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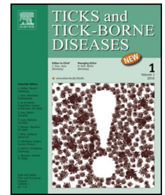

Original article

Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus* Neoehrlichia mikurensis, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*?

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ABSTRACT

In Europe, in addition to *Borrelia burgdorferi* sensu lato and tick-borne encephalitis (TBE) virus, other zoonotic pathogens, like *B. miyamotoi*, a species related to the relapsing fever spirochaetes, *Candidatus* Neoehrlichia mikurensis (*N. mikurensis*), *Rickettsia helvetica*, *Rickettsia monacensis*, and *Anaplasma phagocytophilum* have been reported in the ixodid tick *Ixodes ricinus*. No study was conducted to identify reservoir hosts for these pathogens. Here, we investigated the role played by wild rodents in the natural transmission cycle of *B. miyamotoi*, *N. mikurensis*, *R. helvetica*, *R. monacensis*, and *A. phagocytophilum* in Switzerland. In 2011 and 2012, small mammals were captured in an area where these pathogens occur in questing ticks. *Ixodes ricinus* ticks infesting captured small mammals were analysed after their moult by PCR followed by reverse line blot to detect the different pathogens. Xenodiagnostic larvae were used to evaluate the role of rodents as reservoirs and analysed after their moult. Most of the 108 captured rodents (95.4%) were infested by *I. ricinus* ticks; 4.9%, 3.9%, 24.0%, and 0% of the rodents were infested by *Borrelia*, *N. mikurensis*, *Rickettsia* spp., and *A. phagocytophilum*-infected larvae, respectively. *Borrelia afzelii*, *B. miyamotoi*, *N. mikurensis*, *Rickettsia* spp., and *A. phagocytophilum* were detected in 2.8%, 0.17%, 2.6%, 6.8%, and 0% of the ticks attached to rodents, respectively. *Borrelia afzelii* was transmitted by 4 rodents to 41.2% of the xenodiagnostic ticks, *B. miyamotoi* by 3 rodents to 23.8%, and *N. mikurensis* was transmitted by 6 rodents to 41.0% of the xenodiagnostic ticks. None of the tested rodent transmitted *Rickettsia* spp. or *A. phagocytophilum* to *I. ricinus* xenodiagnostic larvae. This study showed that rodents are reservoir hosts for *B. miyamotoi* and *N. mikurensis* in Europe.

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Introduction

In Europe, the hard tick *Ixodes ricinus* is the vector of pathogens such as viruses, protozoa, and bacteria. During the last decades, new molecular tools revealed an even larger spectrum of tick-borne pathogens in questing ticks, ticks collected from various hosts and in host organs.

The *Borrelia burgdorferi* sensu lato complex is an important group of bacteria transmitted by *I. ricinus*. At least 10 *B. burgdorferi* sensu lato species have been described in *I. ricinus* (*B. burgdorferi* sensu stricto (s.s.), *B. afzelii*, *B. garinii*, *B. bavariensis*, *B. spielmanii*, *B. valaisiana*, *B. finlandensis*, *B. bissettii*, *B. carolinensis*, *B. lusitaniae*). Most of them are known to cause Lyme borreliosis (Rudenko et al., 2011) and have known reservoir hosts (Piesman and Gern, 2004). Recently, another *Borrelia* species, *B. miyamotoi* belonging to the relapsing fever group usually transmitted by soft ticks (Argasidae) has also been reported in hard ticks (Ixodidae). First recognized in *Ixodes persulcatus* in Japan (Fukunaga et al., 1995), *B. miyamotoi* was then described in *Ixodes scapularis* (Scoles et al., 2001), *Ixodes dentatus* (Scoles et al., 2001; Hamer et al., 2012), and *Ixodes pacificus* (Mun et al., 2006) in the USA, and in *I. ricinus* in Europe (Fraenkel et al., 2002; Richter et al., 2003; Gern et al., 2010). *Borrelia miyamotoi* was detected in one tick removed from human in Sweden (Wilhelmsson et al., 2010), and clinical cases associated with *B. miyamotoi* have been recently reported in Russia (Karan et al., 2010; Platonov et al., 2011) and in North America (Gugliotta et al., 2013). *Borrelia miyamotoi* was reported in the mouse *Peromyscus leucopus* in North America (Bunikis and Barbour, 2005; Hamer et al., 2010), in *Apodemus argenteus* (Fukunaga et al., 1995) and *Apodemus speciosus*, *Myodes rufocanus*, *Myodes rutilus* (Taylor et al., 2013) in Japan, and in wild turkey, *Meleagris gallopavo* (Scott et al., 2010), and in ticks removed from the Eastern chipmunk, *Tamias striatus* (Hamer et al., 2010) in North America. In Europe, *B. miyamotoi* was observed in *Cervus elaphus* (Wodecka, 2007) and

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domestic ruminants (Richter and Matuschka, 2010). Ticks infected by *B. miyamotoi* were also collected on 4 species of passerine birds (Fukunaga et al., 1995; Hamer et al., 2012). *Borrelia miyamotoi* is known to be transovarially transmitted from *I. scapularis* females to their progeny, and *P. leucopus* has been identified as a reservoir host in North America (Scoles et al., 2001).

Rickettsiae of the spotted fever group (SFG) are known as obligate intracellular Gram-negative bacteria. Two *Rickettsia* species that belong to the SFG group, *R. helvetica* (Beati et al., 1993) and *R. monacensis* (Simser et al., 2002) are frequently reported in questing *I. ricinus* (e.g., Sprong et al., 2009; Lommano et al., 2012; Overzier et al., 2013), in ticks feeding on various hosts (e.g., Burri et al., 2011; De Sousa et al., 2012; Speck et al., 2013; Overzier et al., 2013) and in host tissues (Sprong et al., 2009; Schex et al., 2011; De Sousa et al., 2012). Whether *R. helvetica* is pathogenic in humans remains unclear, although cardiac and neurologic problems have been reported (Nilsson et al., 1999, 2010). Transovarial transmission of *R. helvetica* in *I. ricinus* has been reported to be very frequent and effective (Burgdorfer et al., 1979), whereas nothing is known on the transovarial transmission of *R. monacensis*.

