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Small risk of developing symptomatic tick-borne diseases following a tick bite in the Netherlands

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Abstract

Background: In The Netherlands, the incidence of Lyme borreliosis is on the rise. Besides its causative agent, *Borrelia burgdorferi* s.l., other potential pathogens like *Rickettsia*, *Babesia* and *Ehrlichia* species are present in *Ixodes ricinus* ticks. The risk of disease associated with these microorganisms after tick-bites remains, however, largely unclear. A prospective study was performed to investigate how many persons with tick-bites develop localized or systemic symptoms and whether these are associated with tick-borne microorganisms.

Results: In total, 297 *Ixodes ricinus* ticks were collected from 246 study participants who consulted a general practitioner on the island of Ameland for tick bites. Ticks were subjected to PCR to detect DNA of *Borrelia burgdorferi s.l.*, *Rickettsia* spp., *Babesia* spp. or *Ehrlichia/Anaplasma* spp. Sixteen percent of the collected ticks were positive for *Borrelia burgdorferi s.l.*, 19% for *Rickettsia* spp., 12% for *Ehrlichia/Anaplasma* spp. and 10% for *Babesia* spp. At least six months after the tick bite, study participants were interviewed on symptoms by means of a standard questionnaire. 14 out of 193 participants (8.3%) reported reddening at the bite site and 6 participants (4.1%) reported systemic symptoms. No association between symptoms and tick-borne microorganisms was found. Attachment duration ≥24 h was positively associated with reddening at the bite site and systemic symptoms. Using logistic regression techniques, reddening was positively correlated with presence of *Borrelia afzelii*, and having 'any symptoms' was positively associated with attachment duration.

Conclusion: The risk of contracting acute Lyme borreliosis, rickettsiosis, babesiosis or ehrlichiosis from a single tick bite was <1% in this study population.

Background

The most prevalent and widespread vector-borne disease of humans and animals in the northern hemisphere is Lyme borreliosis. Early detection of Lyme borreliosis is crucial, as antibiotics are most effective at this stage, preventing the development severe sequelae [1]. Over the last decade, the incidence of Lyme borreliosis has increased significantly in Europe, with up to 16 and 21 cases per 10,000 individuals reported in Scandinavia and Slovenia, respectively [2]. A periodical retrospective study under general practitioners in The Netherlands has shown a continuing and strong increase in general

practitioner (GP) consultations for erythema migrans and hospital admissions in the past 15 years with 22000 cases in 2009. The most straightforward explanation for this increase is the concomitant increase in the number of GP consultations for tick bites [3,4]. Although direct evidence is lacking, the factors responsible for this increase are most probably a combination of higher tick numbers and intensified human recreational behaviour, leading to an increased exposure of the population to tick bites.

The same tick species transmitting the etiologic agents of Lyme disease in Europe, *Ixodes ricinus*, also serves as vector of pathogens causing tick-borne encephalitis, babesiosis, several forms of rickettsioses and anaplasmoses. Incidences and public health risks of tick-borne diseases other than Lyme borreliosis are largely unknown in The Netherlands, but also in other countries. Although Dutch ticks have been shown to have a

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high prevalence of *Rickettsia helvetica* and can contain *Babesia*, so far no endemic disease cases in humans have been observed for these microorganisms [5]. *R. helvetica* is a intracellular bacterium that is suspected of causing acute perimyocarditis, unexplained febrile illness and sarcoidosis [6-11]. Various *Babesia* species are known to cause disease in humans and animals with *Babesia divergens* probably being the most important human pathogenic species in Europe but one European case of *B. microti* infection has been reported as well [12-15]. *Anaplasma* and *Ehrlichia* have also been found in Dutch ticks and a few human cases have been reported in the Netherlands [16,17].

Not only transmitted pathogens but also the tick itself can lead to health impairments. Ticks secrete a complex mixture of bio-active compounds, mainly proteins, during the blood meal [18-20]. These can have local or systemic toxic effects or induce an immune response. Individuals rarely react with intense anaphylaxis but milder allergic reactions are probably more common and easily overlooked [21-23].

