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Potential for Cofeeding Transmission of Tick-Borne Pathogens in Common Voles and Roe Deer – Supportive Molecular Evidence from Field Samples

Christiane Mogl, Philippe Gil de Mendonça*, Andrea Harsch, Johannes Heyl

Institute of Comparative Tropical Medicine and Parasitology, Ludwig Maximilian University, D-80802 Munich, Germany

Abstract: DNA from skin and blood samples from common voles (*Microtus arvalis*) and roe deer (*Capreolus capreolus*) was screened by real-time PCR for *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum*. *Borrelia* sp. was detected in voles only, and *A. phagocytophilum* in deer only. *Borrelia*-positive samples were identified as *Borrelia afzelii.* Prevalence estimate for *B. afzelii* in voles is 20.3% in ear skin whereas in blood it reaches only 4.2%. Prevalence estimate for *A. phagocytophilum* in deer is 85.9% in skin whereas it is 75.5% in blood. The high infection levels of the skin at tick attachment sites suggest that cofeeding might be a significant transmission route for *B. afzelii* in *M. arvalis* and for *A. phagocytophilum* in *C. capreolus*.

Key words: *Microtus arvalis*, *Capreolus capreolus, Ixodes ricinus*, *Borrelia afzelii, Anaplasma phagocytophilum,* real-time PCR

Introduction

Ticks are haematophagous arthropods causing and feeding on subcutaneous haemorrhages. While feeding on a host, they may injest pathogens present in the blood, or inject pathogens into the wound. The immuno-modulatory activity of tick saliva (Jones *et al* 1992) may permit the localized survival of pathogens in the skin of an immune host at the tick bite site. Uninfected ticks (i.e. recipients) feeding in close proximity to an infected tick (i.e. donor) may thus become infected in turn without their host being necessarily systemically infected. This cofeeding transmission process could be observed under laboratory conditions for Tick-Borne Encephalitis Virus, Thogoto Virus, and for the causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato (Gern, Rais 1996, Jones *et al*. 1987, Labuda *et al*. 1993 a, b, 1996, 1997). However, under natural conditions, no evidence could be found in field collected samples to support the theory of cofeeding transmission of Tick-Borne Encephalitis Virus in yellow-necked mice from an endemic area (de Mendonça *et al*. 2011). Further laboratory experiments with *Anaplasma marginale* were unable to demonstrate cofeeding transmission of that pathogen (Kocan, de la Fuente 2003). A related bacterium, *Anaplasma phagocytophilum* is pathogenic to both Humans and domestic animals (Artursson *et al*. 1999, Bermann *et al*. 2002, Chen *et al*. 1994, Johansson *et al*. 1995, Joncour *et al*. 2005, Matsumoto *et al*. 2006, Naranjo *et al*. 2006, Woldehiwet 2006). This bacterium was also reported in wildlife (Alberdi *et al*. 2000, de la Fuente *et al*. 2007, Jenkins *et al*. 2001, Liz *et al*. 2002,

*Corresponding author and current address: P. G. de Mendonça, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd, BG-1000 Sofia, Bulgaria; E-mail: philippe.mendonca@iber.bas.bg

Naranjo *et al*. 2006, Polin *et al*. 2004, Skotarczak *et al*. 2008, Stuen *et al*. 2001, Zeman, Pecha 2008). Prevalence estimates of *A. phagocytophilum* in questing nymphal and adult ticks imply that most ticks become infected through their nymphal blood meal (de Mendonça *et al*. 2008). This very strongly suggests that circling between immature and adult ticks occurs through feeding on the same reservoir host. It was shown that all three active instars of *Ixodes ricinus* are found together, feeding in close proximity on roe deer (Heyl, de Mendonça 2009, 2011). Aggregated distribution of ticks is also observed in common voles, where most ticks are attached to the ear flap (Harsch, de Mendonça unpublished). Both roe deer (*Capreolus capreolus*) and common voles (*Microtus arvalis*) are very abundant mammals of the European landscape (Blant 1995, Meylan 1995, Sempéré *et al* 1996). They thus potentially play an important role in the epidemiology of tick-borne diseases. To date however, very little is known about the part played by these wild mammals in the natural transmission cycles of borreliosis and anaplasmosis.