In the late 1990s a new bacteria, *Candidatus* Neoehrlichia mikurensis (*N. mikurensis*), first named *Ehrlichia*-like belonging to the family of Anaplasmatacae was described in *Ixodes ovatus* in Japan (Kawahara et al., 2004). Thereafter, *N. mikurensis* was detected in field-collected *I. ricinus* ticks first in the Netherlands (Schouls et al., 1999), followed by reports from other European countries (Sanogo et al., 2003; Lommano et al., 2012; Silaghi et al., 2012). *Candidatus* Neoehrlichia *mikurensis* was also isolated from *I. frontalis* ticks fed on one migratory bird (Movila et al., 2013). *Candidatus* Neoehrlichia *mikurensis* appeared as pathogenic for humans and was identified in the blood of febrile patients in different countries in Europe (Fehr et al., 2010; von Loewenich et al., 2010; Welinder-Olsson et al., 2010; Maurer et al., 2013) and in China (Li et al., 2012) as well as from one dog in Germany (Diniz et al., 2011). *Candidatus* Neoehrlichia *mikurensis* was detected in tissues of wild rodents in Japan (Kawahara et al., 2004; Naitou et al., 2006) and in Europe (Beninati et al., 2006; Andersson and Raberg, 2011; Jahfari et al., 2012; Lommano et al., 2012; Silaghi et al., 2012; Vayssier-Taussat et al., 2012). Few investigations on the transovarial transmission of *N. mikurensis* have been undertaken (Jahfari et al., 2012).

Initially known to infect livestock, *Anaplasma phagocytophilum* was later recognized to be pathogenic for humans (Stuen et al., 2013). *Anaplasma phagocytophilum* is genetically diverse, has a broad host range, and has been detected in several tick species (Bown et al., 2003, 2007; Rar and Golovljova, 2011; Baráková, I., Derdáková, M., Carpi, G., Collini, M., Rosso, F., Tagliapietra, V., Hauffe, H. C., Rizzoli, A. (personal communication)). Studies on the reservoir competence of rodents showed that their role remains unclear (Stuen et al., 2013). Transstadial transmission of the bacterium can occur, but transovarial transmission has not been demonstrated (Rar and Golovljova, 2011; Stuen et al., 2013).

Despite the frequent reports of these new pathogens, the natural cycle of most of them remains unknown. More specifically, the identification of reservoir hosts responsible for their maintenance in nature has been neglected. Reservoir hosts for tick-borne pathogens must fulfil different criteria. One criterion is that it must be a source of infection for ticks and allow the pathogen to be transmitted to ticks feeding on them (Kahl et al., 2002). The fact that pathogens are detected in host organs determines carrier hosts and not reservoir hosts since it does not mean that these hosts are infective for ticks (Kahl et al., 2002). Similarly, the detection of pathogens in ticks attached on hosts does not demonstrate unambiguously that pathogens have been transmitted from the hosts to the ticks and hereby their reservoir role. The aim of the present study was to investigate whether wild rodents act as reservoir hosts for *B. miyamotoi*, *R. helvetica*, *R. monacensis*, *N. mikurensis*, and *A. phagocytophilum* in natural cycles.

Materials and methods

Study site

This study was carried out in a mixed deciduous forest named Bois de l'Hôpital, located at 600 m above sea level (Neuchâtel, Switzerland) (47°00′23.67″N; 6°56′50.00″E). *Borrelia miyamotoi*, *B. burgdorferi* s.l., *A. phagocytophilum*, *N. mikurensis*, and *Rickettsia* spp. were reported in questing *I. ricinus* ticks in this forest (Gern et al., 2010; Lommano et al., 2012).

Rodent trapping and tick collection

Rodents were trapped in September, October, and November 2011 and in April and May 2012. For each trapping session, 100 wooden box traps (Czech trap model, http://members.vienna.at/shrew/trapping.html) were used spaced at 5–10 m intervals and baited with seeds, pieces of apple, and hay. Traps were set at sunset and checked early the following morning. The Department of Agriculture and Nature from Canton of Neuchâtel approved the capture of rodents. For maintenance and all experimental procedures, the Department of Economy of Canton Neuchâtel delivered authorization 2/2009. Captured rodents were brought to the laboratory to be identified, sexed, and caged individually over a pan of water until the engorged ticks dropped off. Engorged ticks were collected from water, dried, identified (Cotty, 1985), and placed in tubes at 98% RH and room temperature until moult. One month after moult, ticks were washed in 70% ethanol and stored at −20 °C until investigation for *Borrelia* spp., *N. mikurensis*, *Rickettsia* spp., and *A. phagocytophilum* infection. A maximum of 10 nymphs per captured rodent was analysed.

Xenodiagnosis

After all feeding ticks had dropped off, a xenodiagnosis was performed on rodents to evaluate their reservoir capacity for *Borrelia* spp., *Rickettsia* spp., and *N. mikurensis*. Approximately 50 *I. ricinus* larvae, from our laboratory colony (Graf, 1978) free of pathogen infection, were placed on the head of each rodent and allowed to feed until repletion. Engorged ticks were maintained as described in the "rodent trapping and tick collection" section and were examined as moulted nymphs (maximum of 10 nymphs per rodents) for *Borrelia* spp., *Rickettsia* spp., *N. mikurensis*, and *A. phagocytophilum*. Rodents were then released at the trapping site. To confirm the absence of *Borrelia* spp. and *N. mikurensis* in the ticks used for xenodiagnoses we screened 200 larvae derived from 5 females from the laboratory colony. These larvae were tested in pools containing 20 individuals.

DNA isolation from blood

After tick drop-off, rodents were anaesthetized intramuscularly with 0.03 ml of Xylasol (GRAEUB, Bern, Switzerland) (0.02 ml) and Ketasol-100 (GRAEUB) (0.01 ml), and blood was obtained from the retro-orbital sinus using Pasteur pipettes (1.1 mm diameter). A volume of 125 μl of each blood sample was placed directly on FTA Classic Cards (Whatman®, Buckinghamshire, UK). Cards were left open for 24 h and stored as indicated by the manufacturer. To obtain DNA, 1.2 mm diameter discs were punched into FTA card blood spot by using a Harris Micro-Punch® (Whatman®, Buckinghamshire, UK). Between samples, the tip of the Harris Micro-Punch® was rinsed in bleach and 70% alcohol and dried with sterile wipe. Discs were washed 3 times in 200 μl FTA purification reagent

(Whatman®, Buckinghamshire, UK) and twice in 200 μl house-made TE-1 buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 8.0) in 300-μl tubes as recommended by the manufacturer. Discs were dried at 56 °C in a stove and immediately used for PCR/RLB (reverse line blot) (see below).

