Prevalence of Anaplasma phagocytophilum in small rodents in France

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Abstract

Anaplasma phagocytophilum is an emerging zoonotic tick-borne pathogen affecting a wide range of mammals. Rodents are suspected to be natural reservoirs for this bacterium, but their role in the epidemiologic cycles affecting domestic animals and wild ungulates has not been demonstrated. This study aimed to improve our knowledge on *A. phagocytophilum* prevalence in *Apodemus sylvaticus*, *A. flavicollis* and *Myodes glareolus* using data collected in 2010 in one area in eastern France and in 2012-2013 in two others areas in western France. Rodents were captured in each site and infection was tested using qualitative real-time PCR assays on either blood or spleen samples. Prevalence showed high variability among sites. The highest prevalence was observed in the most eastern site (with an average infection rate of 22.8% across all species), whereas no rodent was found to be PCR positive in the south-west site and only 6.6% were positive in the north-west of France. Finally, a significant increase in prevalence was observed in autumn samples compared to spring samples in the north-west, but no change was found in the other two sites.

Keywords

Tick-borne disease; *Anaplasma*; rodent; prevalence; France

Introduction

Anaplasma phagocytophilum is an emerging tick-borne disease affecting a wide range of mammals including humans (Stuen, 2007). In Europe, *A. phagocytophilum* is one of the most important tick-borne bacteria for domestic animals in terms of economic losses (Stuen, 2007), however the factors driving the epidemiologic cycles of this pathogen are largely unknown.

Prevalence surveys are the initial tools required to determine which mammalian species are involved in the spread of the infectious agent. In Europe, roe deer (*Capreolus capreolus*) and rodents are suspected to act as natural reservoirs for *A. phagocytophilum* (Stuen, 2007). Both groups present high tick burdens and infection prevalence in roe deer can be substantial (up to 90% positive by PCR (Overzier et al., 2013)). The role of small rodents is not fully understood. Prevalence in rodent populations is sparsely documented in Europe and very variable among species and localities (<1% to 19%) (Stuen et al., 2013). In Germany and the Czech Republic, the prevalence of infection in *Myodes glareolus* was similar (around 13%), whereas, it differed strongly in *Apodemus flavicollis*, 0.5% and 15 %, respectively, in the two countries (Hulinska et al., 2004; Hartelt et al., 2008). In France, the prevalence of *A. phagocytophilum* in rodents is poorly documented as only one study has been conducted to date, revealing 2/18 positive *A. sylvaticus* from a single location in the north-west of France (Marumoto et al., 2007).

Our objective in this study was to improve our knowledge on *A. phagocytophilum* prevalence in *Apodemus sylvaticus*, *A. flavicollis* and *M. glareolus* using data collected from three distinct sites in France where the presence of the bacterium has been previously recorded in ticks or in roe deer.

Materials and methods

Rodents were trapped in three locations across France: one site in north-western France in the "Zone Atelier Armorique" (ZA hereafter, N 48°29'22.40",W 1°33'41.48"), one site in south-western France in the region of "Vallons et Coteaux de Gascogne" (VG hereafter, N 43°16'2.64", E 0°51'51.00"), and two sites in eastern France in the Haute-Saône (HS hereafter, N 47°40'24.66", E 6°42'6.00").

In ZA and VG, rodents were trapped in the spring and autumn of 2012 and 2013 (4 trap sessions per site). Twenty-four 100-meter traplines of 32 traps each (INRA live traps, fitted with dormitory boxes) were used. Traps were spaced 3 m apart along the line, with bait, and were checked in the morning 24 and 48 h after setup. Captured rodents were morphologically identified before being euthanized and autopsied. The spleens were removed and stored at -20 °C for detection of *A. phagocytophilum*.

