebrate hosts was reported throughout Europe (Parola, 2004; Gray, 2006). Human cases of HGA in Norway were reported by Bjöersdorff et al. (1999). Tick-borne fever caused by A. phagocytophilum was recorded in moose, red deer, roe deer, and sheep ([Stuen et al., 2003] and [Stuen et al., 2006]). Jenkins et al. (2001) were the first to report Anaplasma spp. in I. ricinus ticks collected from two sites in southern Norway. A preliminary study in Lithuania (Ambrasiene et al., 2004) showed that I. ricinus, mainly known as a vector of tick-borne encephalitis and Lyme borreliosis in this country, also harbored Anaplasma spp and B. divergens. In Lithuania, HGA and babesiosis have not been diagnosed in humans so far.

Investigations of the prevalence of A. phagocytophilum and B. divergens in vector ticks are needed to assess the risk of infection in the human population and for a better understanding of the circulation of these pathogens. The purpose of the present study was to investigate the prevalence of A. phagocytophilum and B. divergens in I. ricinus ticks collected from different locations in Lithuania and Norway using the real-time PCR method.

Material and methods

During spring and summer of 2006, 364 questing I. ricinus ticks were collected in wooded habitats using the standard flagging method by drawing 1 m2 of cotton cloth over the vegetation in 3 locations in Lithuania and in 4 locations in the coastal area of southern Norway (Table 1). Ticks attached to the towel were picked with tweezers and placed into 1.5 ml tubes filled with 70% ethanol. A modified procedure with the ammonium hydroxide solution (2.5%) (Stańczak et al., 1999) was used for DNA extraction. The lysates were stored at −20 °C until use.

Table 1: The prevalence of Anaplasma phagocytophilum and Babesia divergens in Ixodes ricinus ticks in Lithuania and Norway

Collection site	**Number of infected ticks (% infected)/number collected**							
	A. phagocytophilum				**B. divergens**			
	Female	**Male**	**Nymphs**	**Total**	**Female**	**Male**	**Nymphs**	**Total**
Lithuania								
Kaunas 54°87′N, 23°90′E	2 (9)/22	1 (3)/36	0 (0)/12	3 (4)/70	2 (9)/22	0 (0)/36	0 (0)/12	2 (3)/71
Dusetos 55°75′N, 25°87′E	1 (9)/11	-	-	1 (9)/11	0 (0)/11	-	-	0 (0)/11
Kintai 55°42′N, 21°26′E	0 (0)/36	0 (0)/23	-	0 (0)/59	0 (0)/36	1 (4)/23	-	1 (2)/59
Total	3 (4)/69	1 (2)/59	0 (0)/12	4 (3)/140	2 (3)/69	1 (2)/59	0 (0)/12	3 (2)/140
Norway								
Jomfruland 58°52′N, 09°36′E	1 (13)/8	1 (13)/8	6 (8)/75	8 (9)/91	0 (0)/8	0 (0)/8	0 (0)/75	0 (0)/91
Løvøya 59°3′N, 9°43′E	1 (4)/23	1 (4)/23	0 (0)/14	2 (3)/60	0 (0)/23	0 (0)/23	0 (0)/14	0 (0)/60
Hvasser 59°4′N, 10°26′E	0 (0)/8	0 (0)/4	0 (0)/11	0 (0)/23	1 (12.5)/8	0 (0)/4	0 (0)/11	1 (4)/23
Mølen 59°02′N, 10°15′E	0 (0)/18	0 (0)/25	0 (0)/7	0 (0)/50	1 (5.6)/18	0 (0)/25	0 (0)/7	1 (2)/50
Total	2 (4)/57	2 (3)/60	6 (6)/107	10 (4.5)/224	2 (4)/57	0 (0)/60	0 (0)/107	2 (0.9)/224

Detection of Anaplasma phagocytophilum

The species-specific primers ApMSP2f, ApMSP2r, and TaqMan probe ApMSP2p-FAM, as described by Courtney et al. (2004), were used to amplify a 77-bp fragment in the msp2 gene of A. phagocytophilum. PCR was performed by using TaqMan Master Mix in a quantitative thermal cycler (Bio-Rad iCycler). Negative and positive controls were included in all runs.