We therefore aimed to compare the incidence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in skin samples collected from the privileged sites of tick attachment (i.e. sites of localized immuno-modulation) and in blood samples (revealing systemic infection) from roe deer and common voles.

Materials and Methods

Common voles (*Microtus arvalis*) were trapped in Bavaria and Baden-Württemberg (Germany) between April and November 2009. Traps were placed near burrow entrance holes, along vole runways, or buried inside burrow galleries. Voles underwent euthanasia and, for each individual, both blood and skin samples were immediately collected and preserved in 80% ethanol for later molecular processing. The skin sample was taken from the ear flap and blood was collected from the retro-orbital sinus and/or from a kidney using sterile micro-capillaries.

Deer samples were obtained from culled roe deer (*Capreolus capreolus*) from Bavaria, Baden-Württemberg and Thuringia (Germany) between May 2007 and November 2009. For each individual, both blood and skin samples were immediately collected and preserved in 80% ethanol for later molecular processing. Skin samples were collected from the inguinal area where many ticks cluster. Blood was collected by cardiac puncture.

DNA from blood and skin samples was extracted using a QIAamp® DNA Mini Kit (QIAGEN,

Table 1. Primers and probes for the amplification and subsequent hybridization of *Borrelia* DNA. The 5'-amino linker is a *N*-(trifluoroacetamidohexyl-cyanoethyl, *N*,*N*-diisopropylphosphoramidite [TFA])-C6 amino linker.

Primers and probes	Sequence	Source
B-5SBor	5'-biotin-GAGTTCGCGGGAGAGTAGGTTATT-3'	Alekseev *et al*. 2001
23SBor	5'-TCAGGGTACTTAGATGGTTCACTT-3'	Alekseev *et al*. 2001
Borrelia spp.	5'-amino-CTTTGACCATATTTTTATCTTCCA	Rijpkema *et al.* 1995
Borrelia burgdorferi s.s.	5'-amino-AACACCAATATTTAAAAAACATAA	Rijpkema *et al*. 1995
Borrelia garinii	5'-amino-CAAAAACATAAATATCTAAAAACATAA	Poupon *et al*. 2006
Borrelia afzelii	5'-amino-AACATTTAAAAAATAAATTCAAGG	Rijpkema *et al*. 1995
Borrelia valaisiana	5'-amino-TATATCTTTTGTTCAATCCATGT	Poupon *et al*. 2006
Borrelia lusitaniae	5'-amino-TCAAGATTTGAAGTATAAAATAAAA	Poupon *et al.* 2006
Borrelia lusitaniae	5'-amino-CATTCAAAAAAATAAACATTTAAAAACAT	Gern *et al*. 2010
Borrelia lusitaniae	5'-amino-AAATCAAACATTCAAAAAAATAAAC	Gern *et al*. 2010
Borrelia spielmanii	5'-amino-GAATGGTTTATTCAAATAACATA	Gern *et al.* 2010
Borrelia spielmanii	5'-amino-GAATAAGCCATTTAAATAACATA	Gern *et al*. 2010
Borrelia bissettii	5'-amino-AAACACTAACATTTAAAAAACAT	Gern *et al.* 2010
Borrelia bissettii	5'-amino-AACTAACAAACATTTAAAAAACAT	Gern *et al*. 2010
Relapsing fever-like	5'-amino-CTATCCATTGATCAATGC	Gern *et al.* 2010

Table 2. Touch-down PCR program for the amplification of *Borrelia* DNA for subsequent reverse line blot hybridization.

Hot Start Taq activation	95°C	5 min	1 cycle
Denaturation	94°C	20 sec	
Annealing	from 60°C to 52°C	30 sec	1°C/cycle
Extension	72°C	30 sec	
Denaturation	94°C	20 sec	40 cycles
Annealing	52°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	7 min	1 cycle

Table 3. Reaction mix for the amplification of *Borrelia* DNA for subsequent reverse line blot hybridization. Total volume is 50µl, including 10µl template DNA.