DNA isolation from ticks and identification of Borrelia spp., Rickettsia spp., N. mikurensis, and A. phagocytophilum

DNA was isolated from ticks fed on rodents and xenodiagnostic ticks by alkaline hydrolysis (Gern et al., 2010). A polymerase chain reaction (PCR) followed by RLB was used for the identification of *Borrelia* spp., *N. mikurensis*, and *Rickettsia* spp. For *Borrelia* genospecies, the amplification target was the variable spacer region between 2 repeated copies of the *23S* and *5S* ribosomal genes (Alekseev et al., 2001). For *N. mikurensis*, the partial *16S* rRNA gene spanning the V1 region of *Anaplasma* spp. and *Ehrlichia* spp. was amplified according to Schouls et al. (1999) and modified from Bekker et al. (2002). For *Rickettsia* spp., the *23S-5S* internal spacer gene was amplified according to Jado et al. (2006). All PCR amplifications were run in a Tgradient Thermocycler 96 (Whatman Biometra, Göttingen, Germany) by using a touchdown PCR program (for *Borrelia* spp.: Burri et al., 2007; for *Ehrlichia*: Bekker et al., 2002; for *Rickettsia* spp.: Jado et al., 2006). A real-time PCR targeting the *msp2* gene was used to amplify *A. phagocytophilum* according to Courtney et al. (2004). Isolates of *B. garinii* (NE11) and *B. valaisiana* (VS116) were used as positive controls for *Borrelia* spp., *A. phagocytophilum* (Webster strain, kindly provided by Ana Sofia Santos, CEVDI, Portugal) and *R. conorii* (kindly provided by Simona Casati, ICM, Bellinzona, Switzerland) for *N. mikurensis* and *Rickettsia* spp., respectively, and pure water replaced DNA samples for negative controls. For identification of *Borrelia* genospecies by RLB, PCR products were hybridized on 15 oligonucleotides probes (Rijpkema et al., 1995; Poupon et al., 2006; Gern et al., 2010) blotted in line on an activated Biodyne® C membrane (Pall Europe Ltd., Portsmouth, UK) placed in a Miniblotter 45 (Immunetic, Cambridge, MA, USA). Detection and identification of *N. mikurensis* and *Rickettsia* spp. by RLB were performed following the procedure described in Schouls et al. (1999) for *N. mikurensis* and Burri et al. (2011) modified after Jado et al. (2006) for *Rickettsia* spp. Hybridization was visualized by exposure of the membrane with an X-ray film (Hyperfilm, GE Healthcare, UK) after adding chemiluminescence liquid detection (Amersham Biosciences, Europe, Switzerland). All samples were first tested in pools of maximum 10 ticks for *Borrelia* spp., *Rickettsia* spp., and *N. mikurensis*. Ticks from positive pools were further analysed individually. *Candidatus* Neoehrlichia *mikurensis* was also detected using a real-time PCR targeting *GroEsl* gene according to Jahfari et al. (2012). Briefly, primers and dNTPs all 10 μM, 4.8 μl $MgCl_2$ (25 mM), 0.15 μl KapaTaq hotstart (Kapabiosystems by Labgene Scientific) (0.75U per test), 4 μl Kapa Buffer (5×), and 2 μl of DNA sample were used in a reaction volume of 20 μl in an iCycler (Biorad). For each reaction, 2 μl of *N. mikurensis* DNA sample that has been tested positive by RLB were used as a positive control. Pure water was used as a negative control. The cycling conditions started with an initial activation at 95 °C for 5 min followed by 60 cycles at 95 °C for 5 s and 60 °C for 30 s.

DNA sequencing

For *B. miyamotoi* sequencing, a 437-bp product of *flagellin* partial gene was designed to amplify *B. miyamotoi* samples that were detected positive by PCR/RLB. Primers and dNTPs all 10 μM (1 μl Flafor 5′-CAGAYAGAGGTTCTATWCA-3′, 1 μl Flarev 5′-GCTCTTTGATCASTTAYCA-3′, 1 μl dNTPs), 0.25 μl $MgCl_2$ (25 mM), 0.3 μl Taq (Qiagen) (1.5U per test), 4 μl Buffer (10×), and 10 μl of DNA sample were used in a reaction volume of 50 μl. The amplification conditions started with an initial activation at 94 °C for 3 min followed by 50 cycles at 94 °C for 15 s, 50 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. In addition, a 532-bp fragment of the *p66* partial gene was amplified to sequence *B. miyamotoi* (Fomenko et al., 2010). Briefly, 2 sets of external and inner primers, M1F and M2R, M3F and M4R, respectively, were used in a final volume of 25 μl with 3 μl of DNA at the following conditions: 35 cycles at 94 °C for 5 s, 50 °C for 10 s, 72 °C for 30 s. For the nested PCR conditions, the denaturation, annealing, and elongation time was increased to 10 s, 15 s, and 45 s, respectively. For *N. mikurensis*, a 426-bp fragment of the *16S* rRNA partial gene was sequenced. Samples of *B. miyamotoi* and *N. mikurensis* were purified with a kit (Promega, Madison, WI) and sent to Microsynth AG (Balgach, Switzerland) for sequencing. Each obtained sequence was compared with sequences from an international database (NCBI BLAST).

Statistical analysis

Statistical analyses were performed using R 2.15.1 (R Development Core Team, 2012). A 95% confidence interval (CI) was calculated for prevalence of pathogens. A Chi-square test was used to compare the moulting success of ticks infesting captured voles and mice. Fisher's exact test was used to compare infections in voles and mice.

Results

A total of 108 rodents belonging to *A. flavicollis* ($n = 45$), *A. sylvaticus* ($n = 35$), *Apodemus* spp. ($n = 9$), and *M. glareolus* ($n = 19$) was captured. All ticks that detached from the 103 rodents that were infested by ticks (95.4%, 103/108, 95% CI: 91.4–99.3%) were identified as *I. ricinus*. All were larvae except one which was a nymph. The mean infestation level was 12.7 ± 16.5 standard deviation (SD) larvae for *A. sylvaticus*, 8.9 ± 8.9 SD for *A. flavicollis*, 6.6 ± 5.8 SD for undetermined *Apodemus*, and 9.7 ± 13.3 SD larvae for *M. glareolus*.

Moulting success of the 1086 larvae fed on the 103 infested rodents was 81% ($n = 878$) (95% CI: 78.5–83.2%) and was higher for ticks fed on *M. glareolus* (89%, 95% CI: 84–93%) than for those fed on *Apodemus* species (79%, 95% CI: 76.6–81.9%), chi-square test $p = 0.004$. A total of 575 larvae was screened as nymphs for the presence of *Borrelia* spp., *N. mikurensis*, *Rickettsia* spp. and *A. phagocytophilum*. Some of the captured individuals died, hence blood samples from 100 rodents could be collected and screened for pathogens, and xenodiagnosis could be performed on 94 rodents. Moulting success on the 4041 xenodiagnostic larvae fed on 94 rodents was 71% ($n = 2886$) (95% CI: 70–72.8%) and was higher for ticks fed on *Apodemus* species (73%, 95% CI: 71.3–74.1%) than on *M. glareolus* (57%, 95% CI: 51.9–62.4%), chi-square test $p < 0.005$. A total of 841 xenodiagnostic ticks was analysed after their moult. Tables 1–3 show details on rodents that were infected by *Borrelia* spp., *N. mikurensis*, and/or *Rickettsia* spp., respectively, or that carried infected ticks. Screening of 200 unfed larvae from the laboratory colony which were part of batches used for xenodiagnoses showed that none of them were infected.