The risk of developing Lyme borreliosis or any other tick-borne disease after a tick bite depends on many unrelated factors, including the tick species, the number of pathogens per tick, the site and duration of the tick bite, the (genetic) constitution of the pathogen and the individual susceptibility to infection [24-26]. Many, if not all, of these factors may vary geographically and in time. Several prospective studies have estimated the risk of developing Lyme borreliosis following a tick bite, but not in The Netherlands, and rarely for other tick-borne diseases such as spotted fever rickettsiosis, babesiosis, and anaplasmosis [27-30]. Data on Borrelia infection on patients and ticks from the years 2004-2006 were previously reported [31].

Field studies between 1989 and 1993 on the Dutch island of Ameland reported a *B. burgdorferi s.l.* prevalence in ticks of all life stages (n = 521) of 24% (95% CI: 20.7-28.0%) [32,33], which led to an increased awareness of Lyme disease on the island. To estimate the risk of developing symptoms following tick bites and to investigate whether this risk is associated to specific tick-borne pathogens a study was initiated. Patients who consulted one GP on Ameland for a tick bite were invited to participate. They were asked to fill out a questionnaire on the day they visited the GP and were approached again several months later. The ticks were tested for *Borrelia burgdorferi s.l.*, *Rickettsia* spp., *Babesia* spp. and *Ehrlichia/Anaplasma* spp.

Materials and methods
Study participants
Between January 2004 and December 2008 ticks were collected from patients with one or more tick bites that consulted the GP in the village of Ballum on Ameland (53°44'41N, 5°68'43E). Ballum is the smallest village on Ameland with only about 370 citizens but about 500000 tourists visit this village yearly. This is represented in the composition of the study population of which only 20% was a resident of the island. Patients or their guardians were asked for an informed consent for testing the tick for various microorganisms and for collecting data via questionnaires or interviews. In a first questionnaire data were collected concerning, amongst others, the number of tick bites and duration of tick attachment. Approximately six months after the first visit to the GP patients were contacted by phone and interviewed. Some of the patients where only reached after 12 to 18 months. This second interview aimed at identifying possible symptoms related to the tick bite including local redness or erythema migrans and systemic symptoms as fever, malaise, palpitations, joint problems, or neurological symptoms, and if so whether they consulted a GP for this symptoms.

Removal and analysis of ticks
Ticks were carefully removed by the general practitioner and were immersed in 70% ethanol or a lysis buffer (0,5% sodium dodecyl sulfate; 100 mM tris-(hydroxymethyl) aminomethane; 10 mM ethylenediaminetetraacetic acid; 10 mM NaCl; pH: 8,3; 0,5 mg/ml protease K) and sent to the laboratory. Ticks stored in ethanol were determined to species level and DNA was extracted as described earlier [31]. Tick DNA extracts were analyzed by polymerase chain reaction (PCR) followed by reverse line blotting (RLB), as described elsewhere [34]. Primers and probes are described in Table 1.

Statistics
Outcomes were defined as redness on the site of the tick bite, and/or any systemic symptoms such as fever, malaise or pain. Risk factors that were investigated included positive PCR/RLB results for one of the microorganisms (*Borrelia*, *Rickettsia*, *Babesia* or *Ehrlichia/Anaplasma*) in the ticks, any of these (= any microorganism), duration (<24 h, between 24 h and 48 h, >48 h) and number of tick bites.

Firstly, we tested for all possible outcome - risk factor combinations, the strength of association (null hypothesis of unity odds ratio, i.e. no association). To this end, contingency tables were constructed and Fisher's exact tests were performed for each combination. All calculations were performed using R 2.11.1, using the 'Epi' package v1.1.17. For outcomes and binary risk factors (i.e. absence-presence of microorganisms), risk ratios and their 95% confidence intervals were computed. For non-binary risk factors categories were defined. Furthermore, we calculated the exact p-value for the hypothesis of unity odds-ratio.