10x QIAGEN PCR buffer (at 15mM $MgCl_2$)	5.00µl
QIAGEN $MgCl_2$ (at 25mM)	2.00µl
QIAGEN dNTPs (10mM)	1.00µl
Primer 23S Bor (10µM)	1.00µl
Primer B5S Bor (10µM)	1.00µl
H_2O	29.75µl
QIAGEN Hot Start Taq Plus (5U/µl)	0.25µl

Hilden Germany) following the manufacturer's instructions. DNA integrity was checked by PCR using molecular marker pairs p0033/p0049 and p0066/p0067 targeting the vertebrate 18S rRNA gene (modified after Pichon *et al*. 2003). Intact DNA samples were screened for *Borrelia* spp. and *Anaplasma phagocytophilum* by real-time PCR (modified after Courtney *et al*. 2004). Positive and negative controls were included in each PCR run.

Borrelia specific identification was done by reverse line blot hybridization using molecular markers targeting the 5S-23S rRNA inter-genic spacer region of *Borrelia* spp. as described in Table 1. Positive and negative controls were included in each PCR run. For reverse line blot, DNA was amplified using a touch-down protocol (Tables 2 and 3). Reverse line blot hybridization followed the protocol of Rijpkema *et al*. (1995).

Results

Samples from common voles were all negative for *A. phagocytophilum*. No *Borrelia* spp. were detected in any deer sample.

Infection with *Borrelia burgdorferi* sensu lato in common voles (*Microtus arvalis*):

A total of 30 out of 143 voles were found infected with *Borrelia* sp. (overall prevalence estimate: 21.0%; 95% CI: 14.3%-27.7%). All *Borrelia* positive cases were identified as *Borrelia afzelii*. 29 out of 143 vole ear skin samples tested positive for *B. afzelii*. 6 out of 143 vole blood samples tested positive for *B. afzelii*. Of these, 5 voles tested positive for *B. afzelii* for both ear skin and blood (Fig. 1). There is a very highly statistically significant difference in infection levels between skin and blood from common voles (McNemar's test for paired samples corrected for continuity, $X^2=19.360$; df=1; $p<0.001$, based on 286 observations of 143 voles), ear skin being more likely to be infected with *Borrelia afzelii*. Prevalence estimate for *B. afzelii* in ear skin is 20.3% (95% CI: 13.7%-26.9%) whereas prevalence estimate for that pathogen in blood is 4.2% (95% CI: 0.9%-7.5%).

Infection with *Anaplasma phagocytophilum* in roe deer (*Capreolus capreolus*):

A total of 99 out of 106 deer were found infected with *A. phagocytophilum* (overall prevalence estimate: 93.4%; 95% CI: 88.7%-98.1%). 91 out of 106 deer skin samples tested positive for *A. phagocytophilum*. 80 out of 106 deer blood samples tested positive for *A. phagocytophilum* (Fig. 2). Only 7 individuals tested negative for both skin and blood samples. There is no statistically significant difference in infection levels between skin and blood from deer (McNemar's test for paired samples corrected for continuity, $X^2=3.704$; df=1; $p>0.05$, based on 212 observations of 106 deer). Prevalence estimate for *A. phagocytophilum* in skin is 85.9% (95% CI: 79.2%-92.5%) whereas prevalence estimate for that pathogen in blood is 75.5% (95% CI: 67.3%-83.7%).

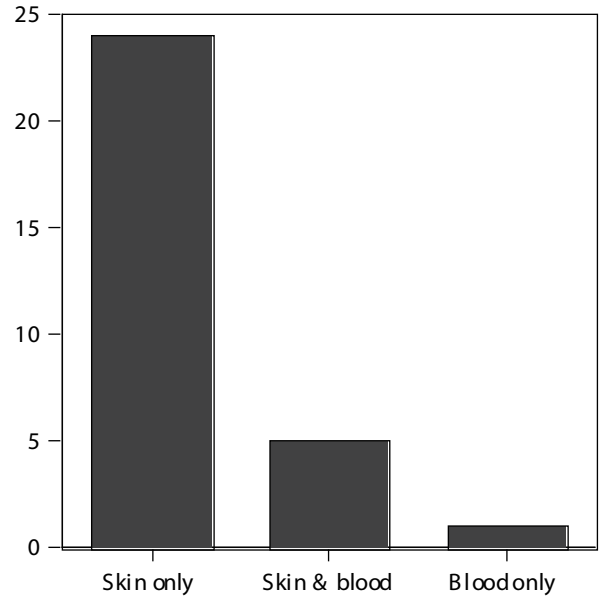

Fig. 1. Number of individual voles which tested positive for *Borrelia afzelii* in skin only, in both skin and blood, and in blood only. Each individual had both skin and blood tested.

