Journal of Infection 81 (2020) 90–97

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Relationship between *Coxiella burnetii* (Q fever) antibody serology and time spent outdoors

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ARTICLE INFO

Article history:
Accepted 13 April 2020
Available online 21 April 2020

Keywords:
Time
Outdoors
Exposure
Outbreak
Q fever
Zoonosis

SUMMARY

Background/aim: From 2007 through 2010, the Netherlands experienced the largest recorded Q fever outbreak to date. People living closer to *Coxiella burnetii* infected goat farms were at increased risk for acute Q fever. Time spent outdoors near infected farms may have contributed to exposure to *C. burnetii*. The aim of this study was to retrospectively evaluate whether hours/week spent outdoors, in the vicinity of previously *C. burnetii* infected goat farms, was associated with presence of antibodies against *C. burnetii* in residents of a rural area in the Netherlands.

Methods: Between 2014-2015, we collected *C. burnetii* antibody serology and self-reported data about habitual hours/week spent outdoors near the home from 2494 adults. From a subgroup we collected 941 GPS tracks, enabling analyses of active mobility in the outbreak region. Participants were categorised as exposed if they spent time within specified distances (500m, 1000m, 2000m, or 4000m) of *C. burnetii* infected goat farms. We evaluated whether time spent near these farms was associated with positive *C. burnetii* serology using spline analyses and logistic regression.

Results: People that spent more hours/week outdoors near infected farms had a significantly increased risk for positive *C. burnetii* serology (time spent within 2000m of a *C. burnetii* abortion-wave positive farm, OR 3.6 (1.2-10.6)), compared to people spending less hours/week outdoors.

Conclusions: Outdoor exposure contributed to the risk of becoming *C. burnetii* serology positive. These associations were stronger if people spent more time near *C. burnetii* infected farms. Outdoor exposure should, if feasible, be included in outbreak investigations.

Introduction

In the years 2007 through 2010, the Netherlands experienced the largest outbreak of Q fever reported to date[1–3]. Over 4000 human cases were identified[4,5] predominantly in the south-eastern part of the country[3], a region with a high density of livestock farming[6,7]. The primary sources of *Coxiella burnetii* infections were abortion-waves in dairy goats, which in the Netherlands are kept in intensive livestock systems[4]. When human Q fever incidence was combined with data about *C. burnetii* status of farms, spatial relationships were identified: with increasing distance from *C. burnetii* positive farms, decreasing human Q fever incidence was observed[8,9]. This relationship has been thoroughly investigated in the past, focussing on environmental conditions[10,11], meteorological conditions[12], and mapping cases in relation to *C. burnetii* positive farms[2,13] as recently reviewed by De Rooij et al[5].

The outbreak was contained by at first, voluntary and later, obligatory vaccination of dairy goats[14,15], introducing mandatory bulk milk checks for *C. burnetii* presence[16] and culling of pregnant goats on bulk milk tank positive farms[17]. Still, in the affected area residual effects remain present to date, with several hundred

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https://doi.org/10.1016/j.jinf.2020.04.013

people still suffering from chronic Q fever after the outbreak[18]. The Q fever outbreak contributed to the interest into the potential effects of livestock production on human health and led to the start of the large "Livestock Farming and Neighbouring Residents' Health" study in 2012 (Dutch acronym: VGO). The main goal of the VGO study is to investigate whether living in the vicinity of livestock farms has an impact on the health of residents[19]. In the VGO study and all previous Q fever analyses, personal exposure was approximated by assigning exposure levels to the home address and for the Q fever analyses both abortion waves and/or bulk milk positivity for *C. burnettii* were used to assign a stable as being *C. burnetii* positive[2,4,10,12,13,9,20]. These approaches are disregarding whether time spent outdoors in close proximity of *C. burnetii* positive farms poses additional risks. Especially, time spent outdoors and active human mobility near *C. burnetii* emitting goat farms, may have affected exposure to *C. burnetii* during the outbreak[2,5,12]. Therefore, as an additional study to the VGO study, the VGO GPS study was initiated in 2014. In this study, participants were asked to log their mobility with a GPS tracker during a whole week. The VGO GPS study took place in the same area where the Q fever outbreak occurred and has provided us with detailed information of residents' daily mobility and average weekly time spent outdoors near the home[7,21].