In HS rodents were captured throughout 2010 during 4 sessions within the framework of a Capture-Mark-Recapture (CMR) survey in two sites, 65 km apart (Bellevaivre and Chérimont). For each session and site, 49 UGGLAN Special No2 live-traps (Grahnab, Gnosjö, Sweden) were set-up in a 7 x 7 grid of 1 ha (100 m x 100 m) with approximately 15 m between traps. Traps were baited and checked over 4 days (3 nights). Individuals were marked by toe clipping. Blood sampling was performed on trapped animals using the retro-orbital method. Blood pellets were separated from serum by centrifugation, and stored at -20 °C. Rodents were morphologically identified and identifications for *A. sylvaticus* and *A. flavicollis* were verified by PCR (Michaux et al., 2001).

DNA in spleen samples were extracted using the kit NucleoSpin Tissue (Macherey Nagel). In the absence of a pre-existing protocol to extract DNA from mammalian blood pellets, we used the "NucleoSpin blood QuickPure" kit and adapted manufacturer instructions by doubling the quantity of proteinase K and BQ1 during the cell lysis step.

DNA of *A. phagocytophilum* was detected by real-time PCR targeting the msp2 gene according to the protocol of Courtney et al. (2004).

As individuals from the HS site could be recaptured, we evaluated the independence of the infectious status of recaptured individuals between two successive months using the Pearson correlation test with a confidence interval based on the Fisher z-transformation.

To increase the number of samples per class and test for seasonal differences in prevalence within rodent populations, we also combined some data. For ZA and VG, data from the two years were combined by month (May 2012 with May 2013 and October 2012 with October 2013). For HS, data from the trap sessions in June-July and in September-October were grouped together to determine mean prevalence in summer and autumn, respectively.

The prevalence of *A. phagocytophilum* at each site was analyzed by generalized linear models (GLM) with rodent species and season as explanatory variables. For significant explanatory variables, an odds-ratio was calculated using the exponential of the GLM coefficients and the confidence intervals. A binomial distribution was used to analyze the prevalence in ZA and a quasi-binomial distribution was used for HS to take into account the dispersion of the data.

All statistic analyses were performed using the R statistical software (version 2.15.1).

Results

In total, 1163 rodents were analyzed including 441 *M. glareolus*, 668 *A. sylvaticus* and 54 *A. flavicollis* (Table 1). In HS, 75 individuals were recaptured and tested at least twice for a positive infection. Among these, 13 individuals negative for *A. phagocytophilum* at first capture became positive the next month, 10 positive individuals recovered from infection, and only 3 remained positive between two successive months. Overall, the infectious status of

individuals between months was therefore statistically independent ($p = 0.59$). Thus, we considered all captures to be independent for the analyses.

No rodents were found infected by *A. phagocytophilum* in VG. In HS, prevalence was high (22.8%) and stable: no significant difference in prevalence was detected among rodent species ($p = 0.93$) or among sampling seasons ($p = 0.84$). In ZA, the prevalence in *M. glareolus* was significantly higher than in *A. sylvaticus* ($p < 0.001$, OR = 4.11, CI 95% = [2.09-8.09]) and the prevalence in autumn was significantly higher than in spring ($p < 0.001$, OR = 7.35, CI 95% = [2.55-21.21]).

Discussion

We studied the prevalence of *A. phagocytophilum* in three rodent species in three sites of France. All species combined, we found a significant difference in prevalence among sites, with a variable pattern of prevalence among rodent species within sites and among seasons.

As in site ZA, higher prevalence of *A. phagocytophilum* in *M. glareolus* than in *A. sylvaticus* has been previously recorded in Switzerland and United Kingdom (Liz et al., 2000; Bown et al., 2003). Although tick infestation rates are generally described to be higher in *Apodemus* spp. than in *M. glareolus* (Kurtenbach et al., 1995; Talleklint and Jaenson, 1997; Perez et al, 2016), *M. glareolus* is considered to be a better reservoir for several tick-borne pathogens (Randolph, 1994; Humair et al., 1999). This interspecific difference could be explained by differences in the ability of their immune systems to eliminate the bacterium. Indeed, Bown et al. (2003) found that *M. glareolus* was more often positive for *A. phagocytophilum* over two consecutive months compared to *A. sylvaticus*. Based on this, these authors suggest that a shorter infection time for *A. sylvaticus* than for *M. glareolus* may explain lower observed prevalence in the former species. Interspecific differences in local prevalence could also be related to the range of *A. phagocytophilum* strains infecting each rodent species. For instance, *M. glareolus* could be infected by a more prevalent strain at the ZA site or be susceptible to more strains than *Apodemus* spp. Finally, another alternative hypothesis could be related to exploitation by different tick species. Two studies have shown that the endophilic tick *I. trianguliceps* is a more important vector of some *A. phagocytophilum* strains specific to small mammals than *I. ricinus* (Blaňarová et al., 2014; Bown et al., 2008). As *M. glareolus* is frequently more heavily parasitized by this tick species than *Apodemus* spp. (Gilot et al., 1976), rodent specific exploitation by different tick vectors could explain among-rodent variation in prevalence.