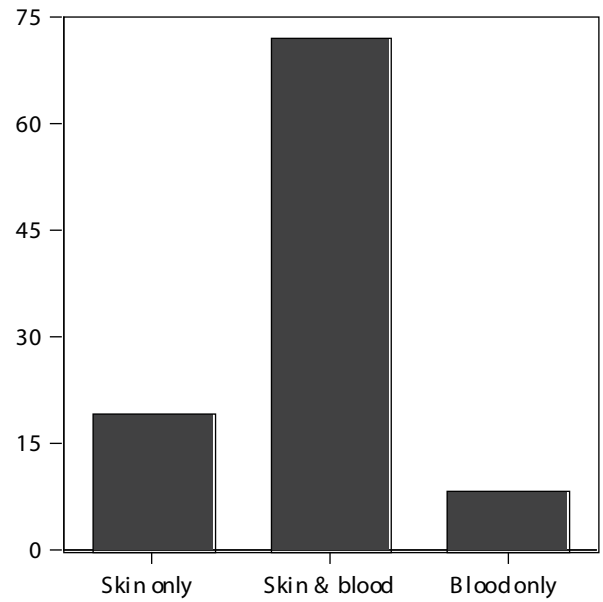

Fig. 2. Number of individual deer which tested positive for *Anaplasma phagocytophilum* in skin only, in both skin and blood, and in blood only. Each individual had both skin and blood tested.

Discussion

To date, only few data on infection with *Borrelia* spp. in *M. arvalis* are available (Paulauskas *et al*. 2008). Our results are the first comparison between skin and blood infection levels for *Borrelia burggdorferi* sensu lato genospecies in Germany. The fact that *B. afzelii* was the sole *Borrelia* genospecies detected in our vole samples comes to no surprise. A similar result was described by Humair *et al*. (1995) for other rodent species. Actually, it was shown that the complement in sera from European rodents very efficiently clears the other *Borrelia* genospecies, thus positively selecting *B. afzelii* (Kurtenbach *et al*. 1998). European rodents thus transmit nearly exclusively *B. afzelii* to feeding ticks (Hanincova *et al*. 2003). Most interestingly, very few voles (barely 4.2%) were systemically infected, although rodents are known to develop systemic infections with *B. afzelii* (P.G. de Mendonça unpublished, Park *et al*. 1999; Randolph *et al*. 2002). Ear skin samples were much more likely to test positive for *B. afzelii* than blood samples. Indeed, approximately 20.3% of the ear flap skin samples we analysed were positive for *B. afzelii*. It might be that the immuno-modulatory activity of tick saliva permits a locally higher survival of *B. afzelii* in vole ear flap. Since most ticks found on common voles are actually attached to the ear flap (Harsch, de Mendonça unpublished), localized infection and cofeeding at the ear flap level might be a significant transmission route for *B. afzelii* in *Microtus arvalis*.

A. phagocytophilum is an obligatory intracellular bacterium found predominantly inside neutrophils and to a lesser extent inside eosinophils and basophils (Woldehiwet 2006). These leukocytes circulate in the blood flow, and infected granulocytes are thus expected to be detected in blood samples from infected animals. The high bacteraemia detected by PCR here (75.5%) comes therefore to no surprise considering the extremely high overall infection rate observed (*A. phagocytophilum* was detected in 93.4% of the individual deer we analysed). The interesting point here, is that skin samples tested positive for *A. phagocytophilum* more often (85.9%) than blood samples (75.5%), although this was barely not statistically significant (p=0.054). Ticks feed from subcutaneous haemorrhages. These haemorrhages are characterized by an immune-cell enriched serum (Randolph *et al*. 2002). It was shown (Allen *et al.* 1977, Brossard, Fivaz 1982, van der Heijden *et al*. 2005) that the infiltrate in the cutaneous reaction at the tick attachment site contains mostly neutrophils, eosinophils and basophils, *i.e*. precisely the cells