For the current study, we aimed at evaluating whether hours/week spent outdoors, an aggregate of self-reported hours/week spent outdoors near the home and GPS measured active mobility in the vicinity of goat farms was associated with the risk of positive *C. burnetii* antibody serology. Furthermore, we assessed whether either self-reported hours/week spent outdoors near the home, or GPS measured active mobility were associated with the risk for positive *C. burnetii* antibody serology.

Methods

Study population: VGO cohort

Study participants of the VGO cohort (N=2494) lived in a rural area in the Netherlands[19]. Farmers and people living on farms were excluded *a priori*, since the focus was on health of non-occupationally exposed neighbouring residents. All cohort members underwent a medical examination in a field study that took place in 2014-2015. During the examination, blood samples were taken and participants were asked to fill in a baseline questionnaire (VGO questionnaire), including questions about demographics, health and lifestyle[19,22]. From the VGO questionnaire, information was available about the home address of participants and the hours/week people spend outdoors near their home.

Study population: GPS group

VGO cohort members that indicated they could be contacted for follow-up research were recruited as participants for the GPS study. We invited 1517 VGO participants to take part in the GPS study and 1014 agreed to participate. All 1014 consenting participants were sent a GPS logger (TracKing Pro Land Air Sea systems Woodstock IL, USA) and were asked to always take it with them during one week before returning it to the study centre. GPS loggers were sent in sixteen batches between September 2014 and February 2016. Included in the package was a questionnaire regarding study adherence and whether participants had logged a 'normal week'. GPSs were set to a logging interval of one second and were equipped with a motion sensor to prevent battery depletion. After data cleaning[7], 941 usable GPS tracks were available (38% of the total VGO cohort), and overall participants had a median of 186 hours of data logged. We used a 60m buffer around the home to assign every logged GPS coordinate as being 'indoors' or 'outdoors', transport modes (walking, biking or motorised transport) were assigned to 'outdoors' coordinates using a previously developed algorithm[21,23]. The 60m buffer around the home, minimizes the chance that time spent outdoors around the home was included to the mobility measurement[21]. Fig. 1 shows a flowchart of the recruitment, data collection and data cleaning process.

Exposure assignment

Since infected goat farms were previously identified as sources in the Dutch Q fever outbreak[1,2,8], we performed analyses with buffers of 500m, 1000m, 2000m, and 4000m around goat farms, in order to test for distance-response relationships. For comparability reasons, we initially evaluated if using a 5000m buffer[8] was feasible, there were however limitations with applying these buffers: using the smaller buffers (500m and 1000m) resulted in too few people exposed to goat farms and using the largest buffers (4000m and 5000m) resulted in too few people unexposed to farms. We therefore decided not to use the 5000m buffer, but used the 4000m buffer as maximum distance and preferred to show the results of the analyses with the 2000m buffers as primary outcomes. See Table 1 for an overview of applied exposure variables and Supplementary Table 1 for an overview of group sizes for the analyses with 500m, 1000m, 2000m and 4000m buffers, an overview of the spatial distribution of the home addresses of participants and the applied buffers, is given in Supp. Fig. 1.

For comparability with previous studies and to evaluate whether farm status ('*C. burnetii* positive' or 'negative') influenced the outcomes, four different definitions were used to describe the *C. burnetii* status of a goat farm:

(a) 'abortion-wave' positive goat farms, these are farms that experienced *C. burnetii* related abortion waves (>5% of animals aborted[1]) between 2007-2009. During these abortion-waves, large amounts of bacteria are excreted[24] and due to the open stables in the Netherlands[4] bacteria can be easily emitted to the direct surroundings of farms. This status was *a priori* defined to represent our primary source of exposure,
(b) 'any *C. burnetii* signal' positive goat farms, 'abortion-wave' and/or 'bulk milk tank' (real-time PCR tests on milk samples, enabling quantification of bacteria[16]) positive, this status was often used in previous Q fever analyses in the Netherlands[2,4,10,12,13,9,20] and we included it for comparability reasons,
(c) goat farms, irrespective of *C. burnetii* status[8],
(d) 'negative' goat farms, all goat farms, excluding farms that were 'any *C. burnetii* signal' positive.

