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A single dose of doxycycline after an *ixodes ricinus* tick bite to prevent Lyme borreliosis: An open-label randomized controlled trial

M.G. Harms[a,*], A. Hofhuis[a], H. Sprong[a], S.C. Bennema[a], J.A. Ferreira[b], M. Fonville[a], A. Docters van Leeuwen[a], W.J.J. Assendelft[c], H.C.P.M. Van Weert[d], W. Van Pelt[a], C.C. Van den Wijngaard[a]

[a] National Institute for Public Health and the Environment, Centre for Infectious Disease Control, PO box 1, Bilthoven 3720 BA, the Netherlands
[b] National Institute for Public Health and the Environment, Department of Statistics, Informatics and Mathematical Modeling, PO box 1, Bilthoven 3720 BA, the Netherlands
[c] Radboud University Medical Center, Department of Primary and Community Care, PO box 9101, Nijmegen 6500 HB, the Netherlands
[d] Amsterdam University Medical Center, Department of Primary Care, PO box 22660, Amsterdam 1100 DD, the Netherlands

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SUMMARY

Objectives: A single dose of doxycycline after a tick bite can prevent the development of Lyme borreliosis in North America, but extrapolation to Europe is hampered by differences in *Borrelia burgdorferi* sensu lato genospecies and tick species. We assessed the efficacy of prophylaxis after a tick bite in Europe.
Methods: We conducted an open-label randomized controlled trial, administering a single dose of 200 mg doxycycline within 72 h after removing an attached tick from the skin, compared to no treatment. Potential participants ≥ 8 years of age who reported a recent tick bite online were invited for the study. After informed consent, they were randomly assigned to either the prophylaxis or the no-treatment group. Participants in the prophylaxis group were asked to visit their general practitioner to administer the antibiotics. All participants were followed up by online questionnaires. Our primary outcome was the development of physician-confirmed Lyme borreliosis in a modified-intention-to-treat analysis. This study is registered in the Netherlands Trial Register (NTR3953) and is closed.
Results: Between April 11, 2013, and June 10, 2015, 3538 potential participants were randomized, of whom 1689 were included in the modified-intention-to-treat analysis. 10 cases of Lyme borreliosis were reported out of 1041 participants (0.96%) in the prophylaxis group, and 19 cases out of 648 no-treatment participants (2.9%), resulting in a relative risk reduction of 67% (95% CI 31 - 84%), and a number-needed-to-treat of 51 (95% CI 29 - 180). No serious adverse events were reported.
Conclusions: This primary care-based trial provides evidence that a single dose of doxycycline can prevent the development of Lyme borreliosis after an *Ixodes ricinus* tick bite.

Introduction

Lyme borreliosis is the most prevalent tick-borne disease in the northern hemisphere.[1] Over the last decades, marked increases in the incidence of Lyme borreliosis have been reported in several Western European countries and the United States (US), with substantial impact on public health.[2–6] Erythema migrans is the most common early skin manifestation of the disease, and responds well to antibiotic treatment.[7] However, erythema migrans is often not observed preceding disseminated Lyme borreliosis manifestations such as neuroborreliosis, Lyme arthritis and Lyme carditis.[7–9]

Antibiotic prophylaxis after a tick bite could reduce the morbidity of Lyme borreliosis, especially in infected people who would develop disseminated Lyme borreliosis without observing a preceding erythema migrans.[10,11] Since 2006, prophylaxis is mentioned in the US guideline for Lyme borreliosis as an optional preventive treatment, based on a study in an endemic area in the US, where a single dose of 200 mg doxycycline prophylaxis was shown to reduce the risk of Lyme borreliosis after a tick bite by *Ixodes scapularis* ticks.[12,13] Medical guidelines in the Netherlands also mention such prophylaxis as a treatment option, besides an

* Corresponding author.
E-mail addresses: margriet.harms@rivm.nl (M.G. Harms), agnetha.hofhuis@rivm.nl (A. Hofhuis), hein.sprong@rivm.nl (H. Sprong), jose.ferreira@rivm.nl (J.A. Ferreira), manoj.fonville@rivm.nl (M. Fonville), arieke.docters.van.leeuwen@rivm.nl (A. Docters van Leeuwen), Pim.Assendelft@radboudumc.nl (W.J.J. Assendelft), h.c.vanweert@amc.uva.nl (H.C.P.M. Van Weert), wilfrid.van.pelt@rivm.nl (W. Van Pelt), kees.van.den.wijngaard@rivm.nl (C.C. Van den Wijngaard).