Borrelia spp. infection

Among ticks fed on the 103 rodents that were infested by ticks, 3% (17/575, 95% CI: 1.6–4.3%) were infected by *Borrelia* spp. (Table 1). *Borrelia afzelii* infected 2.8% (16/575, 95% CI: 1.4–4.1%) of the ticks, and *B. miyamotoi* 0.17% (1/575, 95% CI: 0–0.5%). These *Borrelia*-infected ticks were collected from 5 captured rodents (4.9%, 5/103, 95% CI: 0.7–9%). Ticks from these 5 rodents showed an infection prevalence of 42.5% (17/40, 95% CI: 27.2–57.8%). Only one

Table 1
Apodemus spp. and *Myodes glareolus* individuals displaying *Borrelia* spp. infection in blood, in ticks fed on them, and/or in xenodiagnostic ticks.

Sample ID	Rodent species	Blood sample	Infected ticks fed on rodents in nature/Total ticks analysed	Infected xenodiagnostic ticks/Total ticks analysed	*Borrelia* genospecies
17	*A. sylvaticus*	Negative	5/10	6/10	*B. afzelii*
23	*A. flavicollis*	Negative	4/10	6/10	*B. afzelii*
67	*M. glareolus*	Positive	0/10	1/4	*B. afzelii*
72	*M. glareolus*	Negative	2/6	0/7	*B. afzelii*
92	*M. glareolus*	Negative	5/10	1/10	*B. afzelii*
60	*M. glareolus*	Negative	0/2	3/10	*B. miyamotoi*
77	*A. flavicollis*	Negative	0/10	1/10	*B. miyamotoi*
80	*M. glareolus*	Negative	1/4	1/1	*B. miyamotoi*

Table 2
Apodemus spp. and *Myodes glareolus* individuals displaying *N. mikurensis* infection in blood, in ticks fed on them, and/or in xenodiagnostic ticks. ND, no data.

Sample ID	Rodent species	Blood sample	Infected ticks fed on rodents in nature/Total ticks analysed	Infected xenodiagnostic ticks/Total ticks analysed
13	*Apodemus* spp.	ND	5/10	ND
17	*A. sylvaticus*	Positive	0/10	9/10
18	*A. sylvaticus*	Positive	0/10	0/10
45	*A. sylvaticus*	Positive	0/1	3/10
51	*M. glareolus*	Positive	0/1	7/10
67	*M. glareolus*	Positive	2/10	1/4
73	*M. glareolus*	Positive	7/10	0/10
74	*A. flavicollis*	Positive	0/7	1/10
112	*A. sylvaticus*	Positive	1/4	1/10

rodent displayed a *Borrelia* infection in the blood (Table 1). Xenodiagnosis performed on 94 rodents showed that 7 of them (7.4%, 95% CI: 1.4–11.3%) transmitted *Borrelia* spp. to ticks (Table 1). Four rodents (one *A. flavicollis*, one *A. sylvaticus*, and 2 voles) transmitted *B. afzelii* to 41.2% (14/34, 95% CI: 24.63–57.72) of the xenodiagnostic ticks (Table 1). In addition, 3 rodents (2 *M. glareolus* and one *A. flavicollis*) transmitted *B. miyamotoi* to 23.8% (5/21, 95% CI: 5.6–42%) of the xenodiagnostic ticks (Table 1). Sequencing of the partial *p66* and *flagellin* genes of *B. miyamotoi* isolates from one tick fed on captured rodents and 3 xenodiagnostic ticks from 3 rodents yielded identical sequences. The *p66* gene sequences of *B. miyamotoi* (GenBank accession numbers KF054067, KF054068, KF054069, KF054070) shared 100% homology with the sequence AY363723, and the *flagellin* gene sequences (GenBank accession numbers KF054063, KF054064, KF054065, KF054066) were 100% identical to JF951386.1.

Candidatus Neoehrlichia mikurensis infection

Screening of ticks that were fed on the 103 rodents showed that 2.6% (15/575, 95% CI: 1.3–3.9%) of ticks were infected by *N. mikurensis* (Table 2). Infected ticks were collected from 4 captured rodents (3.9%, 4/103, 95% CI: 0.2–7.6%). Ticks feeding on these 4 rodents showed an infection rate of 44% (15/34, 95% CI: 27.4–60.8%). *Candidatus* Neoehrlichia *mikurensis* was also detected in the blood of 8/100 rodents (Table 2). Among the 94 rodents submitted to

Table 3
Apodemus spp. and *Myodes glareolus* individuals displaying *Rickettsia* spp. infection in blood, in ticks fed on them, and/or in xenodiagnostic ticks. ND, no data.

Sample ID	Rodent species	Blood sample	Infected ticks fed on rodents in nature/total ticks analysed	Infected xenodiagnostic ticks/total ticks analysed	*Rickettsia* species
12	*A. sylvaticus*	Negative	1/10	0/10	*R. helvetica*
13	*Apodemus* spp.	ND	1/10	ND	*R. helvetica*
15	*A. sylvaticus*	Negative	1/10	0/10	*R. monacensis*
16	*A. flavicollis*	Negative	1/3	ND	*R. helvetica*
18	*A. sylvaticus*	Negative	1/10	0/10	*R. helvetica*
21	*A. flavicollis*	Negative	1/9	0/10	*R. helvetica*
26	*A. sylvaticus*	ND	1/7	ND	*R. helvetica*
29	*A. flavicollis*	Negative	1/10	0/10	*R. helvetica*
30	*A. sylvaticus*	Negative	2/10	0/10	*R. helvetica*
31	*A. flavicollis*	Negative	1/10	0/10	*R. helvetica*
33	*M. glareolus*	Negative	1/6	0/10	*R. helvetica*
38	*A. flavicollis*	Negative	1/10	0/10	*R. helvetica*
44	*M. glareolus*	Negative	2/5	0/10	*R. helvetica*
59	*A. sylvaticus*	Negative	1/10	0/10	*R. helvetica*
73	*M. glareolus*	Negative	1/10	0/10	*R. helvetica*
88	*A. sylvaticus*	Negative	1/10	0/10	*R. helvetica*
92	*M. glareolus*	Negative	3/10	0/10	*R. helvetica*
93	*A. sylvaticus*	Negative	1/3	0/10	*R. helvetica*
94	*A. sylvaticus*	Negative	1/7	0/10	*R. helvetica*
95	*M. glareolus*	Negative	4/7	0/10	*R. helvetica*
99	*A. flavicollis*	Negative	1/4	0/10	*R. helvetica*
103	*A. sylvaticus*	Negative	1/4	0/10	*R. helvetica*
104	*A. sylvaticus*	Negative	3/5	0/10	*R. helvetica*
112	*A. sylvaticus*	Negative	3/4	0/10	*R. helvetica*
116	*A. sylvaticus*	Negative	4/4	0/10	*R. helvetica*

xenodiagnoses, 6 (6.4%, 95% CI: 1.4–11.3%) transmitted *N. mikurensis* to 41% (22/54, 95% CI: 27.6–53.8%) of xenodiagnostic ticks (Table 2). Sequencing of the partial *16S* rRNA gene of one blood sample infected with *N. mikurensis* (GenBank accession number KF054062) showed 100% homology with the sequence JQ014620.1.