Data about location of goat farms was obtained from the database (2012) of livestock-keeping farms (Dutch abbreviation: BVB-database). These provincial databases (Limburg and Noord-Brabant) include permit registrations for farms, with information pertaining to location of the farm, animal species and numbers[25,26]. Farms with >50 goats were defined as goat farms, this cut-off was used because intervention steps were mandatory on farms with >50 goats during the outbreak[9,22]. Data concerning abortion-waves occurring on goat farms was provided by GD[27], data about *C. burnetii* positive bulk tank milk testing was available via the Dutch National Institute for Public Health and the Environment (RIVM), but originally collected by the Dutch food and consumer product safety authority[28].

We calculated aggregated hours/week spent outdoors by adding self-reported hours/week spent outdoors near the home (e.g. gardening, care for animals, do-it-yourself activities, sitting in the garden, in hours/week from VGO questionnaire, see Supplement 'VGO questionnaire 'time spent outdoors near the home'' for the

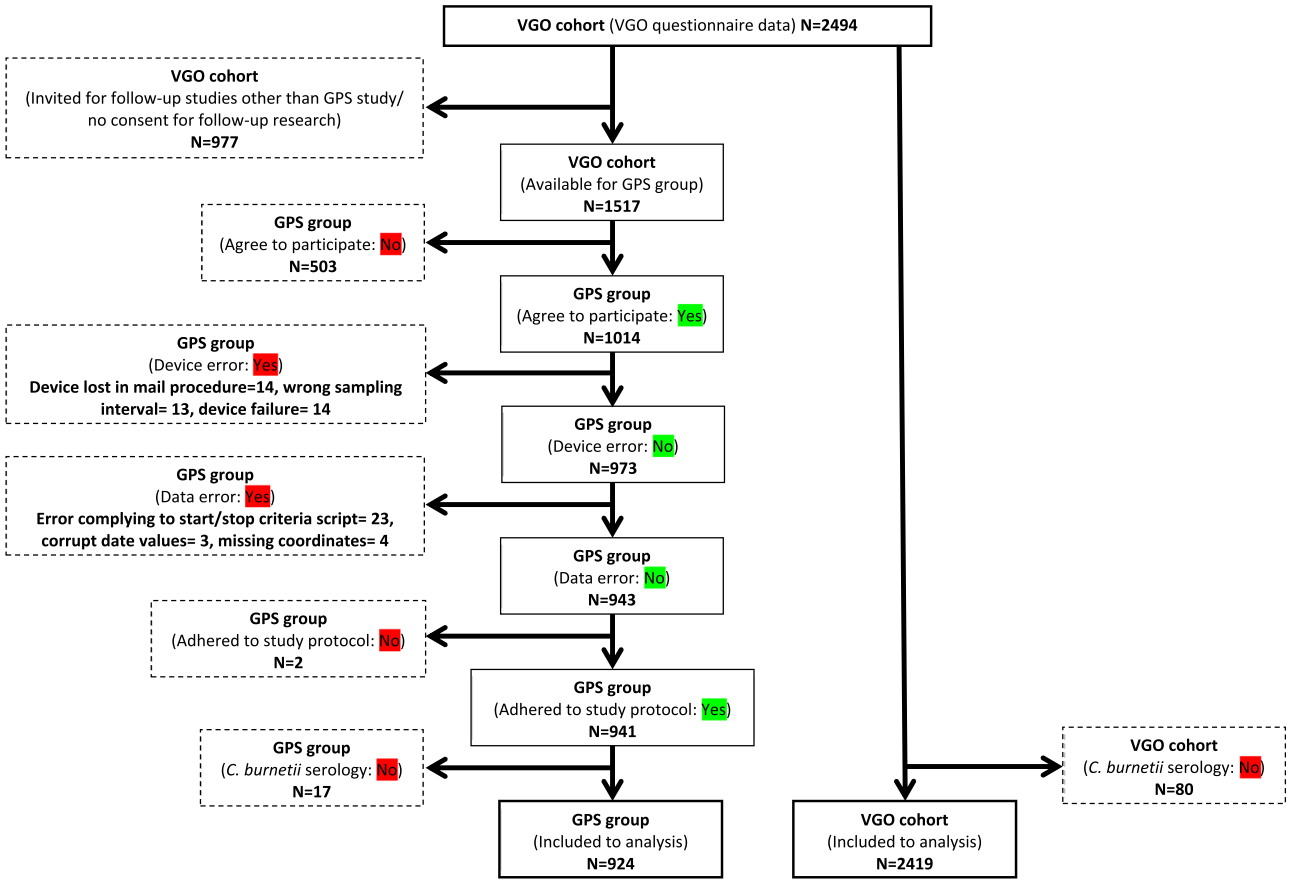

Fig. 1. Flowchart of the recruitment, data collection and data cleaning process in the VGO GPS study.

Table 1
Overview of used exposure variables in the analyses.

Exposure variable	Description	Buffer distances	*C. burnetii* statuses goat farms	Hours/week cut-off for dichotomisation	Performed analyses
GPS group					
Home	Home address within a distance of a goat farm	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	n.a.	Logistic regression Spline analyses*
Aggregated hours/week	Total hours/week spent outdoors within a distance of a goat farm, residential and active mobility aggregated	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	4.6 hours/week (median)	Spline analyses Logistic regression
Residential	Self-reported hours/week spent outdoors near the home address, within a distance of a goat farm	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	1.5 hours/week (median)	Spline analyses Logistic regression
Active mobility	GPS measured hours/week spent outdoors on active mobility, within a distance of a goat farm (walking and biking)	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	See Supp. Table 2 for a detailed overview of applied dichotomisation	Spline analyses Logistic regression
VGO cohort					
Home	Home address within a distance of a goat farm	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	n.a.	Logistic regression Spline analyses*