We found that prevalence of *A. phagocytophilum* increased in autumn in the ZA site, but not in the HS site. A change across seasons could be explained three ways: i) the prevalence of *A. phagocytophilum* in ticks could be higher in autumn, as has been shown in other studies (Bown et al., 2003); ii) the abundance and activity of questing *I. ricinus* could be higher in late spring-early summer (Schulz et al, 2014; Perez et al, 2016), such that the quantity of ticks encountered by rodents and the probability of infection is greater after this period; and/or iii) the age structure of the rodent population changes such that there are more susceptible

individuals in autumn. Recruitment in *M. glareolus* populations begin in June with a peak in autumn (Crawley, 1970). More juvenile rodents are captured during the autumn, and these individuals may not yet have developed resistance to ticks and/or bacteria (Dizij and Kurtenbach, 1995). In HS, rodents were sampled later in the spring than in ZA, such that there were only two months between the two sampling sessions in HS against four months in ZA; this temporal difference could explain the variation found between the two sites. A recent study in Slovakia also found significant seasonal and year-to-year variation in *A. phagocytophilum* prevalence in *I. ricinus* nymphs, but again this variation was not consistent among sites and was therefore difficult to explain (Svitálková et al., 2015).

The difference in prevalence of *A. phagocytophilum* among areas in France needs further investigation. It cannot be attributed to an artifact linked to the type of biological material tested (blood versus spleen), as earlier studies suggest that bacterial detection may be lower in blood pellets (used in HS) than in the spleen (Liz et al., 2000) . Thus, we would therefore expect an underestimation of prevalence in HS compared to the other sites. The site differences could be linked to differences in circulating *A. phagocytophilum* strains or tick species. However, we could not test this hypothesis in the present study because bacterial loads were too low to enable sequencing and we had only limited information on tick presence on the captured rodents. Nonetheless, we might expect strong differences in the presence and abundance of different tick species among the sampled sites because they lie in different climatic biotopes. Indeed, the VG site lies near the distributional limit for *I. trianguliceps,* potentially explaining the absence of *A. phagocytophilum* in rodents of this zone (Gilot et al, 1976; Pérez-Eid, 2007)

The absence of infection in VG rodents was particularly surprising because approximately 75% (56/75) of roe deer and 2% (35/1837) of questing *I. ricinus* nymphs have been found positive in this area (Chastagner, 2014). Recent studies in other European countries have shown that rodents and roe deer can carry specific *A. phagocytophilum* lineages, differing from those described in other mammals (Baráková et al., 2014; Majazki et al., 2013). Thus, it could be that no rodent-specific strain of *A. phagocytophilum* circulates in VG, supporting the hypothesis that rodents do not share the same *A. phagocytophilum* genotypes carried by roe deer. Specific *A. phagocytophilum* genotypes associated with *Ixodes trianguliceps* ticks and *M. glareolus* in Europe (Blaňarová et al., 2014) and with *Myodes* spp., *I. persulcatus* and *I. trianguliceps* in Russia have also been recently described (Rar et al., 2014). Further research is required to investigate the overall diversity and circulation of *A. phagocytophilum* in rodents, especially in *Apodemus* spp. for which few data are currently available.

