



ELSEVIER

EXTENDED ABSTRACT

Different sequence types of the *ank* gene of *Anaplasma phagocytophilum*

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Introduction

Anaplasma phagocytophilum is a Gram-negative, obligately intracellular bacterium that replicates within neutrophils. In Europe, *A. phagocytophilum* is transmitted by *Ixodes ricinus* ticks. It is known as the causative agent of human granulocytic anaplasmosis (HGA), tick-borne fever in cattle and sheep, equine granulocytic ehrlichiosis (EGE), and canine granulocytic ehrlichiosis. Small mammals, roe deer, and red deer have been suggested to serve as reservoir hosts for *A. phagocytophilum* in Europe (Ogden et al., 1998; Alberdi et al., 2000; Liz et al., 2000, 2002; Petrovec et al., 2002, 2003; Bown et al., 2003; Oporto et al., 2003). HGA was first described in the United States in 1994 (Chen et al., 1994), and more than 1000 cases have been reported since then in the US. However, in Europe, HGA is a rare disease and the clinical course seems to be less severe (Blanco and Oteo, 2002). We therefore asked whether European *A. phagocytophilum* strains differ from those circulating in North America.

Materials and methods

Sequence analysis of the 16S rRNA, *groESL* and *ank* genes of *A. phagocytophilum* DNA detected in 42 of 1022 *I. ricinus* ticks from Germany was performed as described (von Loewenich et al., 2003a). Furthermore, blood or tissue samples from humans, dogs, horses, sheep, cattle, European bison, roe deer, and red deer from Germany, Slovenia, Norway, Spain and Poland were analyzed for the presence of *A. phagocytophilum* DNA by nested PCR. In positive samples the *ank* gene was amplified and sequenced bidirectionally (Massung et al., 2000; von Loewenich et al., 2003a).

Results and discussion

The 16S rRNA and *groESL* gene sequences from German ticks were as much as 100% identical to sequences from strains that caused diseases in humans or animals in other European countries and in the US. Fifteen *ank* sequences were $\geq 99.4\%$ identical to sequences derived from humans with HGA in Europe and from a horse with EGE from Germany (von Loewenich et al., 2003a, b). These 15 sequences were also highly identical to those reported from the US. Thus, German *I. ricinus* ticks harbor *A. phagocytophilum* strains that most likely are closely related to strains which can cause disease in humans. Nine additional *ank* sequences were clearly different from known *ank*

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sequences and have never been detected in cases of HGA or granulocytic ehrlichiosis in animals. Eight of these sequences clustered together. To test the hypothesis whether strains with a variation within the *ank* gene are associated with a certain host or reservoir we investigated 29 human and animal samples from different European countries. Preliminary results show that *ank* sequences derived from humans, dogs, and horses cluster together, whereas *ank* sequences from roe deer show independently of the geographic origin of the sample the same variation of the *ank* gene previously detected in 8 strains from ticks from Germany. To further characterize strains with variation in the *ank* gene in terms of their pathogenicity in vivo, the cultivation of such strains and the investigation in animal models is needed.

References

- Alberdi, M.P., Walker, A.R., Urquhart, K.A., 2000. Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*. *Epidemiol. Infect.* 124, 315–323.
- Blanco, J.R., Oteo, J.A., 2002. Human granulocytic ehrlichiosis in Europe. *Clin. Microbiol. Infect.* 8, 763–772.
- 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.
- Chen, S.-M., Dumler, J.S., Bakken, J.S., Walker, D.H., 1994. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J. Clin. Microbiol.* 32, 589–595.
- 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.
- Liz, J.S., Sumner, J.W., Pfister, K., Brossard, M., 2002. PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). *J. Clin. Microbiol.* 40, 892–897.
- Massung, R.F., Owens, J.H., Ross, D., Reed, K.D., Petrovec, M., Bjöersdorff, A., Coughlin, R.T., Beltz, G.A., Murphy, C.I., 2000. Sequence analysis of the *ank* gene of granulocytic ehrlichiae. *J. Clin. Microbiol.* 38, 2917–2922.
- Ogden, N.H., Bown, K., Horrocks, B.K., Woldehiwet, Z., Bennett, M., 1998. Granulocytic ehrlichia infection in ixodid ticks and mammals in woodlands and uplands of the U.K. *Med. Vet. Entomol.* 12, 423–429.
- Oporto, B., Gil, H., Barral, M., Hurtado, A., Juste, R.A., Garcia-Perez, A., 2003. A survey on *Anaplasma phagocytophila* in wild small mammals and roe deer (*Capreolus capreolus*) in northern Spain. *Ann. N. Y. Acad. Sci.* 990, 98–102.
- Petrovec, M., Bidovec, A., Sumner, J.W., Nicholson, W.L., Childs, J.E., Avšič-Županc, T., 2002. Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotypic lineages. *Wien. Klin. Wschr.* 114, 641–647.
- Petrovec, M., Sixl, W., Schweiger, R., Mikulasek, S., Lebeth, E., Wüst, G., Marth, E., Strasek, K., Stünzner, D., Avšič-Županc, T., 2003. Infections of wild animals with *Anaplasma phagocytophila* in Austria and the Czech Republic. *Ann. N. Y. Acad. Sci.* 990, 103–106.
- von Loewenich, F.D., Baumgarten, B.U., Schröppel, K., Geißdörfer, W., Röllinghoff, M., Bogdan, C., 2003a. High diversity of *ankA* sequences of *Anaplasma phagocytophilum* among *Ixodes ricinus* ticks in Germany. *J. Clin. Microbiol.* 41, 5033–5040.
- von Loewenich, F.D., Stumpf, G., Baumgarten, B.U., Röllinghoff, M., Dumler, J.S., Bogdan, C., 2003b. A case of equine granulocytic ehrlichiosis provides molecular evidence for the presence of pathogenic *Anaplasma phagocytophilum* (HGE agent) in Germany. *Eur. J. Clin. Microbiol. Infect. Dis.* 22, 303–305.