Residential	Self-reported hours/week spent outdoors near the home address, within a distance of a goat farm	500m 1000m 2000m 4000m	'abortion-wave' positive 'any *C. burnetii* signal' positive 'goat farm' 'negative'	1.5 hours/week (median)	Spline analyses Logistic regression

* *Note,* these spline analyses were performed using the shortest distance between the home and closest goat farm and were considered as secondary analyses. All other spline analyses were performed with 'exposed' hours/week spent outdoors and considered as primary analyses.

used question) and hours/week spent on active mobility (measured with GPS loggers). Aggregated hours/week spent outdoors were dichotomised into 'not often outdoors' and 'often outdoors' using the median hours/week spent outdoors (4.6h/week). This frequency categorisation was combined with information about the goat farms to which people were exposed ('abortion-wave' positive farm within 2000m of home and/or GPS track).

In line with previous analyses, we defined 'at home exposed' if a participant lived within 2000m distance of an 'abortion-wave' positive goat farm. We assigned exposure to self-reported hours/week spent outdoors near the home (from VGO questionnaire). Here, we dichotomised self-reported hours/week into 'not often outdoors' and 'often outdoors' using the median hours/week spent outdoors near home (1.5h/week). Exposure during these hours/week spent outdoors was defined in line with 'at home exposed'.

Next, data from the GPS group was used to evaluate the associations between hours/week spent outdoors on active mobility near 'abortion-wave' positive farms and *C. burnetii* antibody serology responses. We used GPS coordinates assigned to one of the active modes (walking and biking), that fell within 2000m distance around an 'abortion-wave' positive farm. The number of 'exposed' GPS coordinates (one per second) were added, thus providing an estimate of the total hours/week 'exposed' while being actively mobile. Participants were indicated as 'exposed while mobile' if their total logged 'exposed' hours/week exceeded the 20th percentile of 'exposed' hours/week of the group that was actively mobile within the 2000m buffer (for 'abortion-wave' positive farms the cut-off was 116 seconds). Participants that logged less than the 20th percentile and those who were actively mobile outside of the used buffers were assigned to the 'unexposed while mobile' reference group. See Supp. Table 2 for an overview of the used time cut-offs.