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References

Baráková, I., Derdáková, M., Carpi, G., Rosso, F., Collini, M., Tagliapietra, V., Ramponi, C., Hauffe, H.C., Rizzoli, A., 2014. Genetic and ecologic variability among *Anaplasma phagocytophilum* strains, northern Italy. Emerg. Infect. Dis. 20, 1082-1084.

Blaňarová, L., Stanko, M., Carpi, G., Miklisová, D., Víchová, B., Mošanský, L., Bona, M., Derdáková, M., 2014. Distinct *Anaplasma phagocytophilum* genotypes associated with *Ixodes trianguliceps* ticks and rodents in Central Europe. Ticks Tick-borne Dis. 5, 928-938.

Bown, K. J., Begon, M., Bennett, M., Woldehiwet, Z., Ogden, N. H., 2003. Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. Emerg. Infect. Dis. 9, 63-70.

Bown, K. J., Lambin, X., Telford, G. R., Ogden, N. H., Telfer, S., Woldehiwet, Z., Birtles, R. J., 2008. Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. Appl. Environ. Microbiol. 74, 7118-7125.

Chastagner, A. P., 2014. Etude des cycles épidémiologiques d'*Anaplasma phagocytophilum* en France: apport des approches de caractérisation génétique. Thesis: University Blaise Pascal Clermont-Ferrand (France).

Courtney, J. W., Kostelnik, L. M., Zeidner, N. S., Massung, R. F., 2004. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. J. Clin. Microbiol. 42, 3164-3168.

Crawley, M. C., 1970. Some population dynamics of the Bank vole, *Clethrionomys glareolus* and the Wood mouse, *Apodemus sylvaticus* in mixed woodland. J. Zool. 160, 71-89.

Dizij, A., Kurtenbach, K., 1995. *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *lxodes ricinus* L, the main European vector of *Borrelia burgdorferi*. Parasite Immunol. 17, 177-183.

Gilot, B., Beaucournu, J.C., Pautou, G., Fayard, A., Moncada, E., 1976. Contribution à la connaissance de la répartition et de l'écologie d'*Ixodes trianguliceps* en France, et plus particulièrement dans le Sud-Est. Acta Trop. 33, 254–286.

Hartelt, K., Pluta, S., Oehme, R., Kimmig, P., 2008. Spread of ticks and tick-borne diseases in Germany due to global warming. Parasitol. Res. 103, 109-116.

Hulínská, D., Langrová, K., Pejčoch, M., Pavlásek, I., 2004. Detection of *Anaplasma phagocytophilum* in animals by real-time polymerase chain reaction. Apmis. 112, 239-247.

Humair, P. F., Rais, O., Gern, L., 1999. Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. Parasitol. 118, 33-42.

Kurtenbach, K., Kampen, H., Dizij, A., Arndt, S., Seitz, H. M., Schaible, U. E., Simon, M. M., 1995. Infestation of rodents with larval *Ixodes ricinus* (Acari: Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi sl* in German woodlands. J. Med. Entomol. 32, 807-817.

Liz, J. S., Anderes, L., Sumner, J. W., Massung, R. F., Gern, L., Rutti, B., Brossard, M., 2000. PCR detection of granulocytic ehrlichiae in *Ixodes ricinus* ticks and wild small mammals in western Switzerland. J. Clin. Microbiol. 38, 1002-1007.

Majazki, J., Wüppenhorst, N., Hartelt, K., Birtles, R., von Loewenich, F. D., 2013. *Anaplasma phagocytophilum* strains from voles and shrews exhibit specific *ankA* gene sequences. BMC Vet. Res. 9, 235-241.

Marumoto, K., Joncour, G., Lamanda, P., Inokuma, H., Brouqui, P., 2007. Detection of *Anaplasma phagocytophilum* and *Ehrlichia sp.* HF strains in *Ixodes ricinus* ticks in Brittany, France. Clin. Microbiol. Infect. 13, 338-341.

Michaux, J. R., Kinet, S., Filippucci, M. G., Libois, R., Besnard, A., Catzeflis, F., 2001. Molecular identification of three sympatric species of wood mice (*Apodemus sylvaticus, A. flavicollis, A. alpicola*) in western Europe (Muridae: Rodentia). Mol. Ecol. Notes. 1, 260-263.