Table 1 Primers and probes used in this study for PCR and RLB

Name	Sequence (5' - 3')	Type	Target	Species	Reference
5S borSeq	GAGTTCGCGGGAGAGTAGGTTATTGCC [1]	Primer	23S-5S IGS	*B. burgdorferi* sensu lato	[38]
23S borSeq	TCAGGGTACTTAGATGGTTCACTTCC	Primer	23S-5S IGS	*B. burgdorferi* sensu lato	[38]
A-borsl1	CTTTGACCATATTTTTATCTTCCA	Probe	23S-5S IGS	*B. burgdorferi* sensu lato	[39]
A-borsl2	CTTCCATCTCTATTTAGCCAATTT	Probe	23S-5S IGS	*B. burgdorferi* sensu lato	[38]
A-borsl3	TATTTTTATCTTCCATCTCTATTTT	Probe	23S-5S IGS	*B. burgdorferi* sensu lato	[38]
B31-A-s.stricto	AACACCAATATTTAAAAAACATAA	Probe	23S-5S IGS	*B. burgdorferi* sensu stricto	[39]
Ga2-garinii	AACATGAACATCTAAAAACATAAA	Probe	23S-5S IGS	*B. garinii*	[39]
Vs46lN2afzelii	AACATTTAAAAAATAAATTCAAGG	Probe	23S-5S IGS	*B. afzelii*	[39]
Vsll62 val	CATTAAAAAAATATAAAAAATAAATTTAAGG	Probe	23S-5S IGS	*B. valaisiana*	[39]
A-Ruski	GAATAAAACATTCAAATAATATAAAC	Probe	23S-5S IGS	*B. afzelii (variant ruski)*	[40]
A-LusiP	CAAAAAAATGAACATTTAAAAAC	Probe	23S-5S IGS	*B. lusitaniae*	[41]
B-GA1b	CGGGATCCCGAGTTTGCCGGGACTTCTTCT [1]	Primer	*16SrRNA*	*Ehrlichia/Anaplasma*	[42]
16S8Fe	GGAATTCAGAGTTGGATCMTGGYTCAG	Primer	*16SrRNA*	*Eubacteria*	[43]
Ehr-all	TTATCGCTATTAGATGAGCC	Probe	*16SrRNA*	*Anaplasma* genus	[42]
A-HGE	GCTATAAAGAATAGTTAGTGG	Probe	*16SrRNA*	HGE agent	[42]
A-Eqph	TTGCTATAAAGAATAATTAGTGG	Probe	*16SrRNA*	*A. phagocytophilum*	[42]
A-dHGE	GCTATGAAGAATAGTTAGTG	Probe	*16SrRNA*	HGE agent (variant)	[42]
A-dPh	TTGCTATGAAGAATAATTAGT	Probe	*16SrRNA*	*A. phagocytophilum variant*	[38]
A-E.Schot	GCTGTAGTTTACTATGGGTA	Probe	*16SrRNA*	*A. schotti* (variant)	[42]
A-murisT	AGCTATAGGTTTGCTATTAGT	Probe	*16SrRNA*	*E. muris* T variant	[40]
A-Chaff	ACCTTTTGGTTATAAATAATTGTTA	Probe	*16SrRNA*	*E. chaffeensis*	[42]
A-can	TCTGGCTATAGGAAATTGTTA	Probe	*16SrRNA*	*E. canis*	[42]
A-Wolbach	CTACCAAGGCAATGATCTA	Probe	*16SrRNA*	*Wolbachia*	[38]
Rick-16S rev	ACTCACTCGGTATTGCTGGA [1]	Primer	*16SrRNA*	*Rickettsia* genus	[41]
Rick-16S for	AACGCTATCGGTATGCTTAACA	Primer	*16SrRNA*	*Rickettsia* genus	[41]
A-Rickall	TTTAGAAATAAAAGCTAATACCG	Probe	*16SrRNA*	*Rickettsia* genus	[41]
A-Rhelv2	GCTAATACCATATATTCTCTATG	Probe	*16SrRNA*	*R. helvetica*	[41]
A-Rconor	CTTGCTCCAGTTAGTTAGT	Probe	*16SrRNA*	*R. conorii*	[41]
A-16SRickIRS	GTATATTCTCTACGGAAAAAA	Probe	*16SrRNA*	*Rickettsia* IRS3	[41]
A-RProwaz	CGGATTAACTAGAGCTCGCT	Probe	*16SrRNA*	*Rickettsia prowazekii*	[34]
A-RTyphi	CGGATTAATTAGAGCTTGCT	Probe	*16SrRNA*	*Rickettsia typhi*	[34]
A-NonHelv A	AATACCGTATATTCTCTACGGA	Probe	*16SrRNA*	Non- *Rickettsia helvetica*	[34]
A-NonHelv B	AATACCGTATATTCTCTGCGGA	Probe	*16SrRNA*	Non- *Rickettsia helvetica*	[34]
BATH-Rn	TAAGAATTTCACCTCTGACAGTTA [1]	Primer	*18SrRNA*	*Babesia* genus	[44]
BATH-Fn	ACACAGGGAGGTAGTGACAAG	Primer	*18SrRNA*	*Babesia* genus	[44]
Catch all 2	GTAATGGTTAATAGGARCRGTT	Probe	*18SrRNA*	*Babesia* genus	[44]
Ba-div	GTTAATATTGACTAATGTCGAG	Probe	*18SrRNA*	*B. divergens*	[45]
Ba-mic 1	CCGAACGTTATTTTATTGATTT	Probe	*18SrRNA*	*B. microti*	[34]
Ba-mot	GCTTGCTTTTTTGTTACTTTG	Probe	*18SrRNA*	*B. motasi*	[44]
Ba-mic 2	GRCTTGGCATCWTCTGGA	Probe	*18SrRNA*	*B. microti*	[44]
Ba-EU1	CTGCGTTATCGAGTTATTG	Probe	*18SrRNA*	*B. EU1*	[34]