Serology

Participants were considered *C. burnetii* antibody positive, if levels of IgG antibodies to *C. burnetii* phase II antigen were above 30 International Units/ml (IU/ml) or between 20-30 IU/ml ('borderline' positive). Levels below 20 IU/ml were considered 'negative', according to the manufacturer's standards (Serion ELISA classic, Virion/Serion, Würzburg, Germany)[20,22].

Statistical analysis

We previously tested whether the GPS group was a representative sample of the VGO cohort[21], but repeated the analyses specified for this study. Chi-square tests of independence were performed for *C. burnetii* antibody serology status, gender, education level and smoking status. Age distributions were compared with a Wilcoxon rank sum test.

We used splines to explore the shape of the association between the different exposure variables (Table 1) and *C. burnetii* serology. Penalised regression splines were used applying the (default) 'thin plate' basis of the R package mgcv (mixed generalised additive model computation vehicle). Due to the group size limitations (Supp. Table 1), we preferred to show the results for the 2000m buffers, spline plots using the other buffers are provided in Supp. Figs. 2,3,4.

We used logistic regression to evaluate associations between *C. burnetii* serology and the different exposure variables (Table 1) adjusting for age, gender, educational level (low, medium, high) and smoking status (current, former, never). The analyses for living near a farm and self-reported hours/week spent outdoors near the home were subsequently repeated in the full VGO cohort.

Sensitivity analysis

In addition, we used splines in a number of sensitivity analyses to assess whether:

I. The distance between the home address and nearest 'abortion-wave' positive farm was associated with positive serology for *C. burnetii*[29].
II. The case definition influenced the shape of the associations. For this analysis participants indicated as 'borderline' positive (*C. burnetii* antibody serology: 20-30 IU/ml) were assumed to be false positive and thus assigned to the reference group instead of the positive case group.
III. Logging a normal week during the GPS measurements influenced the shape of the associations. For all GPS group members, we had self-reported information whether people had had a 'normal week' during the GPS measurement. We excluded participants that reported not having had a 'normal week' during GPS logging.
IV. Analysis I. was repeated in the full VGO cohort.

All analyses were repeated with the other *C. burnetii* statuses of goat farms ('any *C. burnetii* signal' positive farm, 'goat farm' and 'negative' farm) and buffer sizes (500m, 1000m, and 4000m).

All statistical analyses were performed using R (3.4.3), and all GIS analyses were performed with ArcGIS ArcMap 10.5 (ESRI, Redlands, CA, USA) and automated using Python 2.7.

Results

Participants without *C. burnetii* serology data were excluded from the analyses and 924 (98%) participants remained in the GPS group, of which 32 (3.5%) were seropositive, 19 (2.1%) were borderline positive and 873 (94.5%) were seronegative. In the VGO cohort, 93 participants (3.8%) were serology positive, 53 (2.2%) were borderline positive and 2273 (94%) serology negative. The distributions of age and percentages of serology positive participants, gender, education levels and smoking status displayed similar distribution among the GPS group and VGO cohort (Table 2).

Hours/week spent outdoors near goat farms and positive serology

Spending more aggregated hours/week outdoors within 2000m of 'abortion-wave' and 'any *C. burnetii* signal' positive farms was associated with a statistically significant increased risk for positive *C. burnetii* serology (OR 3.6, 95%CI (1.2-10.6) and OR 4.9, 95%CI (1.9-12.4), respectively, see Table 3). No increased risks were observed for aggregated hours/week spent outdoors within 2000m of 'goat farms' or 'negative' farms (OR 1.0 95%CI (0.4-2.2) and OR 1.0 95%CI (0.4-2.5), respectively, see Table 3). Spline plots for aggregated hours/week spent outdoors within 2000m of farms (Fig. 2a-d) confirmed these trends.