Rickettsia spp. infection

Finally, 6.8% (39/575, 95% CI: 4.7–8.8%) of ticks fed on the 103 infested rodents were infected with *Rickettsia* spp., most of them with *R. helvetica* (n = 38) and one with *R. monacensis* (Table 3). *Rickettsia*-infected ticks were collected from 25 captured rodents (24%, 25/103, 95% CI: 16–32.5%). Ticks fed on these individuals showed an infection prevalence of 20.7% (39/188, 95% CI: 14.9–26.5%). No *Rickettsia* spp. was detected in the blood samples of the 100 examined rodents (Table 3). None of the rodents transmitted *R. helvetica* or *R. monacensis* to xenodiagnostic ticks (Table 3).

Anaplasma phagocytophilum infection

Anaplasma phagocytophilum infection was detected neither in ticks that were fed on captured rodents nor in xenodiagnostic ticks. Blood of rodents was not investigated for *A. phagocytophilum* because FTA cards could not be processed in the RT-PCR system available in our laboratory.

Co-infections with different pathogens

Co-infections with more than one pathogen species were observed in the blood, in ticks feeding on captured rodents, and in xenodiagnostic ticks. One *M. glareolus* (Sample ID 67) displayed a co-infection with *B. afzelii* and *N. mikurensis* in the blood (Tables 1 and 2). Two ticks attached on 2 captured rodents (Sample ID 13 and 112) were co-infected with *N. mikurensis* and *R. helvetica* (Tables 2 and 3), and 2 ticks fed on another rodent (Sample ID 92) were co-infected with *R. helvetica* and *B. afzelii* (Tables 1 and 3). Finally, one *A. sylvaticus* (Sample ID 17) transmitted both *B. afzelii* and *N. mikurensis* to xenodiagnostic ticks (Tables 1 and 2).

In the studied rodent population, 53% of *M. glareolus* voles (10/19) versus 28% *Apodemus* spp. mice (25/89) displayed infection by at least one pathogen in their blood and/or in ticks that fed on them. The difference was almost significant (Fisher's exact test p = 0.057).

Discussion

Currently, many studies on tick-borne pathogens focus mainly on the identification of emerging pathogens, like *B. miyamotoi*, *R. helvetica*, *R. monacensis*, *N. mikurensis*, and *A. phagocytophilum* in ticks, organs, or blood of different hosts, but very few have considered the identification of hosts acting as reservoirs in nature. Classically, 2 methods are used to identify reservoir hosts of tick-borne pathogens. The first approach consists in comparison of the infection rate of questing larvae with that of larvae fed on suspected hosts in the same habitat. A higher infection rate in larvae feeding on tested hosts is a good indication that the host is a reservoir. However, this method is not strong enough particularly for pathogens that are transovarially transmitted from the female to the eggs, which gives rise to infected questing larvae. In contrast, the second approach, xenodiagnosis, used in this study, appears more appropriate. It consists of feeding uninfected ticks, usually larvae from a laboratory colony that is pathogen-free, on the tested host and in analysing them for pathogens after their moult to the next stage (Gern and Humair, 2002). This method gives information on the reservoir role of the tested host.

Since small mammals are important hosts for immature stages of *I. ricinus*, we evaluated their capacity to transmit emerging pathogens to ticks. We performed xenodiagnoses on rodents captured in an area where *Borrelia* spp., *N. mikurensis*, *A. phagocytophilum* and *Rickettsia* spp. were found in questing nymphs (Gern et al., 2010; Lommano et al., 2012). Captured rodents carried ticks infected by *B. afzelii*, *B. miyamotoi*, *N. mikurensis*, *R. helvetica*, and *R. monacensis*. Using xenodiagnoses, we confirmed that *Apodemus* spp. mice and *M. glareolus* transmit *B. afzelii* to ticks (Humair et al., 1999; Pérez et al., 2012). In addition, *Apodemus* spp. mice and *M. glareolus* transmitted *B. miyamotoi* to 23.8% of xenodiagnostic ticks. Nevertheless, it is known that *B. miyamotoi* can be transmitted transovarially in ticks (Scoles et al., 2001; Rollend et al., 2013) and that laboratory colonies of ticks may be infected by this relapsing fever-related spirochaete (Scoles et al., 2001). Control of 200 larvae from our colony as well as regular controls of this colony (data not shown) showed that it is not contaminated by *B. miyamotoi*. In addition, previous examination by immunofluorescence of more than 1300 larvae from the same colony was negative (Bellet-Edimo et al., 2005). We were not successful in the detection of *B. miyamotoi* in blood using PCR/RLB, however observation of blood smears stained with Giemsa allowed observation of spirochaetes in 5 captured rodents, one of which transmitted *B. miyamotoi* to xenodiagnostic ticks (data not shown). Sequencing of *B. miyamotoi* showed that all samples belonged to the European type (Geller et al., 2012).

Xenodiagnosis also allowed us to show that rodents transmitted *N. mikurensis* to uninfected ticks. Only 6.4% of rodents were infective for ticks, but some individuals were very efficient in their infectivity for ticks that reached 70–90%. Eight rodents (8%) were harbouring *N. mikurensis* in their blood, which confirms previous observations (Beninati et al., 2006; Andersson and Raberg, 2011; Silaghi et al., 2012), and 7 of them transmitted *N. mikurensis* either to ticks fed on them when they were captured or to xenodiagnostic ticks. These results suggest that transmission of *N. mikurensis* to ticks is high when rodents are infected. Jahfari et al. (2012) could not detect *N. mikurensis* in questing larvae and, in the present study, examination of unfed larvae from the laboratory colony used for xenodiagnoses showed that none was infected by *N. mikurensis*. These results suggest that transovarial transmission of *N. mikurensis* does not occur.