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expectant policy, when a patient presents with a tick bite with more than 24 h attachment time.[14]

In Europe however, the main vector for Lyme borreliosis is *I. ricinus,* which transmits *B. burgdorferi* sensu lato (s.l.) earlier after attachment than *I. scapularis,* the main vector in the US.[15–18] Moreover the predominant *B. burgdorferi* s.l. genospecies in Europe are also different from those in the US,[19] which raises the question to what extent results for the US can be extrapolated to a European setting. Therefore, the objective of this study was to evaluate the efficacy of prophylactic treatment with a single dose of 200 mg doxycycline to prevent Lyme borreliosis after an *Ixodes ricinus* tick bite compared to no treatment.

Methods

Study design and participants

We conducted an open-label randomized controlled trial in the Netherlands for which participants were recruited through the citizen science website www.tekenradar.nl, where each year around 9.000 tick bites are reported.[20] Individuals of at least 8 years old were eligible for the study if they reported a tick bite within 72 h after removal and collection of the tick, and if they did not report any other tick bites in the past three months, nor reported any contraindications for treatment with doxycycline such as pregnancy or allergies.

After online invitation and informed consent for the study, invitees printed and signed their written informed consent form and sent this to us by regular mail, together with their tick. The study was approved by the medical ethics committee Noord-Holland (CCMO registration number NL42713), and was performed according to the Declaration of Helsinki.

Randomization

Participants were randomly allocated to receive either antibiotic prophylaxis or no treatment. Randomisation was computerised within the online study website, with a 1:1 ratio between the prophylaxis and the no-treatment group. This ratio was adapted stepwise to a 7:3 ratio to maintain study power as during the study we observed a higher crossover from the prophylaxis to the no-treatment group – i.e. failure to take prophylaxis – as well as a higher loss to follow-up in the prophylaxis group.

Procedures

At inclusion, participants were asked to fill out an online questionnaire regarding the date of their tick bite, duration of tick attachment, development of erythema migrans or other possible manifestations of Lyme borreliosis,[7] the use of any antibiotics or other medications in the past two weeks and comorbidities in the previous year. Participants in the prophylaxis group were then asked to visit their general practitioner with an information letter in which we requested the prescription of a single dose of 200 mg doxycycline (or with a body weight below 50 kg a lower dose of 4 mg/kg body weight) to be taken within 72 h after tick removal, after checking for contra-indications. For adequate treatment, if needed, we instructed all participants (prophylaxis and no-treatment group) to contact their general practitioner if symptoms possibly related to Lyme borreliosis occurred. One week and one month after inclusion participants filled out online follow-up questionnaires inquiring about the use and timing of antibiotic prophylaxis, development and antibiotic treatment of possible Lyme borreliosis, and development of adverse events. Three months after inclusion, and every subsequent three months until 18 months after inclusion, participants received further follow-up questionnaires about development and treatment of Lyme borreliosis, and other tick bites since inclusion. With written permission of the participants, we verified any online report of possible development and treatment of Lyme borreliosis with a paper questionnaire to the participant's general practitioner to confirm the diagnosis.

Collected ticks

Ticks were sent by regular mail either taped directly to the written informed consent form, or in case of more engorged ticks in an Eppendorf vial supplied to the participant. Upon reception, ticks were stored at -20 °C to be analysed in batches. Tick species, developmental stage, gender and engorgement were examined by microscope. Degree of engorgement was determined visually and set in 4 categories, from flat (score 0) to substantially engorged (score 3).[21] To isolate DNA, ticks with engorgement scores of 0 or 1 were boiled in ammonium hydroxide and for more engorged ticks the Qiagen (Valencia CA. USA) blood and tissue DNA extraction kit was used.[22,23] Presence of *B. burgdorferi* s.l. DNA was determined with a duplex quantitative QPCR using fragments of the outer membrane protein A (OspA) gene and the flagellin B (FlaB) gene as targets.[22]

Outcome measures

Our primary outcome measure was development of Lyme borreliosis within 6 months after inclusion. Lyme borreliosis was defined in two categories similar to Hofhuis et al.:[21] (1)"physician-confirmed erythema migrans" or (2)"physician-confirmed disseminated Lyme borreliosis", in line with the clinical case definitions for Lyme borreliosis described by Stanek et al.[7] As secondary outcome measures, tick factors that may predict the risk of Lyme borreliosis were assessed, including duration of tick attachment, tick engorgement and tick infection with *Borrelia burgdorferi* s.l.[21] For patient safety, all incoming questionnaires were monitored weekly for reports of adverse events.