Overzier, E., Pfister, K., Herb, I., Mahling, M., Böck, G., Silaghi, C., 2013. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks infesting roe deer in southern Germany. Ticks Tick-borne Dis. 4, 320-328.

Perez, G., Bastian, S., Agoulon, A., Bouju-Albert, A., Durand, A., Faille, F., Lebert, I., Rantier, Y., Plantard, O., Butet, A., 2016. Effect of landscape features on the relationship between *Ixodes ricinus* ticks and their small mammal hosts. Parasit.Vectors. 9, 20-39.

Pérez-Eid, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. TEC & DOC EMinter, Lavoisier, Paris, France, pp. 314.

Randolph, S. E., 1994. Density-dependent acquired resistance to ticks in natural hosts, independent of concurrent infection with *Babesia microti*. Parasitol. 108, 413-419.

Rar, V. A., Epikhina, T. I., Yakimenko, V. V., Malkova, M. G., Tancev, A. K., Bondarenko, E. I., Ivanov, M. K., Tikunova, N. V., 2014. Genetic variability of *Anaplasma phagocytophilum* in ticks and voles from *Ixodes persulcatus/Ixodes trianguliceps* sympatric areas from Western Siberia, Russia. Ticks Tick-borne Dis. 5, 854-863.

Schulz, M., Mahling, M., Pfister, K. (2014). Abundance and seasonal activity of questing *Ixodes ricinus* ticks in their natural habitats in southern Germany in 2011. J. Vector Ecol. 39, 56-65.

1 Stuen, S., 2007. *Anaplasma phagocytophilum* - the most widespread tick-borne infection in animals in
2 Europe. Vet. Res. Commun. 31, 79-84.

3 Stuen, S., Granquist, E. G., Silaghi, C., 2013. *Anaplasma phagocytophilum*—a widespread multi-host
4 pathogen with highly adaptive strategies. Front. Cell. Infect. Microbiol. 3, 31-71.

5 Svitálková, Z., Haruštiaková, D., Mahríková, L., Berthová, L., Slovák, M., Kocianová, E.,
6 Kazimírová, M., 2015. *Anaplasma phagocytophilum* prevalence in ticks and rodents in an urban and
7 natural habitat in South-Western Slovakia. Parasit.Vectors. 8, 276-287.

8 Talleklint, L., Jaenson, T. G., 1997. Infestation of mammals by *Ixodes ricinus* ticks (Acari: Ixodidae)
9 in south-central Sweden. Exp. Appl. Acarol. 21, 755-771.

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12 Table

13 Table 1. Prevalence of *A. phagocytophilum* detected by real-time PCR (number of positive
14 samples/number of analyzed samples (prevalence %))

	Zone Atelier Armorique (ZA)			Vallons de Gascogne (VG)			Haute-Saôr		
	May	October	Total	May	October	Total	June	July	Septem
M. glareolus	3/65 (4.6)	17/80 (21.2)	20/145 (13.8)[a]	0/23 (0)	0/21 (0)	0/45 (0)	13/44 (29.5)	24/80 (30)	12/7 (15.2
A. sylvaticus	1/173 (0.6)	18/271 (6.6)	19/444 (4.3)[a]	0/80 (0)	0/138 (0)	0/218 (0)	0/2 (0)	1/1 (100)	-
A. flavicollis	-	-	-	-	-	-	1/8 (12.5)	2/34 (5.9)	6/8 (75)
Total	4/238 (1.7)[b]	35/351 (10)[b]	39/589 (6.6)	0/103 (0)	0/159 (0)	0/263 (0)	14/54 (25.9)	27/115 (23.5)	18/8 (20.7

15 GLM results

16 [a] *A. phagocytophilum* prevalence in *M. glareolus* was significantly higher than in *A. sylvaticus*
17 (OR = 4.11, CI 95 % = [2.09-8.09]).

18 [b] *A. phagocytophilum* prevalence in autumn was significantly higher than in spring (OR =
19 7.35, CI 95 % = [2.55-21.21]).

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