We found that with more hours/week spent outdoors near the home while living within 2000m of an 'abortion-wave' (OR 2.1, 95%CI (0.6-7.4)), 'any *C. burnetii* signal' (OR 2.6, 95%CI (1.0-6.9)) positive or 'goat farm' (OR 1.4, 95%CI (0.6-3.3)), the risk for positive *C. burnetii* serology increased (Table 3). These associations were confirmed in the spline analyses for hours/week spent outdoors near the home (Fig. 3a-d). For weekly routine active mobility, we observed that people in general, only spent short periods within the specified buffers around (*C. burnetii* positive) goat farms (Supplementary Table 3). The splines showed that overall, (the limited periods of) active mobility alone was not associated with an increased risk for positive status of *C. burnetii* antibody serology (Fig. 3e-h). Logistic regression analyses suggested a marginal, not statistically significant, positive association for active mobility within 2000m of 'abortion-wave' positive goat farms (OR 1.2,

Table 2
General characteristics study population, subset and statistical comparison.

Variable	VGO cohort	GPS group	P-value
Total participants in population (N=)	2494	941	n.a.
Participants, with Q fever serology data (N=(% of total population))	2419 (97.0%)	924 (98.2%)	n.a.
Q fever IgG serology positive (N= (%)) Yes (>30 EU/ml)	93 (3.8%)	32 (3.5%)	0.85[a]
Borderline (20-30 EU/ml)	53 (2.2%)	19 (2.1%)	
No (<20EU/ml)	2273 (94%)	873 (94.5%)	
Age (years, median (range))	59 (20-72)	59 (20-72)	0.22[b]
Gender (N females= (%))	1315 (54.4%)	508 (55.0%)	0.78[a]
Education (N= (%)) Low	609 (25.2%)	221 (23.9%)	0.75[a]
Medium	1079 (44.6%)	419 (45.3%)	
High	731 (30.2%)	284 (30.7%)	
Smoking (N= (%)) Never	1024 (42.3%)	373 (40.4%)	0.10[a]
Former	1157 (47.8%)	478 (51.7%)	
Current	221 (9.1%)	70 (7.6%)	
No data	17 (0.7%)	3 (0.3%)	

Table 3
Group sizes and risks for positive serology for *C. burnetii* antibodies associated with aggregated hours/week spent outdoors, hours/week spent outdoors near the home address and hours/week of active mobility within 2000m of goat farms. Please note, that serology-positive individuals were considered cases and serology-negative individuals controls.

Q fever status	'Abortion-wave' positive farms			'Any *C. burnetii* signal' positive farms			Goat farms			Negative farms		
	cases	controls	OR (95%CI)	cases	controls	OR (95%CI)	cases	controls	OR (95%CI)	cases	controls	OR (95%CI)
Exposure while outdoors near the home address and in mobility (aggregated time)												
Farm near home and GPS track, often outdoors	5	23	3.6 (1.2-10.6)	9	42	4.9 (1.9-12.4)	17	189	1.0 (0.4-2.2)	12	156	1.0 (0.4-2.5)
Farm near home and GPS track, not often outdoors	1	22	0.9 (0.1-6.7)	2	42	1.2 (0.3-5.7)	6	155	0.5 (0.2-1.3)	5	132	0.6 (0.2-1.7)
Farm near home only, often outdoors	0	0	-	0	0	-	1	0	-	0	1	-
Farm near home only, not often outdoors	0	1	-	0	1	-	1	11	1.1 (0.1-9.2)	1	7	1.9 (0.2-17.5)
Farm near GPS track only, often outdoors	1	65	0.3 (<0.1-2.1)	4	144	0.7 (0.2-2.1)	8	185	0.5 (0.2-1.3)	8	188	0.6 (0.2-1.6)
Farm near GPS track only, not often outdoors	4	42	1.3 (0.4-4.5)	9	83	2.3 (0.9-5.9)	3	91	0.4 (0.1-1.4)	4	98	0.6 (0.2-2.0)
No farm near home or GPS track, often outdoors	19	349	0.9 (0.5-1.8)	14	258	1.2 (0.6-2.7)	3	116	0.3 (0.1-1.0)	9	132	0.9 (0.4-2.4)
No farm near home or GPS track, not often outdoors	21	371	Ref.	13	303	Ref.	12	126	Ref.	12	159	Ref.
Exposure while outdoors near the home address												
Farm near home, often outdoors	3	24	2.1 (0.6-7.4)	6	42	2.6 (1.0-6.9)	10	139	1.4 (0.6-3.3)	5	112	0.8 (0.3-2.3)
Farm near home, not often outdoors	3	22	2.9 (0.8-10.5)	5	43	2.5 (0.9-7.0)	15	216	1.5 (0.7-3.3)	13	184	1.5 (0.7-3.2)
No farm near home, often outdoors	19	355	1.0 (0.5-1.8)	16	337	0.9 (0.5-1.7)	12	240	1.0 (0.5-2.3)	17	267	1.3 (0.6-2.6)
No farm near home, not often outdoors	26	472	Ref.	24	451	Ref.	14	278	Ref.	16	310	Ref.
Exposure in active mobility												
Farm near GPS track	11	152	1.2 (0.6-2.5)	24	311	1.6 (0.9-2.9)	34	620	0.9 (0.5-1.6)	29	574	0.7 (0.4-1.7)
No farm near GPS track	40	721	Ref.	27	562	Ref.	17	253	Ref.	22	299	Ref.