Statistical analysis

With a planned sample size of 2500 (1250 prophylaxis; 1250 no treatment), our study was designed to detect a 58% relative risk reduction for the development of Lyme borreliosis in the prophylaxis group assuming a Lyme borreliosis incidence in the no-treatment group of at least 2%, as found in earlier studies (power 80%, alpha=0.05).[21,24]

We performed both a modified-intention-to-treat and a per-protocol analysis.

For the modified-intention-to-treat analysis participants were excluded if they: a) reported chronic complaints attributed to Lyme borreliosis at $t=0$; b) did not timely finish their questionnaire at $t=0$; c) missed both of the questionnaires at $t=1$ week and $t=1$ month; d) missed both of the questionnaires at $t=3$ and 6 months; e) reported new tick bites within 3 months after inclusion unless Lyme borreliosis developed before these new tick bites; f) at $t=0$ reported medication use – other than the prescribed study prophylaxis – which might have had an effect on the development of Lyme borreliosis, such as immunosuppressants, other antibiotic prescriptions than the study prophylaxis, or erroneously prescribed study prophylaxis (i.e. other antibiotics than doxycycline, wrong dosage or taking the prophylaxis more than 72 h after removing the tick); g) at $t=0$ reported medication use that possibly had an effect on the efficacy of the prophylaxis such as antacids and antiepileptics. See also Fig. 1 and supplementary material Table S1.

For the per-protocol analysis, we additionally excluded all participants that reported crossover between study groups. Some of the participants in the prophylaxis group reported crossover to the