Detection of Babesia divergens

For detection, the primers BdiF (CAG CTT GAC GGT AGG GTA TTG G), BdiR (TCG AAC CCT AAT TCC CCG TTA), and TaqMan probe BdiT (6-FAM-CGAGGCAGCAACGG-MGB) were used to amplify a 62-bp fragment in the 18S rRNA gene of B. divergens. PCR was performed in a reaction volume of 30 µl by using TaqMan Master Mix. The PCR conditions were initial denaturation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles with a 15-s denaturation at 95 °C followed by a 1-min annealing–extension step at 60 °C. Finial extension was at 72 °C for 2 min. Negative and positive controls were included in all runs.

Results and discussion

The overall A. phagocytophilum infection rates of I. ricinus ticks were 3% (4 out of 140) in Lithuania and 4.5% (10 out of 224) in Norway. The infection rates varied in different locations from 0% to 9% in Lithuania and in Norway (Table 1).

The prevalence of A. phagocytophilum in European I. ricinus tick populations varies. Our study indicated a low overall prevalence of infection in ticks. In a previous study in Norway, Jenkins et al. (2001) found 33 out of 341 (9.7%) ticks screened by PCR with primers Ehr521–Ehr747 positive for Anaplasma spp (formerly Ehrlichia spp.). We, however, found a lower level of A. phagocytophilum. A reason for this may be our use of species-specific primers.

Similar infection rates as in our study were noted in north-western and west-central Poland with 4.5% and 4.1%, respectively ([Skotarczak et al., 2003] and [Skotarczak et al., 2006]). In studies conducted in mid-eastern, northern, and north-eastern Poland, the observed prevalences of A. phagocytophilum in I. ricinus were higher with 13.1%, 16%, and 19.2%, respectively (Grzeszczuk et al., 2002; Stańczak et al., 2002; Tomasiewicz et al., 2004). In a study in Switzerland, the prevalence of infection was found to be 0.8% (Pusterla et al., 1998), in eastern Slovakia 13.3% (Derdakova et al., 2003), and in Bulgaria, it was quite high with 34% (Christova et al., 2001).

The prevalence of A. phagocytophilum infection varies between different life stages of I. ricinus ticks. In the present study, we detected A. phagocytophilum in Lithuania only in adult ticks (4 of 128). Females were more frequently infected than males. In Norway, both the nymphal and the adult stage were infected, and a higher prevalence was found in nymphs (6%) than in adults (3%). The infection rates in females and males were almost the same (Table 1). The investigated numbers of ticks, however, were too small to draw clear conclusions. Swedish and Italian studies showed a higher infection rate in nymphs than in adult ticks (von Stedingk et al., 1997; Cinco et al., 1997). In Bulgaria, a much higher infection rate was observed in adult ticks than in nymphs with 34% versus 2%, respectively, but no difference was observed between males and females (Christova et al., 2001). In Poland, a much higher prevalence was found in females (21.3% in northern and 45.7% in mid-eastern Poland) than in males (6.5% in northern and 4.5% in mid-eastern Poland) but a lower value was observed in nymphs (0.94% in the mid-eastern part) (Stańczak et al., 2002; Tomasiewicz et al., 2004). It is difficult to explain the differences between the infection rates. Explanations may be the differences in the use of species-specific primers for A. phagocytophilum and variations in habitats and microclimate.

The transmission cycle of A. phagocytophilum is not fully understood. In Europe, I. ricinus is the recognized vector of HGA, but the dynamics of transmission to mammals have not been completely elucidated (Parola et al., 2005). In Europe, in addition to large animals (horses, cattle, sheep, goats, dogs, cats) small rodents like Apodemus flavicollis, Ap. sylvaticus, and Myodes glareolus (formerly

Clethrionomys glareolus) have been shown to harbor A. phagocytophilum and suggested these rodents as potential reservoirs (Liz et al., 2000; Bown et al., 2003; Halinska et al., 2004).

Three out of 140 (2%) ticks from Lithuania and two out of 224 (0.9%) ticks from Norway were infected with B. divergens (Table 1). The prevalence of B. divergens varied in different locations: from 0% to 4% in Norway and from 0% to 3% in Lithuania. In both countries, B. divergens was detected exclusively in adult ticks. In Norway, only females (4%) harbored B. divergens. A similar rate of infected ticks was found in Poland where the overall prevalence was 3.0%. Females had a higher infection rate (6.7%) than males (4.5%), while larvae and nymphs were not infected (Skotarczak and Cichocka, 2001).

The results of present study confirm the presence of A. phagocytophilum and B. divergens in natural populations of I. ricinus in Lithuania and Norway and the possible risk of transmitting these infections to humans.

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