95%CI (0.6-2.5)) or 'any *C. burnetii* signal' positive goat farms (OR 1.6, 95%CI (0.9-2.9)) (Table 3).

The sensitivity analyses showed that with increasing distance to the nearest 'abortion-wave' positive, 'any *C. burnetii* signal' positive and 'goat farms' the risk for positive *C. burnetii* antibody serology decreased (I.) in the GPS group and the whole VGO cohort (IV). For 'negative' goat farms no such associations were found (Supp. Fig. 5). These associations showed the same tendencies when looking at the increasing buffer distances and types of *C. burnetii* status of the farms: higher ORs were found for risk of serology positivity if 'abortion-wave' or 'any *C. burnetii* signal' positive goat farms were in closer proximity to the home address (Supp. Table 1). Using the

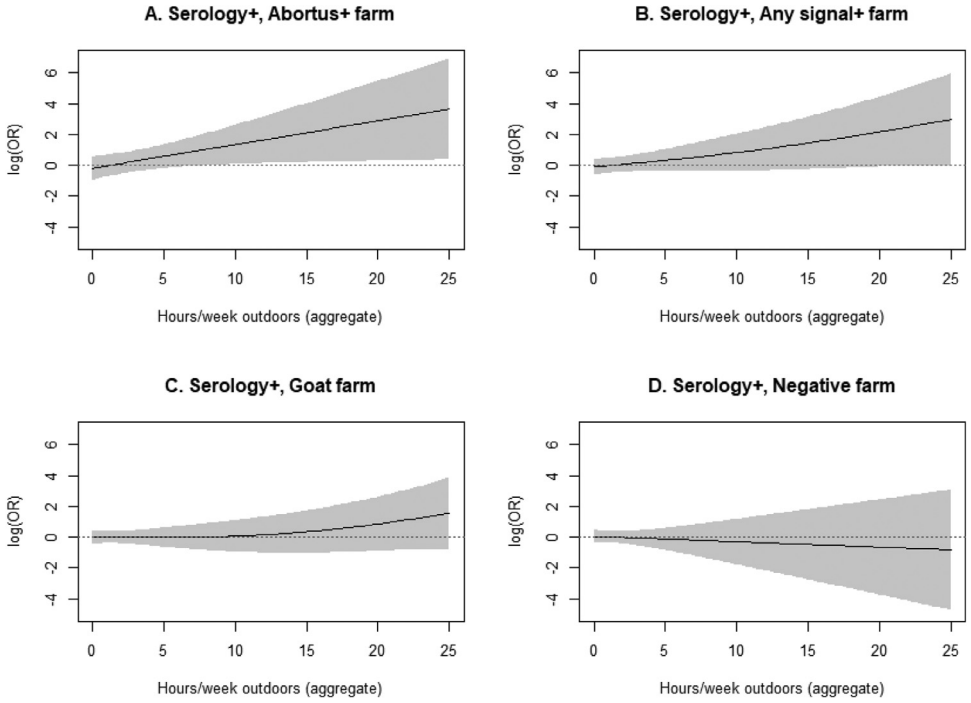

Fig. 2. Spline analysis of risk for positive *C. burnetii* serology (log(OR)) and aggregated hours/week spent outdoors, time spent outdoors near the home and active mobility, within 2000m of former (*C. burnetii* positive) goat farms. (A) hours/week spent outdoors near 'abortion-wave' positive farms. (B) hours/week spent outdoors near 'any *C. burnetii* signal' positive farms (abortion-wave and/or bulk milk tank positive goat farms). (C) hours/week spent outdoors near goat farms. (D) hours/week spent outdoors near *C. burnetii* 'negative' goat farms.

stricter case definition (II.) or reducing our data set to participants reporting to have had a 'normal week' (III.) during the GPS measurement did not materially change effects in the spline analyses (Supp. Fig. 6 and 7).

Discussion

Our analyses indicated that spending more hours/week outdoors near former *C. burnetii* positive farms, significantly increased the risk of being *C. burnetii* serology positive. To a lesser extent, these associations were observed for self-reported hours/week spent outdoors in the vicinity of the home only. Routine hours/week of active mobility near former *C. burnetii* positive goat farms only marginally increased the risk for positive *C. burnetii* serology.

The main driver of the increased risk for positive *C. burnetii* serology were self-reported hours/week spent outdoors near the home, while living near farms that were *C. burnetii* positive during the Dutch Q fever outbreak[1]. This is in line with recent observations in this study population where we observed an increase in pneumonia risk for people living near goat farms that reported to spent more hours/week outdoors near the home[7].