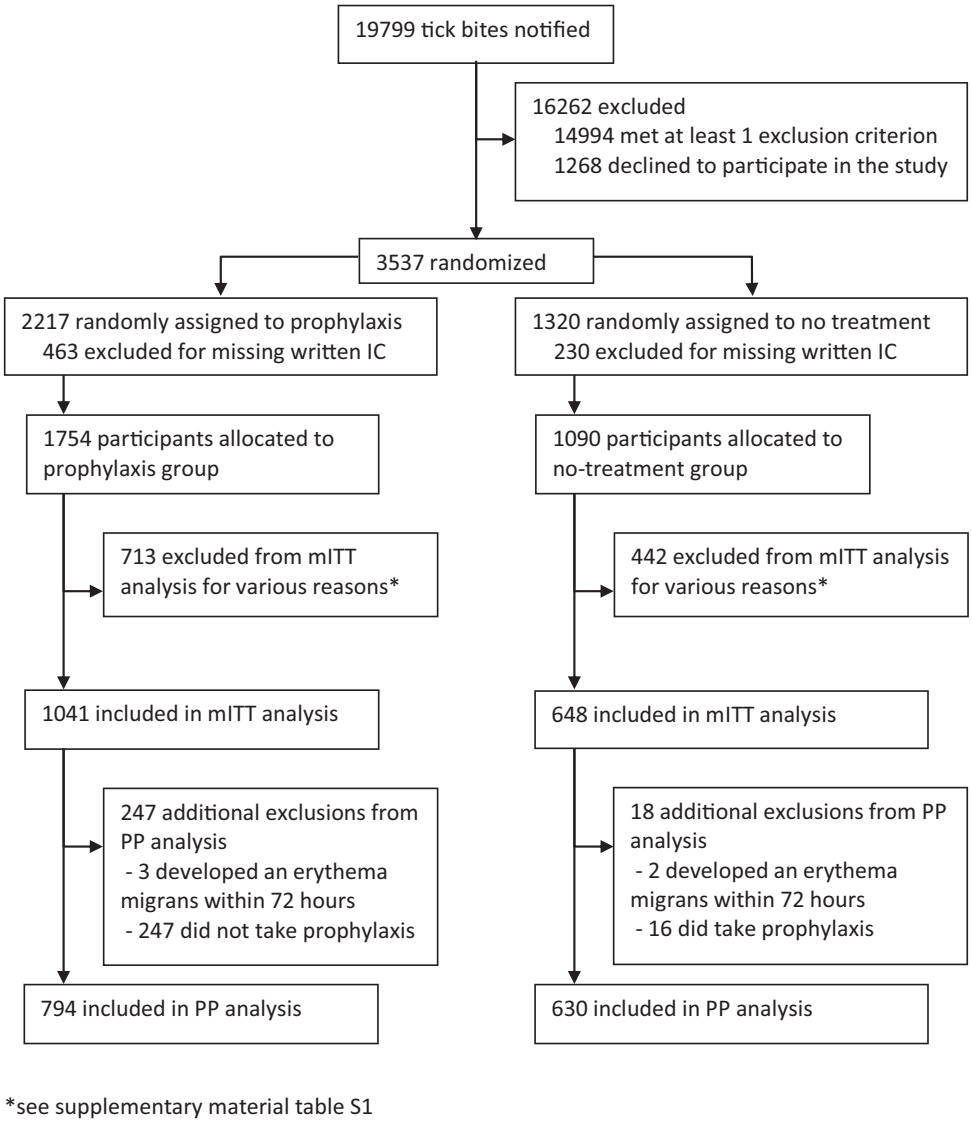

Fig. 1. Trial profile. mITT = modified intention-to-treat. PP = per-protocol. IC = informed consent *see supplementary material Table S1 for a complete list of exclusions

no-treatment group due to erythema migrans developed within 72 h after tick removal, which called for an immediate full antibiotic treatment instead of the study prophylaxis. To balance the per-protocol study groups, we therefore excluded all participants diagnosed with Lyme borreliosis within 72 h after tick removal.

For both the modified-intention-to-treat and per-protocol analysis, we used the Newcombe-Wilson method to estimate the absolute risk in both groups, relative risk, relative risk reduction and number-needed-to-treat to prevent one case of Lyme borreliosis.[25] Since post-randomization exclusions may have biased the study groups, we also used a permutation test of overall treatment effect with the data stratified over potential confounders in both the modified-intention-to-treat and per-protocol populations to correct for possible biases. To explore ways of lowering the number-needed-to-treat, we performed subgroup analyses for participants with a higher risk of Lyme borreliosis according to their baseline tick characteristics, such as an engorged tick, reported tick attachment time of more than 24 h and *B. burgdorferi* s.l. positive ticks.

R version 3.4.3 and the R packages coin and epitools were used for the statistical analyses. This study was monitored by a clinical research associate and is registered with the Netherlands Trial Register, number NTR3953.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Fig. 1 shows that from April 11th, 2013 to June 10th, 2015 a total of 19,799 individuals notified a tick bite on the website www.tekenradar.nl of whom 4805 fulfilled the inclusion criteria and were invited to participate in the study. Of these, 3537 individuals provided digital informed consent of whom 2217 were randomly allocated to the prophylaxis group and 1320 to the no-treatment (control) group. After online randomization, a total of 693 individuals failed to supply written informed consent and could thus not be analysed in this study. The participants' baseline characteristics age, sex, number of tick bites, and tick infection were well balanced for both study groups in the remaining 2844 participants available for analyses (Table 1).

Table 1
Characteristics of participants in both study groups for the randomized participants, modified-intention-to-treat analysis and the per-protocol analysis.