Probes were 5'-amino-labeled. [1] Reverse primer 5'-labeled with biotine tetraethyleneglycol.

For purposes of logistic regression, it is convenient to know what factors are associated at the 70% significance level [35]. Significant factors and categorical data (number of bites, number of days the tick was attached) were included in backwards logistic regressions for each outcome variable. Any combination of microorganism and days of attachment was also tested in the logistic regression.

Results

Ticks were collected from 246 study participants. In total 297 ticks were removed ranging from 1 to 18 ticks per individual with an average of 1.2 ticks per individual. All ticks that were identified to species level were *Ixodes ricinus*. Life stages of 236 ticks could be determined microscopically. Of these ticks, 65 (28%) were adults, 133 (56%) were nymphs and 38 (16%) were larvae.

At least 53 participants were bitten by adult ticks, 96 by nymphs and 16 by larvae. Two hundred-ninety-four ticks were tested for *Borrelia burgdorferi s.l., Rickettsia* spp. and *Babesia* spp., 286 ticks were also tested for *Ehrlichia/Anaplasma* spp. One-hundred-ninety-three (78.5%) participants were reached for a second interview, 51 participants were lost to follow-up. For epidemiological analysis only data of the responding participants were used.

Of all tested ticks 58% were negative for all microorganisms tested for. 16% were positive for *B. burgdorferi s.l.*, 19% for *Rickettsia* spp., 10% for *Babesia* spp. and 12% for *Ehrlichia/Anaplasma* spp. The overall infection rate with *B. burgdorferi* s.l. was 16% (n = 294, CI 12.1-20.5%), which is significantly lower (p = 0.005) than in the early 90s (24% (n = 521, CI 20.7-28.0%) [32]. Different sub-species of *B. burdorferi* s.l. were found during this study of which *B. afzelii* was the most common one. *Rickettsiae* that were identified to species level were *Rickettsia helvetica* and *Rickettsia monacensis*. All but one Babesia species were identified as *Babesia microti*. The *Ehrlichia/Anaplasma* species identified were mainly *Ehrlichia* sp. schotti variant (recently named "*Candidatus* Neoehrlichia mikurensis"), one tick contained *Anaplasma phagocytophilum* and a last one could not be determined to species level. An overview on the identified microorganisms is given in Table 2. No larvae were found positive for *Babesia* and only one for *Borrelia*. Infection rates of the different life stages were calculated and are presented in figure 1.