which are invaded by *A. phagocytophilum*. The immuno-modulation at the tick attachment site by tick saliva actually influences the transfer of *A. phagocytophilum* between the vector tick and the reservoir host (Borjesson *et al*. 2003). The extravasation and thus highly increased number of *Anaplasma* infected granulocytes in the skin at the tick attachment site explain the particularly high infection levels observed for skin samples. The combination of (1) clustering of high numbers of ticks in the inguinal area, and (2) high infection levels with *A. phagocytophilum* of the skin at tick attachment sites, suggests that cofeeding might be a significant transmission route for *A. phagocytophilum* in *Capreolus capreolus*.

In conclusion, the high infection levels of the skin at tick attachment sites suggest that cofeeding might be a significant transmission route for *B. afzelii* in *M. arvalis* and for *A. phagocytophilum* in *C. capreolus*.

Individual contributions

Collection of deer samples : JH, PGM, CM & AH.
Collection of vole samples : AH & PGM.
DNA extraction : CM, PGM & AH.
Molecular analyses : CM, PGM & AH.

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References

Alberdi M. P., A. R. Walker, K. A. Urquhart 2000. Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*. – *Epidemiology and Infection*, **124**: 315-323.

Alekseev A. N., H.V. Dubinina, I. Van De Pol, L. M. Schouls 2001. Identification of *Ehrlichia* spp. and *Borrelia burgdorferi* in *Ixodes* Ticks in the Baltic Regions of Russia. – *Journal of Clinical Microbiology*, **39**: 2237-2242.

Allen J. R., B.M. Doube, D. H. Kemp 1977. Histology of bovine skin reaction to *Ixodes holocyclus*, Neuman. *Canadian* – *Journal of Comparative Medicine*, **41**: 26-35.

Artursson K., A. Gunnarsson, U.-B. Wikström, E. Olsson Engvall 1999. A serological and clinical follow-up in horses with confirmed equine granulocytic ehrlichiosis. – *Equine Veterinary Journal*, **31**:473-477.

Bermann F., B. Davoust, P.E. Fournier, A.V. Brisou-Lapointe, P. Brouqui 2002. *Ehrlichia equi* (*Anaplasma phagocytophila*) infection in an adult horse in France. – *Veterinary Record*, **150**: 787-788.

Blant M. 1995. *Capreolus capreolus*. L., 1758. – In: Hausser J. (Ed.): Mammifères de la Suisse, Mémoires de l'Académie Suisse des Sciences Naturelles, **103**: 328-333.

Borjesson D. L., S. I. Simon, E. Hodzic, H. E. V. DeCock, C. M. Ballantyne, S. W. Barthold 2003. Roles of neutrophil beta 2 integrins in kinetics of bacteremia, extravasation, and tick acquisition of *Anaplasma phagocytophila* in mice. – *Blood,* **101**: 3257-3264.

Brossard M., V. Fivaz 1982. *Ixodes ricinus* L.: mast cells, basophils and eosinophils in the sequence of cellular events in the skin of infested or reinfested rabbits. – *Parasitology*, **85**: 583-592.

Chen S.-M., S. Dumler, J. S. Bakken, D. H. Walker 1994. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. – *Journal of Clinical Microbiology*, **32**: 589-595.

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

de la Fuente J., F. Ruiz-Fons, V. Naranjo, A. Torina, O. Rodrigez, C. Gortazar 2007. Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. – *Research in Veterinary Science*, **84** : 382-386.

Gern L.,V. Douet, Z. López, O. Rais, F. Morán Cadenas 2010. Diversity of *Borrelia* genospecies in *Ixodes ricinus* ticks in a Lyme borreliosis endemic area in Switzerland identified by using new probes for reverse line blotting. – *Ticks and Tick-borne Diseases*, **1**: 23-29.

Gern L., O. Rais 1996. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). – *Journal of Medical Entomology*, **33:** 189-192.

Hanincova K., S. M. Schäfer, S. Etti, H.-S. Sewell, V. Taragelova, D. Ziak, M. Labuda, K. Kurtenbach 2003. Association of *Borrelia afzelii* with rodents in Europe. – *Parasitology*, **126**: 11-20.