	Randomized population		Modified-intention-to-treat population		Per-protocol population	
	Prophylaxis group ($n=1754$)	No-treatment group ($n=1090$)	Prophylaxis group ($n=1041$)	No-treatment group ($n=648$)	Prophylaxis group ($n=794$)	No-treatment group ($n=630$)
Age, years*	45. 8 (17.7)	45.4 (18.0)	44.8 (17.6)	44.7(18.2)	45.0 (17.1)	44.6(18.1)
Sex						
Male	865 (49%)	538 (49%)	515 (49%)	330 (51%)	398 (50%)	322 (51%)
Female	887 (51%)	552 (51%)	526 (51%)	318 (49%)	396 (50%)	308 (49%)
Unknown	2 (0%)	0 (0%)				
History of Lyme borreliosis						
no previous episodes	1593 (91%)	969 (89%)	964 (93%)	603 (93%)	727 (92%)	587 (93%)
fully recuperated from previous episode	142 (8%)	106 (10%)	77 (7%)	45 (7%)	67 (8%)	43 (7%)
still suffering Lyme borreliosis symptoms	19 (1%)	15 (1%)				
Number of tick bites						
1 tick	1490 (93%)	916 (93%)	970 (93%)	608 (94%)	738 (93%)	593 (94%)
multiple ticks	119 (7%)	68 (7%)	71 (7%)	40 (6%)	56 (7%)	37 (6%)
Tick stage**						
Larva	19 (1%)	16 (2%)	15 (1%)	10 (2%)	11 (1%)	9 (1%)
Nymph	1117 (72%)	696 (73%)	756 (73%)	469 (72%)	589 (74%)	461 (73%)
Female	400 (26%)	235 (25%)	261 (25%)	164 (25%)	188 (24%)	156 (25%)
Male	6 (0%)	2 (0%)	3 (0%)	1 (0%)	2 (0%)	1 (0%)
Unknown	11 (1%)	5 (1%)	6 (1%)	4 (1%)	4 (1%)	3 (0%)
Engorgement**						
0 - flat	671 (42%)	403 (41%)	453 (44%)	274 (42%)	352 (44%)	268 (43%)
1 - slightly engorged	341 (21%)	202 (21%)	221 (21%)	138 (21%)	162 (20%)	134 (21%)
2 - engorged	299 (19%)	183 (19%)	210 (20%)	121 (19%)	163 (21%)	118 (19%)
3 - severely engorged	207 (13%)	151 (15%)	135 (13%)	104 (16%)	102 (13%)	101 (16%)
Unknown	91 (6%)	45 (5%)	22 (2%)	11 (2%)	15 (2%)	9 (1%)
Tick attachment time						
less than 12 h	380 (22%)	239 (22%)	213 (20%)	151 (23%)	156 (20%)	148 (23%)
12 to 24 h	333 (19%)	251 (23%)	193 (19%)	148 (23%)	142 (18%)	147 (23%)
over 24 h	558 (32%)	339 (31%)	345 (33%)	193 (30%)	268 (34%)	183 (29%)
Unknown	483 (28%)	261 (24%)	290 (28%)	156 (24%)	228 (29%)	152 (24%)
Follow-up at 1 week and 1 month						
neither week nor month questionnaire	100 (6%)	63 (6%)				
either week or month questionnaire	186 (11%)	122 (11%)	106 (10%)	63 (10%)	40 (5%)	61 (10%)
both week and month questionnaire	1468 (84%)	905 (83%)	935 (90%)	585 (90%)	754 (95%)	569 (90%)
Follow-up at 3 and 6 months						
neither 3 nor 6 month questionnaires	113 (6%)	81 (7%)				
either 3 or 6 month questionnaires	142 (8%)	101 (9%)	71 (7%)	54 (8%)	38 (5%)	51 (8%)
both 3 and 6 month questionnaires	1499 (85%)	908 (83%)	970 (93%)	594 (92%)	756 (95%)	579 (92%)
Tick infection with *B. burgdorferi* s.l.						
Negative in PCR	1222 (76%)	759 (77%)	825 (79%)	522 (81%)	644 (81%)	509 (81%)
Positive in PCR	313 (19%)	183 (19%)	216 (21%)	126 (19%)	150 (19%)	121 (19%)
Unknown	74 (5%)	42 (4%)				

* Mean age (standard deviation).
** In case multiple ticks were sent in, the batch was classified as the tick with the highest risk of Lyme borreliosis.

For modified-intention-to-treat analysis, 1041 participants were included in the prophylaxis group and 648 in the no-treatment group (Fig. 1). A total of 1155 participants was excluded because of the acquisition of other tick bites in the first 3 months of the study, lost to follow-up in the 6 months after inclusion, and/or various other reasons (supplementary material Table S1). Baseline characteristics for both study groups remained similar (Table 1).

For the per-protocol analysis 794 participants were included in the prophylaxis group and 630 in the no-treatment group, after additional exclusion of 265 participants because of crossover between study groups and/or development of erythema migrans within 72 h after tick removal (Fig. 1).