Table 2 Different tick-borne microorganisms found in ticks collected from humans

Species	positive ticks
Borrelia burgdorferi s.l.	47/297
B. afzelii	33
B. garinii	6
B. valaisiana	1
undetermined	7
Rickettsia spp.*	55/297
R. helvetica	40
R. monacensis	11
undetermined	5
Ehrlichia/Anaplasma spp.	33/289
Ehrlichia sp. schotti variant	31
A. phagocytophilum	1
undetermined	1
Babesia spp.	28/297
B. microti	27
undetermined	1

Microorganisms in tick lysates were detected and identified by PCR followed by RLB.
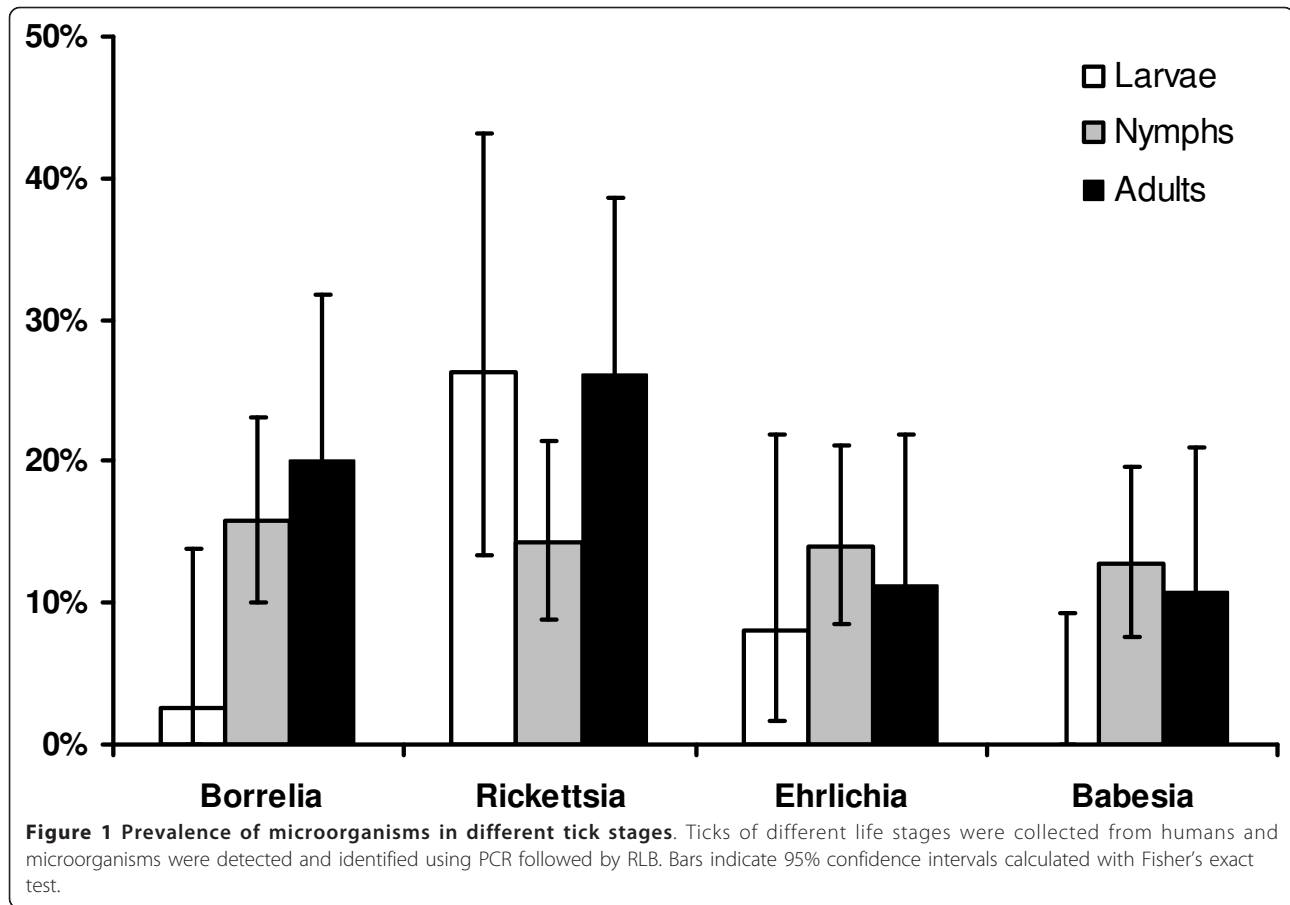*one tick had a double infection with *R. helvetica* and *R. monacensis*.