Recently, some authors reported the detection of *R. helvetica* in the blood of roe deer and wild boar (Sprong et al., 2009), whereas others were not able to detect *R. helvetica* in the spleen and blood of roe deer (Overzier et al., 2013). One study reported the presence of *R. helvetica* in the blood of 29% of mice in the Netherlands (Sprong et al., 2009), but unfortunately the authors did not specify the mouse species, which might be important as mentioned below. In our study, blood samples from all rodents were negative for *R. helvetica* and *R. monacensis*. Interestingly, although 20.7% of larvae attached on captured rodents were infected by *R. helvetica*, none of the xenodiagnostic ticks fed on rodents were found infected by *R. helvetica* or *R. monacensis*, suggesting that rodents do not represent an important source of infection for ticks. To our knowledge, only one study investigated the reservoir role of rodents for *R. helvetica* using xenodiagnosis (Péter, 1984). This author showed that *A. sylvaticus* and *M. glareolus* did not transmit *R. helvetica* to ticks feeding on them, whereas *A. flavicollis* transmitted it very rarely, probably due to a very fast and intense immune answer according to this author. Since transovarial transmission in ticks has been described for *R. helvetica* (Burgdorfer et al., 1979), the infection rate (20.7%) observed in larvae fed on captured rodents was most probably the result of either an extremely short rickettsiaemia or of transovarial transmission. Due to high transovarial transmission of *R. helvetica*, the tick itself is most probably the main reservoir host for this pathogen, as also suggested by Sprong et al. (2009).

In the present study, we assessed wild rodent infectivity for ticks using xenodiagnosis. We showed that *M. glareolus* and *A. flavicollis* transmitted *B. miyamotoi* to uninfected ticks and that these hosts are reservoirs for *B. miyamotoi*. Similarly, we identified *M. glareolus*, *A. sylvaticus*, and *A. flavicollis* as reservoirs for *N. mikurensis*. However, although *R. helvetica* and *R. monacensis* were detected in ticks fed on captured rodents, none of the rodents transmitted these *Rickettsia* species to xenodiagnostic ticks confirming that the investigated rodent species are not important reservoirs for *R. helvetica* (Péter, 1984).

Finally, *A. phagocytophilum* was detected neither in *I. ricinus* ticks infesting captured rodents nor in xenodiagnostic ticks. This is interesting because this pathogen was detected in questing *I. ricinus* ticks in the studied area (Lommano et al., 2012) and because a similar situation has been reported in other sites in Switzerland (Burri et al., 2011). This suggests that questing *I. ricinus* ticks in the studied area have not acquired *A. phagocytophilum* while feeding on *Apodemus* and *Myodes* rodents and that probably other hosts were the source of their infection. However, because the method used to collect blood in this study (FTA cards) revealed to be incompatible with our RT-PCR system, we were unable to find out if rodent blood was infected. Hence, we cannot exclude that rodents in the area are reservoirs for subpopulations of *A. phagocytophilum* maintained by another tick species, like *I. trianguliceps* (Bown et al., 2003, 2009).

In the studied rodent population, *M. glareolus* voles were slightly more often found infected with the studied pathogens in their blood and/or in ticks that fed on them than mice suggesting that voles play an important role in the maintenance of the diversity of these pathogens (unknown for *A. phagocytophilum*) in the studied area.

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References

Alekseev, A.N., Dubinina, H.V., Van de Pol, I., Schouls, L.M., 2001. Identification of *Ehrlichia* spp. and *Borrelia burgdorferi* in *Ixodes* ticks in the Baltic regions of Russia. J. Clin. Microbiol. 39, 2237–2242.

Andersson, M., Raberg, L., 2011. Wild rodents and novel human pathogen *Candidatus* Neoehrlichia mikurensis, southern Sweden. Emerg. Infect. Dis. 17, 1716–1718.

Beati, L., Péter, O., Burgdorfer, W., Aeschlimann, A., Raoult, D., 1993. Confirmation that *Rickettsia helvetica* sp. nov. is a distinct species of the spotted fever group of Rickettsiae. Int. J. Syst. Bacteriol. 43, 521–526.

Bekker, C.P., de Vos, S., Taoufik, A., Sparagano, O.A., Jongejan, F., 2002. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Vet. Microbiol. 89, 223–238.

Bellet-Edimo, R., Betschart, B., Gern, L., 2005. Frequency and efficiency of transovarial and subsequent transstadial transmissions of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks. Bull. Soc. Neuch. Sci. Nat. 128, 117–125.

Beninati, T., Piccolo, G., Rizzoli, A., Genchi, C., Bandi, C., 2006. Anaplasmataceae in wild rodents and roe deer from Trento Province (northern Italy). Eur. J. Clin. Microbiol. Infect. Dis. 25, 677–678.

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., Ogden, N.H., Petrovec, M., Shaw, S.E., Woldehiwet, Z., Birtles, R.J., 2007. High resolution genetic fingerprinting of European strains of *Anaplasma phagocytophilum* by use of multilocus variable-number tandem-repeat analysis. J. Clin. Microbiol. 45, 1771–1776.

Bown, K.J., Lambin, X., Ogden, N.H., Begon, M., Telford, G., Woldehiwet, Z., Birtles, R.J., 2009. Delineating *Anaplasma phagocytophilum* ecotypes in coexisting, discrete enzootic cycles. Emerg. Infect. Dis. 15, 1948–1954.

Bunikis, J., Barbour, A.G., 2005. Third *Borrelia* species in white-footed mice. Emerg. Infect. Dis. 11, 1150–1151.

Burgdorfer, W., Aeschlimann, A., Péter, O., Hayes, S.F., Philip, R.N., 1979. *Ixodes ricinus*: vector of a hitherto undescribed spotted fever group agent in Switzerland. Acta Trop. 36, 357–367.

Burri, C., Morán Cadenas, F., Douet, V., Moret, J., Gern, L., 2007. *Ixodes ricinus* density and infection prevalence of *Borrelia burgdorferi* sensu lato along a North-facing altitudinal gradient in the Rhone Valley (Switzerland). Vector Borne Zoonotic Dis. 7, 50–58.

Burri, C., Dupasquier, C., Bastic, V., Gern, L., 2011. Pathogens of emerging tick-borne diseases, *Anaplasma phagocytophilum*, *Rickettsia* spp., and *Babesia* spp., in *Ixodes* ticks collected from rodents at four sites in Switzerland (Canton of Bern). Vector Borne Zoonotic Dis. 11, 939–944.

Cotty, A., 1985. Clé de détermination des Ixodidae et Amblyommidae de Suisse. University Neuchâtel, Switzerland, Ph.D. thesis.

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.

De Sousa, R., Lopes de Carvalho, I., Santos, A.S., Bernardes, C., Milhano, N., Jesus, J., Menezes, D., Nuncio, M.S., 2012. Role of the lizard *Teira dugesii* as a potential host for *Ixodes ricinus* tick-borne pathogens. Appl. Environ. Microbiol. 78, 3767–3769.

Diniz, P.P., Schulz, B.S., Hartmann, K., Breitschwerdt, E.B., 2011. *Candidatus* Neoehrlichia mikurensis infection in a dog from Germany. J. Clin. Microbiol. 49, 2059–2062.