In the modified-intention-to-treat analysis, a total of 29 participants reported Lyme borreliosis, of which 28 (97%) were erythema

migrans and 1 (3%) disseminated Lyme borreliosis (see case list in supplementary material Table S2). Of these 29 cases, 10 out of 1041 (0.96%) were reported in the prophylaxis group and 19 out of 648 (2.9%) in the no-treatment group, resulting in a relative risk of 3.1 (95% CI 1.4 6.5, p-value = 0.003) and a number-needed-to-treat of 51 (95% CI 29 - 180) to prevent one case of Lyme borreliosis. This corresponds with a relative risk reduction of the prophylactic treatment of 67% (95% CI 31 – 84%, Table 2). In the per-protocol analysis ($n = 1424$) a total of 22 participants reported development of Lyme borreliosis of which 21 (96%) erythema migrans and 1 (4%) disseminated Lyme borreliosis (supplementary material, Table S2). Five out of 794 (0.6%) were reported amongst the participants who had taken prophylaxis according to the study protocol, and 17 out of 630 (2.7%) amongst the participants who did not take the treatment, resulting in a relative risk of 4.3 (95% CI 1.59 - 11.55, p-value = 0.002), a number-needed-to-treat of 48 (95% CI 28 - 150), and a relative risk reduction of 77% (95% CI 39 – 91%, Table 2).

Table 1 shows that the tick bite characteristics of participants in the modified-intention-to-treat population were mostly similar in the two study groups. We only observed a higher fraction of 12–24 h reported duration of tick attachment ($p = 0.038$) and a higher fraction of more severely engorged ticks in the no-treatment group ($p = 0.090$). When tested with the permutation test with the data stratified over these two variables, the difference in risk between the study groups in the modified-intention-to-treat analysis remained significant ($p = 0.014$).

In the participants included in the per-protocol analysis most tick bite characteristics were similar in the two study groups as well; however, the difference in the distribution of tick attachment duration was more marked than in the population for modified-intention-to-treat analysis (p-values ranging from 0.013 to 0.090). Tick engorgement still showed a possible non-random distribution and additionally the no-treatment group also had a higher fraction of missing questionnaires ($p = 0.001$, in short-term follow-up and $p = 0.014$ for 3-month follow-up). Despite these differences, we found evidence for a treatment effect when the difference between the prophylaxis and no-treatment group was tested with the permutation test with the data stratified over missing questionnaires (short and long term follow-up), tick engorgement and observed duration of tick attachment ($p = 0.006$).

In both the modified-intention-to-treat and the per-protocol subgroup analyses, the risk difference between study groups remained similar for participants with engorged ticks (modified-intention-to-treat: a relative risk of 3.12 (95% CI 1.27 - 7.65) and number-needed-to-treat of 38 (95% CI 20 - 224); per-protocol: a relative risk of 5.65 (95% CI 1.64 19.49) and number-needed-to-treat of 31 (95% CI 18 - 99); Table 2). For participants with *B. burgdorferi* s.l. PCR positive ticks the point estimate of the relative risk was higher and the number-needed-to-treat lower (modified-intention-to-treat: a relative risk of 8.00, 95% CI 2.34 - 27.30, number-needed-to-treat of 10, 95% CI 6 - 25), likewise for the subgroup of participants that had an engorged and *B. burgdorferi* s.l. PCR positive tick (modified-intention-to-treat: a relative risk of 9.20, 95% CI 2.11–40.11, number-needed-to-treat of 6, 95% CI 4 - 17). However, for the people reporting a tick attachment time of over 24 h, a significant risk difference was found in the per-protocol analysis (a relative risk of 8.79, 1.07 – 72.37 p-value = 0.018, Table 2), but not in the modified-intention-to-treat analysis.

When restricting the analyses to cases with ticks that tested positive for *B. burgdorferi* s.l. in PCR, the difference between study groups became even more marked in the modified-intention-to-treat analysis (a relative risk of 7.5, 95% CI 2.17 – 26.01 *p*-value <0.001) and in the per-protocol analysis the efficacy becomes 100% (95% CI 77–100%, Table 2). Of all erythema migrans cases, the participants in the prophylaxis group tend to notice development of

Table 2
Differences between study groups for modified-intention-to-treat analysis and per-protocol analysis as calculated as absolute risk, relative risk reduction, number-needed-to-treat and relative risk, for all study participants as well as several subsets of participants as characterized by their individual characteristics.