In total 22 study participants reported symptoms of which 14 reported reddening around the tick bite site and 6 reported systemic symptoms and 2 reported both. This corresponds to an absolute risk of 11.4% for developing symptoms and 8.3% and 4.1%, respectively, for local reddening and systemic symptoms after a tick bite. Reddening at the bite site did not show the pattern of erythema migrans in any of the cases. Systemic symptoms included fever (n = 3), malaise (n = 3), fatigue (n = 3), panic attacks (n = 1), muscle pain (n = 1), joint pain (n = 1) or stiffness of the neck (n = 1). Three study participants reported symptoms but did not specify them further. Local reddening occurred and systemic symptoms occurred most frequently in participants bitten by an adult tick (n = 8 and n = 3, respectively) followed by those bitten by nymphs (n = 4 and n = 2) and larvae (n = 0 and n = 1). For the remaining cases life stages of ticks were not determined. Three 171 participants reported no symptoms. Eighty-four percent of the participants reported that the tick had been attached less than 24 hours while 4.7% reported that the tick had been attached for more than 48 hours. The occurrence of symptoms or reddening at the bite site was not correlated with the infectious state of the ticks (Table 3, 4, 5). However, a significant positive correlation (at 95% significance) was found between attachment duration of ticks (≥24 h) and symptoms. This was the case for redness (p = 0.08) and systemic symptoms (p = 0.02) analyzed individually and also when symptoms were analyzed conjointly (p = 0.009).

Fisher's exact test were significant (p < 0.30) at the 70% level for local redness with attachment duration of more than 24 h, more than 48 h, more than two tick bites and *B. afzelii*, for systemic symptoms with >24 h, *Ehrlichia* spp. and *B. garinii* infection of the tick and for any symptom with >24 h, >48 h, any infection of the tick, *B. afzelii* and *Borrelia burgdorferi s.l.* Logistic regression with these factors and categorical data yielded no significant result for systemic symptoms. For redness, or any symptoms the following significant regression was found,

$$R = \frac{e^{0.79d}e^{1.1A}}{36.6 + e^{0.79d}e^{1.1A}},$$

$$G = \frac{e^{0.8d}}{21.8 + e^{0.8d}},$$

where *R* is the probability of redness at *d* days of tick presence and *A* indicates absence ('0') or presence ('1') of *B. afzelii*. *G* is the probability of systemic symptoms depending again on the number of days *d*. Logistic regression indicated the presence of one *B. burgdorferi*

Figure 1 Prevalence of microorganisms in different tick stages. Ticks of different life stages were collected from humans and microorganisms were detected and identified using PCR followed by RLB. Bars indicate 95% confidence intervals calculated with Fisher's exact test.

sub-species, *B. afzelii*, to be associated with local reddening (Figure 2). For example, 4 days of *B. afzelii* presence yields a probability of redness of 66%.

Discussion

Tick bites may cause serious and lasting effects due to transmitted pathogens. Symptoms, especially localized ones, can also be due to reaction towards the tick itself. During this study several risk factors for developing

Table 3 Relative risks of developing any symptom correlated to various risk factors

	Exposed individuals	Any symptom	Relative risk (95% CI)	P value
>24 h	30/193	8/30	3.10 (1.43 - 6.75)	0.0092*
Borrelia	43/190	7/43	1.60 (0.70 - 3.66)	0.2841
Rickettsia	41/190	6/41	1.36 (0.57 - 3.26)	0.5809
Ehrlichia	27/182	4/27	1.44 (0.52 - 3.97)	0.5059
Babesia	26/190	2/26	0.63 (0.16 - 2.54)	0.7439
Any infection	98/190	14/98	1.64 (0.72 - 3.73)	0.2625

Relative risks were calculated by dividing incidence rates of exposed by incidence rates of unexposed participants. Confidence intervals and p-values were calculated using Fisher's exact test. P-values below 0.05 (*) were regarded as significant.

localized and systemic symptoms were investigated. Ticks were tested for four different groups of microorganisms. Statistical analysis showed none of the micro-organisms to be significantly associated to systemic symptoms but logistic regression indicated that *B. afzelii* might be associated with local reddening. In this study, only DNA of microorganisms was detected and identified in the ticks. This does not proof the presence of viable microorganisms neither does it tell something about their pathogenicity to humans. Therefore some of the PCR positive ticks might not have carried infectious agents at all and this could contribute to