Fehr, J.S., Bloemberg, G.V., Ritter, C., Hombach, M., Lüscher, T.F., Weber, R., Keller, P.M., 2010. Septicemia caused by tick-borne bacterial pathogen *Candidatus* Neoehrlichia mikurensis. Emerg. Infect. Dis. 16, 1127–1129.

Fomenko, N.V., Livanova, N.N., Borgoiakov, V., Kozlova, I.V., Shulaikina, I.V., Pukhovskaia, N.M., Tokarevich, K.N., Livanov, S.G., Doroshchenko, E.K., Ivanov, L.I., 2010. Detection of *Borrelia miyamotoi* in ticks *Ixodes persulcatus* from Russia. Parazitologiia 44, 201–211.

Fraenkel, C.J., Garpmo, U., Berglund, J., 2002. Determination of novel *Borrelia* genospecies in Swedish *Ixodes ricinus* ticks. J. Clin. Microbiol. 40, 3308–3312.

Fukunaga, M., Takahashi, Y., Tsuruta, Y., Matsushita, O., Ralph, D., McClelland, M., Nakao, M., 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int. J. Syst. Bacteriol. 45, 804–810.

Geller, J., Nazarova, L., Katargina, O., Järvekülg, L., Fomenko, N., Golovljova, I., 2012. Detection and genetic characterization of relapsing fever spirochete *Borrelia miyamotoi* in Estonian ticks. PLoS One 7, e51914.

Gern, L., Humair, F., 2002. Ecology of *Borrelia burgdorferi* sensu lato in Europe. In: Gray, J., Kahl, O., Lane, R.S., Stanek, G. (Eds.), Lyme Borreliosis: Biology, Epidemiology and Control. CAB International, Oxon, United Kingdom, pp. 149–174.

Gern, L., Douet, V., López, Z., Rais, O., Morán Cadenas, F., 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 Tick-borne Dis. 1, 23–29.

Graf, G.F., 1978. Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae), 1ère partie. Bull. Soc. Entomol. Suisse 51, 89–97.

Gugliotta, J.L., Goethert, H.K., Berardi, V.P., Telford 3rd, S.R., 2013. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. N. Engl. J. Med. 368, 240–245.

Hamer, S.A., Tsao, J.I., Walker, E.D., Hickling, G.J., 2010. Invasion of the Lyme disease vector *Ixodes scapularis*: implications for *Borrelia burgdorferi* endemicity. EcoHealth 7, 47–63.

Hamer, S.A., Hickling, G.J., Keith, R., Sidge, J.L., Walker, E.D., Tsao, J.I., 2012. Associations of passerine birds, rabbits, and ticks with *Borrelia miyamotoi* and *Borrelia andersonii* in Michigan, U.S.A. Parasite Vector 5, 231.

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. Parasitology 118, 33–42.

Jado, I., Escudero, R., Gil, H., Jimenez-Alonso, M.I., Sousa, R., Garcia-Perez, A.L., Rodriguez-Vargas, M., Lobo, B., Anda, P., 2006. Molecular method for identification of *Rickettsia* species in clinical and environmental samples. J. Clin. Microbiol. 44, 4572–4576.

Jahfari, S., Fonville, M., Hengeveld, P., Reusken, C., Scholte, E.J., Takken, W., Heyman, P., Medlock, J., Heylen, D., Kleve, J., Sprong, H., 2012. Prevalence of *Neoehrlichia mikurensis* in ticks and rodents from North-west Europe. Parasite Vector 5, 74.

Kahl, O., Gern, L., Eisen, L., Lane, R.S., 2002. Ecological research on *Borrelia burgdorferi* sensu lato: terminology and some methodological pitfalls. In: Gray, J., Kahl, O., Lane, R.S., Stanek, G. (Eds.), Lyme Borreliosis: Biology, Epidemiology and Control. CAB International, Oxon, United Kingdom, pp. 29–46.

Karan', L.S., Koliasnikova, N.M., Toporkova, M.G., Makhneva, M.A., Nadezhdina, M.V., Esaulkova, A.Iu., Romanenko, V.V., Arumova, E.A., Platonov, A.E., Maleev, V.V., 2010. Usage of real-time polymerase chain reaction for diagnostics of different tick-borne infections. Zh. Mikrobiol. Epidemiol. Immunobiol. 3, 72–77 (in Russian).

Kawahara, M., Rikihisa, Y., Isogai, E., Takahashi, M., Misumi, H., Suto, C., Shibata, S., Zhang, C., Tsuji, M., 2004. Ultrastructure and phylogenetic analysis of *Candidatus* Neoehrlichia mikurensis in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. Int. J. Sys. Evol. Microbiol. 54, 1837–1843.

Li, H., Jiang, J.F., Liu, W., Zheng, Y.C., Huo, Q.B., Tang, K., Zuo, S.Y., Liu, K., Jiang, B.G., Yang, H., Cao, W.C., 2012. Human infection with *Candidatus* Neoehrlichia mikurensis, China. Emerg. Infect. Dis. 18, 1636–1639.

Lommano, E., Bertaiola, L., Dupasquier, C., Gern, L., 2012. Infections and co-infections of questing *Ixodes ricinus* ticks by emerging zoonotic pathogens in Western Switzerland. Appl. Environ. Microbiol. 78, 4606–4612.

Maurer, F.P., Keller, P.M., Beuret, C., Joha, C., Achermann, Y., Gubler, J., Bircher, D., Karrer, U., Fehr, J., Zimmerli, L., Bloemberg, G.V., 2013. Close geographic association