Heyl J, P. G. de Mendonça 2009. Contribution of roe deer to the epidemiology of anaplasmosis – new methodology and preliminary results. – *Beiträge zur Jagd- und Wildforschung,* **34** : 461-464.

Heyl J, P. G. de Mendonça 2011. Tick infestation in roe deer (*Capreolus capreolus*) from Thuringia (Germany). – *Acta zoologica bulgarica*, **63** (3) 313-317.

Humair P. F., O. Peter, R. Wallich, L. Gern 1995. Strain variation of Lyme spirochetes isolated from *Ixodes ricinus* ticks and rodents collected in two endemic areas in Switzerland. – *Journal of Medical Entomology*, **32**: 433-438.

Jenkins A., K. Handeland, S. Stuen, L. Schouls, I. van de Pol, R.-T. Meen, B.-E. Kristiansen 2001. Ehrlichiosis in a moose calf in Norway. – *Journal of Wildlife Diseases*, **37**: 201-203.

Johansson K.-E., B. Pettersson, M. Uhlén, A. Gunnarsson, M. Malmqvist, E. Olsson 1995. Identification of the causative agent of granulocytic ehrlichiosis in Swedish dogs and horses by direct solid phase sequencing of PCR products

from the 16S rRNA gene. – *Research in Veterinary Science*, **58**: 109-112.

Joncour G., E. Collin, B. Courtay 2005. Dairy cows as bio-indicators of *Anaplasma phagocytophilum* prevalence, agent of tick-borne fever, in France. Presentation to the 5th International Conference on Ticks and Tick-borne Pathogens, University of Neuchâtel, Switzerland, 29.8-2.9.2005.

Jones L. D., C. R. Davies, G. M. Steele, P. A. Nuttall 1987. A novel mode of arbovirus transmission involving a nonviremic host. – *Science*, **237:** 775-777.

Jones L., W. R. Kaufman, P. A. Nuttall 1992. Modification of the skin feeding site by tick saliva mediates virus transmission. – *Experientia*, **48:** 779-782.

Kocan K. M., J. de la Fuente 2003. Co-feeding studies of ticks infected with *Anaplasma marginale*. – *Veterinary Parasitology*, **112**; 295–305.

Kurtenbach K., H.-S. Sewell, N. H. Ogden, S. E. Randolph, P. A. Nuttal 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. – *Infection and Immunity*, **66**: 1248-1251.

Labuda M., J. M. Austyn, E. Zuffova, O. Kozuch, N. Fuchsberger, J. Lysy, P.A. Nuttall 1996. Importance of localized skin infection in tick-borne encephalitis virus transmission. – *Virology*, **219:** 357-366.

Labuda M., O. Kozuch, E. Zuffova, E. Eleckova, R. S. Hails, P. A. Nuttall 1997. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. – *Virology*, **235:** 138-143.

Labuda M., L. D. Jones, T. Williams, V. Danielova, P. A. Nuttall 1993. Efficient transmission of tick-borne encephalitis virus between cofeeding ticks. – *Journal of Medical Entomology*, 30**:** 295-299.

Labuda M., P. A. Nuttall, O. Kozuch, E. Eleckova, T. Williams, E. Zuffova, A. Sabo 1993. Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. – *Experientia*, **49:** 802-805.

Liz J. S., J. W. Sumner, K. Pister, M. Brossard 2002. PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). – *Journal of Clinical Microbiology*, **40**: 892-897.

Matsumoto K., G. Joncour, B. Davoust, P.-H. Pitel, A. Chauzy, E. Collin, H. Morvan, N. Vassalo, P. Brouqui 2006. *Anaplasma phagocytophilum* infection in cattle in France. – *Annals of the New York Academy of Sciences*, **1078**: 491-494.

de Mendonça P. G., A.-M. Benedek, M. Jurčovičová 2011. Molecular screening of European wild rodents for tick-borne encephalitis virus. – *Acta zoologica bulgarica*, **63** (2): 195-197.

de Mendonça P. G., A. Kupca, J. Raczynski, M. Rinder, K. Pister 2008. Novel approaches to the epidemiology of anaplasmosis. Presentation to the Annual Parasitology Meeting of the German Veterinary Society, Celle, 9-11.7.2008.