Table 4 Relative risks of developing redness at the bite site correlated to various risk factors

	Exposed individuals	Redness on bite site	Relative risk (95% CI)	P value
>24 h	30/193	5/30	2.47 (0.92 - 6.60)	0.0801
Borrelia	43/190	5/43	1.55 (0.57 - 4.22)	0.3647
Rickettsia	41/190	3/41	0.84 (0.25 - 2.80)	1
Ehrlichia	27/182	2/27	0.96 (0.23 - 4.04)	1
Babesia	26/190	2/26	0.90 (0.22 - 3.74)	1
Any infection	98/190	10/98	1.56 (0.59 - 4.13)	0.4382

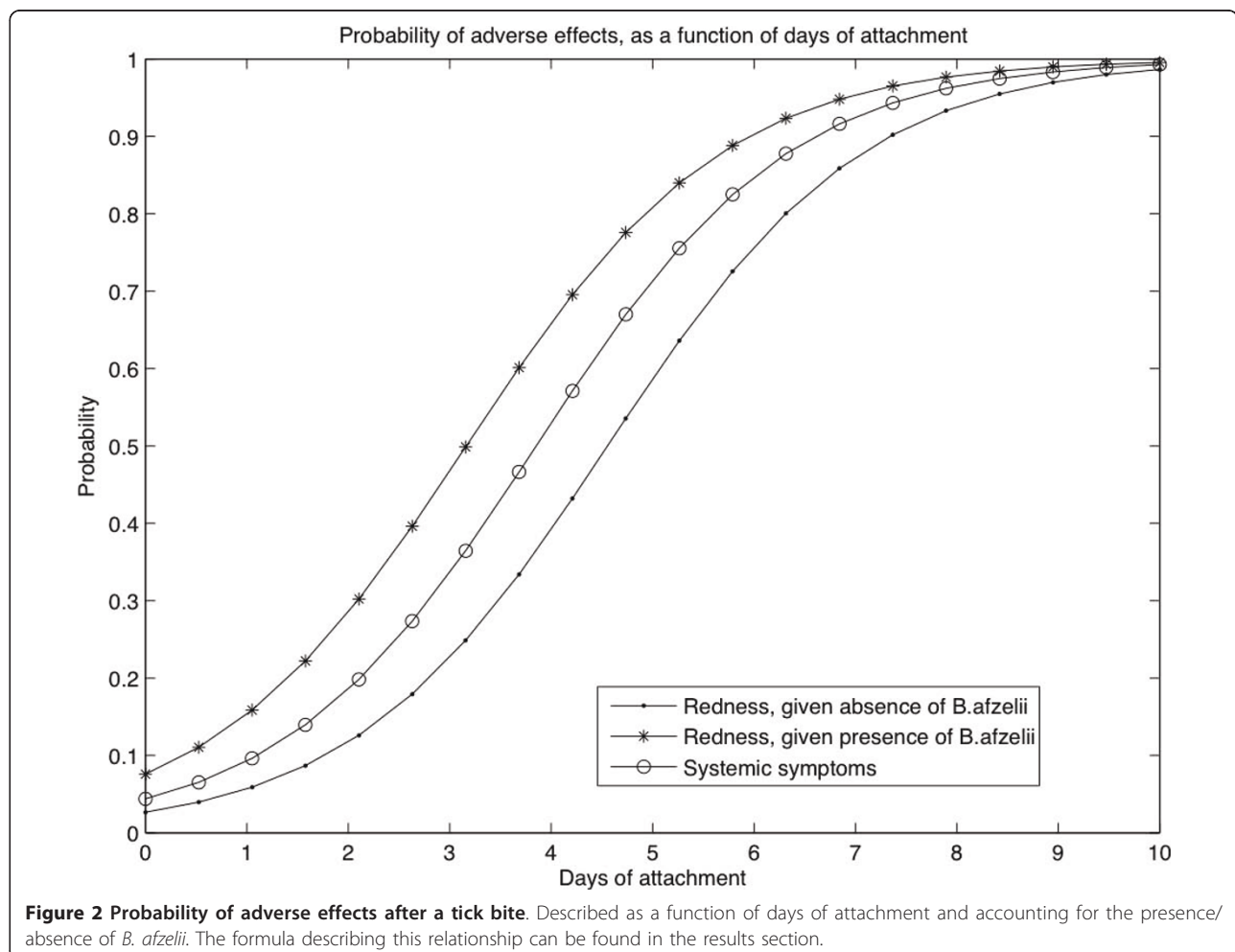**Table 5 Relative risks of developing systemic symptoms correlated to various risk factors**