of human neoehrlichiosis and tick populations carrying *Candidatus* Neoehrlichia mikurensis in eastern Switzerland. J. Clin. Microbiol. 51, 169–176.
Movila, A., Toderas, I., Uspenskaia, I., Conovalov, J., 2013. Molecular detection of tick-borne pathogens in *Ixodes ricinus* from Moldova collected in 1960. Ticks Tick Borne Dis. 4, 359–361.
Mun, J., Eisen, R.J., Eisen, L., Lane, R.S., 2006. Detection of a *Borrelia miyamotoi* sensu lato relapsing-fever group spirochete from *Ixodes pacificus* in California. J. Med. Entomol. 43, 120–123.
Naitou, H., Kawaguchi, D., Nishimura, Y., Inayoshi, M., Kawamori, F., Masuzawa, T., Hiroi, M., Kurashige, H., Kawabata, H., Fujita, H., Ohashi, N., 2006. Molecular identification of *Ehrlichia* species and *Candidatus* Neoehrlichia mikurensis from ticks and wild rodents in Shizuoka and Nagano Prefectures, Japan. Microbiol. Immunol. 50, 45–51.
Nilsson, K., Lindquist, O., Pahlson, C., 1999. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet 354, 1169–1173.
Nilsson, K., Lindquist, O., Pahlson, C., 2010. *Rickettsia helvetica* in patient with meningitis, Sweden, 2006. Emerg. Infect. Dis. 16, 490–492.
Overzier, E., Pfister, K., Herb, I., Mahling, M., Bock Jr., 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. 13, 559–564.
Pérez, D., Kneubühler, Y., Rais, O., Gern, L., 2012. Seasonality of *Ixodes ricinus* ticks on vegetation and on rodents and *Borrelia burgdorferi* sensu lato genospecies diversity in two Lyme borreliosis-endemic areas in Switzerland. Vector Borne Zoonotic Dis. 12, 633–644.
Péter, O., 1984. Contribution à la connaissance de l'écologie de la Rickettsie suisse transmise par la tique *Ixodes ricinus* L., 1758 (Ph.D. thesis). University of Neuchâtel, Switzerland.
Piesman, J., Gern, L., 2004. Lyme borreliosis in Europe and North America. Parasitology 129 (Suppl.), S191–S220.
Platonov, A.E., Karan, L.S., Kolyasnikova, N.M., Makhneva, N.A., Toporkova, M.G., Maleev, V.V., Fish, D., Krause, P.J., 2011. Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg. Infect. Dis. 17, 1816–1823.
Poupon, M.A., Lommano, E., Humair, P.F., Douet, V., Rais, O., Schaad, M., Jenni, L., Gern, L., 2006. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. Appl. Environ. Microbiol. 72, 976–979.
R Development Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rar, V., Golovljova, I., 2011. *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia mikurensis" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect. Genet. Evol. 11, 1842–1846.
Richter, D., Matuschka, F.-R., 2010. Elimination of Lyme disease spirochetes from ticks feeding on domestic ruminants. Appl. Environ. Microbiol. 76, 7650–7652.
Richter, D., Schlee, D.B., Matuschka, F.-R., 2003. Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg. Infect. Dis. 9, 697–701.
Rijpkema, S.G., Molkenboer, M.J., Schouls, L.M., Jongejan, F., Schellekens, J.F., 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. J. Clin. Microbiol. 33, 3091–3095.
Rollend, L., Fish, D., Childs, J.E., 2013. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: a summary of the literature and recent observations. Ticks Tick Borne Dis. 4, 46–51.
Rudenko, N., Golovchenko, M., Grubhoffer, L., Oliver Jr., J.H., 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. Ticks Tick Borne Dis. 2, 123–128.
Sanogo, Y.O., Parola, P., Shpynov, S., Camicas, J.L., Brouqui, P., Caruso, G., Raoult, D., 2003. Genetic diversity of bacterial agents detected in ticks removed from asymptomatic patients in northeastern Italy. Ann. N. Y. Acad. Sci. 990, 182–190.
Schex, S., Dobler, G., Riehm, J., Müller, J., Essbauer, S., 2011. *Rickettsia* spp. in wild small mammals in Lower Bavaria, South-Eastern Germany. Vector Borne Zoonotic Dis. 11, 493–502.
Schouls, L.M., Van De Pol, I., Rijpkema, S.G., Schot, C.S., 1999. Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. J. Clin. Microbiol. 37, 2215–2222.
Scoles, G.A., Papero, M., Beati, L., Fish, D., 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1, 21–34.
Scott, M.C., Rosen, M.E., Hamer, S.A., Baker, E., Edwards, H., Crowder, C., Tsao, J.I., Hickling, G.J., 2010. High-prevalence *Borrelia miyamotoi* infection among wild turkeys (*Meleagris gallopavo*) in Tennessee. J. Med. Entomol. 47, 1238–1242.
Silaghi, C., Woll, D., Mahling, M., Pfister, K., Pfeffer, M., 2012. *Candidatus* Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks *Ixodes ricinus* and *Dermacentor reticulatus*, Germany. Parasite Vector 5, 285.
Simser, J.A., Palmer, A.T., Fingerle, V., Wilske, B., Kurtti, T.J., Munderloh, U.G., 2002. *Rickettsia monacensis* sp. nov., a spotted fever group *Rickettsia*, from ticks (*Ixodes ricinus*) collected in a European city park. Appl. Environ. Microbiol. 68, 4559–4566.
Speck, S., Perseke, L., Petney, T., Skuballa, J., Pfäffle, M., Taraschewski, H., Bunnell, T., Essbauer, S., Dobler, G., 2013. Detection of *Rickettsia helvetica* in ticks collected from European hedgehogs (*Erinaceus europaeus*, Linnaeus, 1758). Ticks Tick Borne Dis. 4, 222–226.
Sprong, H., Wielinga, P.R., Fonville, M., Reusken, C., Brandenburg, A.H., Borgsteede, F., Gaasenbeek, C., van der Giessen, J.W., 2009. *Ixodes ricinus* ticks are reservoir hosts for *Rickettsia helvetica* and potentially carry flea-borne *Rickettsia* species. Parasite Vector 2, 41.
Stuen, S., Granquist, E., Silaghi, C., 2013. *Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies. Front. Cell. Infect. Microbiol. 3, 31.
Taylor, K.R., Takano, A., Konnai, S., Shimozuru, M., Kawabata, H., Tsubota, T., 2013. *Borrelia miyamotoi* infections among wild rodents show age and month independence and correlation with *Ixodes persulcatus* larval attachment in Hokkaido, Japan. Vector Borne Zoonotic Dis. 13, 92–97.
Vayssier-Taussat, M., Le Rhun, D., Buffet, J.P., Maaoui, N., Galan, M., Guivier, E., Charbonnel, N., Cosson, J.F., 2012. *Candidatus* Neoehrlichia mikurensis in bank voles, France. Emerg. Infect. Dis. 18, 2063–2065.
von Loewenich, F.D., Geissdörfer, W., Disqué, C., Matten, J., Schett, G., Sakka, S.G., Bogdan, C., 2010. Detection of *Candidatus* Neoehrlichia mikurensis in two patients with severe febrile illnesses: evidence for a European sequence variant. J. Clin. Microbiol. 48, 2630–2635.
Welinder-Olsson, C., Kjellin, E., Vaht, K., Jacobsson, S., Wennerås, C., 2010. First case of human *Candidatus* Neoehrlichia mikurensis infection in a febrile patient with chronic lymphocytic leukemia. J. Clin. Microbiol. 48, 1956–1959.
Wilhelmsson, P., Fryland, L., Borjesson, S., Nordgren, J., Bergström, S., Ernerudh, J., Forsberg, P., Lindgren, P.E., 2010. Prevalence and diversity of *Borrelia* species in ticks that have bitten humans in Sweden. J. Clin. Microbiol. 48, 4169–4176.
Wodecka, B., 2007. Significance of red deer (*Cervus elaphus*) in the ecology of *Borrelia burgdorferi* sensu lato. Wiad. Parazytol. 53, 231–237.