Meylan A. 1995. *Microtus arvalis*. (Pallas, 1778). – In: Hausser J. (Ed.): Mammifères de la Suisse, Mémoires de l'Académie Suisse des Sciences Naturelles, **103**: 328-333.

Naranjo V, Ruiz-Fons F, Höfle U, Fernández de Mera IG, Villanúa D, Almazán C, Torina A, Caracappa S, Kocan KM, Gortázar C, de la Fuente J 2006. Molecular epidemiology of Human and bovine anaplasmosis in Southern Europe. – *Annals of the New York Academy of Sciences*, **1078**: 95-99.

Park KH, Lim JA, Kim JH, Park EU 1999. Study on pathogenicity of *Borrelia burgdorferi* sensu lato isolated in Korea. – *Journal of the Korean Society of Microbiology*, **34**: 471-478.

Paulauskas A., D. Ambrasiene, J. Radzijevskaja, O. Rosef, J. Turcinaviciene 2008. Ticks, rodents and tick-borne diseases in Lithuania and Norway. – *Parasitology Research*, **103** (s. 1): 154.

Pichon B., D. Egan, M. Rogers, J. Gray 2003. Detection and identification of pathogens and host DNA in unfed host-seeking *Ixodes ricinus* L. (Acari: Ixodidae). – *Journal of Medical Entomology*, **40**: 723-731.

Polin H., P. Hufnagl, R. Haunschmid, F. Gruber, G. Ladurner 2004. Molecular evidence of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks and wild animals in Austria. – *Journal of Clinical Microbiology*, **42**: 2285-2286.

Poupon M.-A., E. Lommano, P.-F. Humair, V. Douet, O. Rais, M. Schaad, L. Jenni, L. Gern 2006. Prevalence of *Borrelia burgdorferi* Sensu Lato in Ticks Collected from Migratory Birds in Switzerland. – *Applied and Environmental Microbiology*, **72** (1): 976-979.

Randolph S. E., C. Chemini, C. Furlanello, C. Genchi, R. S. Hails, P. J. Hudson, L. D. Jones, G. Medley, R. A. Norman, A. P. Rizzoli, G. Smith, E. J. Woolhouse 2002. The ecology of tick-borne infections in wildlife reservoirs. – In: Hudson P. J., A. P. Rizzoli, B. T. Grenfell, H. Heesterbeek, A. P. Dobson (Eds.): The ecology of wildlife diseases. Oxford University Press, Oxford, 119-138.

Rijpkema S. G. T., M. J. C. H. Molkenboer, L. M. Schouls, F. Jongejan, J. F. P. Schellekens 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. – *Journal of Clinical Microbiology*, **33**:3091-3095.

Sempéré A. J., V. E. Sokolov, A. A. Danilkin 1996. *Capreolus capreolus*. – *Mammalian Species*, **538**: 1-9.

Skotarczak B., M. Adamska, M. Sawczuk, A. Maciejewska, B. Wodecka, A. Rymaszewska 2008. Coexistence of tick-borne pathogens in game animals and ticks in western Poland. – *Veterinarni Medicina*, **53**: 668-675.

Stuen S., E. Olsson Engvall, I. van de Pol, L. M. Schouls 2001. Granulocytic Ehrlichiosis in a Roe Deer Calf in Norway. – *Journal of Wildlife Diseases*, **37**: 614-616.

van der Heijden K. M., M. P. J. Szabo, M. I. Egami, M. Campos Pereira, E. R. Matushima 2005. Histopathology of tick-bite lesions in naturally infested capybaras (*Hydrochoerus hydrochaeris*) in Brazil. – *Experimental and Applied Acarology*, **37**: 245-255.

Woldehiwet Z. 2006. *Anaplasma phagocytophilum* in ruminants in Europe. – *Annals of the New York Academy of Sciences*, **1078**: 446-460.

Zeman P., M. Pecha 2008. Segregation of genetic variants of *Anaplasma phagocytophilum* circulating among wild ruminants within a Bohemian forest (Czech Republic). – *International Journal of Medical Microbiology*, **298**: 203-210.

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