	Exposed individuals	Systemic symptoms	Relative risk (95% CI)	P value
>24 h	30/193	4/30	5.43 (1.44 - 20.54)*	0.0215*
Borrelia	43/190	2/43	1.14 (0.24 - 5.44)	1
Rickettsia	41/190	3/41	2.18 (0.54 - 8.75)	0.3729
Ehrlichia	27/182	2/27	2.30 (0.47 - 11.24)	0.2779
Babesia	26/190	1/26	0.90 (0.12 - 7.03)	1
Any infection	98/190	5/98	1.56 (0.38 - 6.36)	0.7218

the absence of disease cases during this study. Future studies should aim to investigate whether these microorganisms, especially the less well studied Rickettsiaceae and *Babesia* species, are an emerging risk to public health.

Tick attachment duration was found to be strongly associated with an increased risk for developing localized reddening other than erythema migrans at the bite site as well as systemic symptoms. This association is most probably due to a direct response towards the tick or substances it secreted into the wound. Ticks secrete a complex mixture of compounds, some of which are potentially toxic, and which accumulate during the feeding process. Castelli *et al.* [36] described local reactions as result of tick bites. These included nodules, erythema and alopecia. A longer attachment period is likely to increase the risk of developing localized or systemic symptoms due to excreted tick proteins and also due to pathogens [37]. Timely removal of a tick is therefore a major factor to reduce transmission of potentially toxic tick excretions and tick-borne pathogens and our study supports that it significantly lowers the risk of developing symptoms.

Many microorganisms that do not live symbiotically in ticks need vertebrate hosts to maintain themselves in a tick population. However, transovarial transmission can contribute more or less to the maintenance of microorganisms in ticks, which seems to play a minor role for

Figure 2 Probability of adverse effects after a tick bite. Described as a function of days of attachment and accounting for the presence/absence of *B. afzelii*. The formula describing this relationship can be found in the results section.

Ehrlichia/Anaplasma and a major role for *Rickettsia* spp. The prevalence of these bacteria in larvae was already 8.1% and 26%, respectively. For *Rickettsia* spp. the prevalence was similar in adults, nymphs and larvae. This was observed in an earlier study as well and indicates that *Rickettsiae* are transmitted transovarially at a high degree and therefore rely only a little on vertebrate hosts [5]. It was observed that the prevalence of *B. burgdorferi s.l.* in ticks decreased significantly from 1989/1993 to 2004/2008. This seems to contradict findings that Lyme borreliosis is on the rise in the Netherlands [3,4]. However, an increase in disease incidence is more likely due to an increased exposure of people to infected ticks. This can also be caused by a change in recreational behaviour or in the number of ticks present in the environment. Although these factors are difficult to measure and have not been measured during this study it seems likely that these and not an increase in infection rate are responsible for an increase in Lyme borreliosis incidence.

Conclusion
In our study, the overall risk of developing symptoms after a tick bite is 11.4% and most of these symptoms are restricted to local reactions. The risk of contracting symptoms of Lyme borreliosis after a single tick bite, even if the tick is infested with potential pathogens, is lower than 1%. Based upon the data collected in this study none of the participants developed symptomatic rickettsiosis, babesiosis or ehrlichiosis. This means that the risk of contracting overt symptoms one of these diseases was lower than 0.5% in this study population. The study shows that prompt removal of ticks reduces the risk of developing symptoms after a tick bite. Thorough checking for ticks together with appropriate clothing, tick avoiding behavior and use of insect repellents, is therefore the most powerful measure to prevent tick-borne diseases. Although the risk of developing symptoms after a tick bite is very low, timely removal of the tick is of an essence and this message should be promoted more clearly and with emphasis to the public.

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