	prophylaxis group			no-treatment group				relative risk	relative risk reduction	number-needed-to-treat
	n	events	absolute risk (%)	n	events	absolute risk (%)	p-value	estimate (95% CI)	estimate (95% CI)	estimate (95% CI)
All participants										
modified-intention-to-treat	1041	10	0.96	648	19	2.93	0.003	3.05 (1.43 - 6.52)	67% (31% - 84%)	51 (29 - 180)
per-protocol	794	5	0.63	630	17	2.70	0.002	4.29 (1.59 - 11.55)	77% (39% - 91%)	48 (28 - 150)
Subset of participants sending in an engorged tick (Engorgement category 1 + 2 + 3, see Table 1)										
modified-intention-to-treat	566	7	1.24	363	14	3.86	0.010	3.12 (1.27 - 7.65)	68% (23% - 87%)	38 (20 - 224)
per-protocol	427	3	0.70	353	14	3.97	0.002	5.65 (1.64 - 19.49)	82% (43% - 95%)	31 (18 - 99)
Subset of participants sending in a *B. burgdorferi* s.l. positive tick										
modified-intention-to-treat	216	3	1.39	126	14	11.11	<0.001	8 (2.34 - 27.30)	88% (60% - 96%)	10 (6 - 25)
per-protocol	150	0	0.00	121	13	10.74	<0.001		100% (77% - 100%)	9 (6 - 20)
Subset of participants sending in an engorged (Engorgement category 1 + 2 + 3, see Table 1) and *B. burgdorferi* s.l. positive tick										
modified-intention-to-treat	102	2	1.96	61	11	18.03	<0.001	9.20 (2.11 - 40.11)	89% (58% - 97%)	6 (4 - 17)
per-protocol	65	0	0.00	59	11	18.64	<0.001		100% (70% - 100%)	5 (4 - 13)
Subset of participants reporting an attachment time of >24 h										
modified-intention-to-treat	345	4	1.16	193	6	3.11	0.173	2.68 (0.77 - 9.39)		
per-protocol	268	1	0.37	183	6	3.28	0.018	8.79 (1.07 - 72.38)	89% (29% - 98%)	34 (17 - 913)
Only cases caused by *B. burgdorferi* s.l. positive ticks										
modified-intention-to-treat	1034	3	0.29	643	14	2.18	<0.001	7.5 (2.17 - 26.01)	87% (57% - 96%)	53 (32 - 140)
per-protocol	789	0	0.00	626	13	2.08	<0.001		100% (77% - 100%)	48 (31 - 109)

their erythema migrans earlier after the tick bite than those in the no-treatment group, although the difference is only significant in the per-protocol-analysis (Wilcoxon ranked sum test with continuity correction; modified-intention-to-treat: mean 10.4 vs. 14.6 days, p-value = 0.079, per-protocol: mean 5.2 vs. 16.3 days, *p*-value = 0.003, see Table S2).

No serious adverse events or suspected unexpected serious adverse reactions were reported. However, some treatment-related complaints were notified by the 1188 participants that reported intake of prophylaxis. Most frequently reported were nausea and diarrhoea, both by 5% of the treated participants. Other complaints mentioned by at least 1% of the treated participants were headache, stomach-ache, dizziness and fatigue. 84.76% did not report any adverse reaction to the study medication.

Discussion

This open-label randomized controlled trial shows that a single prophylactic dose of 200 mg doxycycline is effective in reducing the risk of Lyme borreliosis after an *Ixodes ricinus* tick bite. We found a relative risk reduction (or efficacy) of 67% in the modified-intention-to-treat population with a confidence interval (31% to 84%), largely overlapping with the confidence intervals of the efficacy reported in earlier US studies for prophylaxis after an *Ixodes scapularis* tick bite.[10,12] Our results indicate that in a European setting, a single prophylactic dose of doxycycline has a similar efficacy in preventing Lyme borreliosis as in endemic areas in North America, despite differences in transmission dynamics due to different tick vectors and other predominant *Borrelia burgdorferi* s.l. genospecies. Evidence-based use of prophylaxis for prevention of Lyme borreliosis can thus be extended to the European setting.

This study was designed as an open-label trial to facilitate inclusion through an online platform, which means that after randomization, participants were aware of their allotted study group. This has caused some selection bias by crossover, or drop-out rates that were associated to the randomized allocation (Fig. 1 and supplementary material Table S1). For example, participants that took prophylaxis in the no-treatment group often had a longer tick-attachment time. However, after randomization most baseline characteristics remained similar in both the modified-intention-to-treat and per-protocol study groups (Table 1). Moreover, when we performed analyses stratified over characteristics that did suggest possible selection bias, the risk difference between the study groups persisted. Also, the similarities in the results between the modified-intention-to-treat and per-protocol analyses, especially when stratified over variables that may have been unbalanced between study groups, further support our findings.

In addition, the use of an online inclusion platform enabled us to include far more participants as well as positive endpoint measurements (cases who developed Lyme borreliosis) than any of the studies done previously. While the largest study to date had 482 participants, of which 9 developed an erythema migrans,[12] we were able to evaluate 1689 participants of which 28 developed an erythema migrans and one acrodermatitis chronica atrophicans, enabling us to look at several subgroups with respect to tick and participant characteristics.

The study prophylaxis was prescribed by the participants own general practitioner, which approaches the administering of prophylaxis in a routine primary-care setting. The current open-label study is therefore largely a pragmatic trial and its result indicative for the efficacy of prophylaxis in primary care.[26,27] Besides actual treatment of the tick bite, the visit to the general practitioner in itself may make people more alert to the possible development of erythema migrans after a tick bite, since participants that visited their general practitioner for prescription of the study prophylaxis were often quicker to notice their erythema migrans.

Even though prophylactic treatment leads to a statistically significant reduction in the risk of Lyme borreliosis, the number-needed-to-treat in both the current study and the US studies remains relatively high, at around 50. Whether or not this justifies prescribing prophylaxis after each tick bite needs further evaluation. In the Netherlands for instance, with 1.5 million tick bites per year this could lead to a substantial increase in primary-care consultations and use of antibiotics. Moreover, the 25.500 erythema migrans cases per year that result from these tick bites can be recognized relatively easily and can be cured with a full course of doxycycline. When looking solely at disseminated Lyme borreliosis (in the Netherlands 1500 cases per year), we observed only one case in the no-treatment group, which is insufficient for calculating prophylaxis efficacy specifically for disseminated Lyme.

In the future, the number-needed-to-treat can possibly be reduced by only treating the patients with the highest risk of Lyme borreliosis using tick-screening selection criteria such as *Borrelia* infection of the tick and tick engorgement.[16,21,28] For instance, we found a number-needed-to-treat of 10 in the participants with a *B. burgdorferi* s.l. positive tick, making prophylaxis a much more effective treatment option in this subset of patients. Unfortunately, in a study by Sprong et al. a commercially available point-of-care tick test did not predict development of Lyme borreliosis.[29] To our knowledge, since then no reliably validated *Borrelia* tick test has become available for tick screening in a point-of-care setting.

Not all ticks of the 29 participants in which Lyme borreliosis developed tested positive for *Borrelia* (Table 2, modified-intention-to-treat analysis). There could be several explanations for this phenomenon, perhaps some of the diagnoses of erythema migrans in clinical practice are either misdiagnosis or possibly not due to *Borrelia burgdorferi* s.l. infection, or perhaps the PCR test is not 100% sensitive or participants failed to notice a secondary tick bite.

Although all treatment-related complaints reported in this study lie within the known spectrum of side-effects for doxycycline, an alternative preventive treatment with less possible side-effects than oral prophylaxis, could be topical prophylaxis.[11,30] A study with a murine model showed protection for *Borrelia* infection by topical azithromycin,[30] and subgroup analyses in a clinical trial in Germany and Austria suggested that topical azithromycin reduces erythema migrans after an *Ixodes ricinus* tick bite.[11]

Our study confirms that treating a tick bite with a prophylactic dose of 200 mg of doxycycline within 72 h after removing the tick significantly reduces the risk of Lyme borreliosis in a European setting. However, we found a fairly high number-needed-to-treat, similar to US studies, which can be reduced if objective point-of-care measurements for tick infection or feeding time would become available to restrict prophylactic treatment to high-risk tick bites.

Contributors

MH, AH, HS, JF, WA, HVW, WVP and CVDW conceived and designed the analysis. MH, SB and CVDW collected the data. HS, MF and ADVL did the laboratory analysis. MH, AH, SB, JF, WVP and CVDW did the statistical analysis. MH and CVDW wrote the article. All authors commented on and approved the final manuscript.

Declaration of Competing Interest

We declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.06.032.

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