It has been questioned whether mobility played a role in the exposure to, and uptake of, *C. burnetii* bacteria in people moving through the area during the 2007-2009 Q fever outbreak[2,5,12]. Our analyses showed that active mobility as such only marginally increased the risk of becoming serology positive for *C. burnetii* antibodies. In an earlier analysis we did not find such an association for pneumonia[7]. When active mobility (in hours/week) was aggregated with the self-reported hours/week spent outdoors, the spline plots displayed narrower error margins. This indicates that the risk of becoming *C. burnetii* serology positive is more accurately calculated when active mobility was considered as well.

In line with previous studies[2,8,9,20], we also identified a distance-risk association between positive *C. burnetii* antibody serology in residents and living near previously *C. burnetii* infected goat farms, in our GPS subgroup and the full VGO cohort. We showed that the source of exposure seems to have played a role in the distance-risk associations, since living near 'abortion-wave' positive farms, 'any *C. burnetii* signal' positive farms and, to a lesser extent, just 'goat farms' increased the risk for positive *C. burnetii* antibody serology. These three *C. burnetii* statuses all included farms that had experienced abortion-waves during the Dutch outbreak[1].

With kidding and abortions of infected pregnant goats[30], large amounts of *C. burnetii* bacteria are excreted to the environment[24]. While in the environment, *C. burnetii* bacteria are exceptionally durable against dehydration and chemical agents. *C. burnetii* bacteria remain viable and infectious for a long period outside of a host organism[31]. Also adding to the risk of infection is that *C. burnetii* bacteria are extremely infectious to humans[32]. Given the potentially excreted amount and infectivity of emitted *C. burnetii* bacteria during the outbreak, spending time outdoors within close distance to an emitting farm appears to have contributed to *C. burnetii* exposure and infection in the years 2007 through 2009.

Strengths and limitations

A strength of our study is that main analyses were based on measurements from a large study group (GPS group, N=941), living in a rural area where between 2007 and 2009 a large Q fever outbreak occurred. In addition, we had detailed information about medical-, occupational- and spatial characteristics of our study participants. GPS group members were recruited from the larger VGO study cohort (N=2494)[7,19,22] and part of the VGO study was a serology screening for Q fever antibodies[20,22]. Although nearly 6% of the GPS group were (borderline-) positive for *C. burnetii* antibodies, we were limited in our ability to explore the risks for positive *C. burnetii* antibody serology.

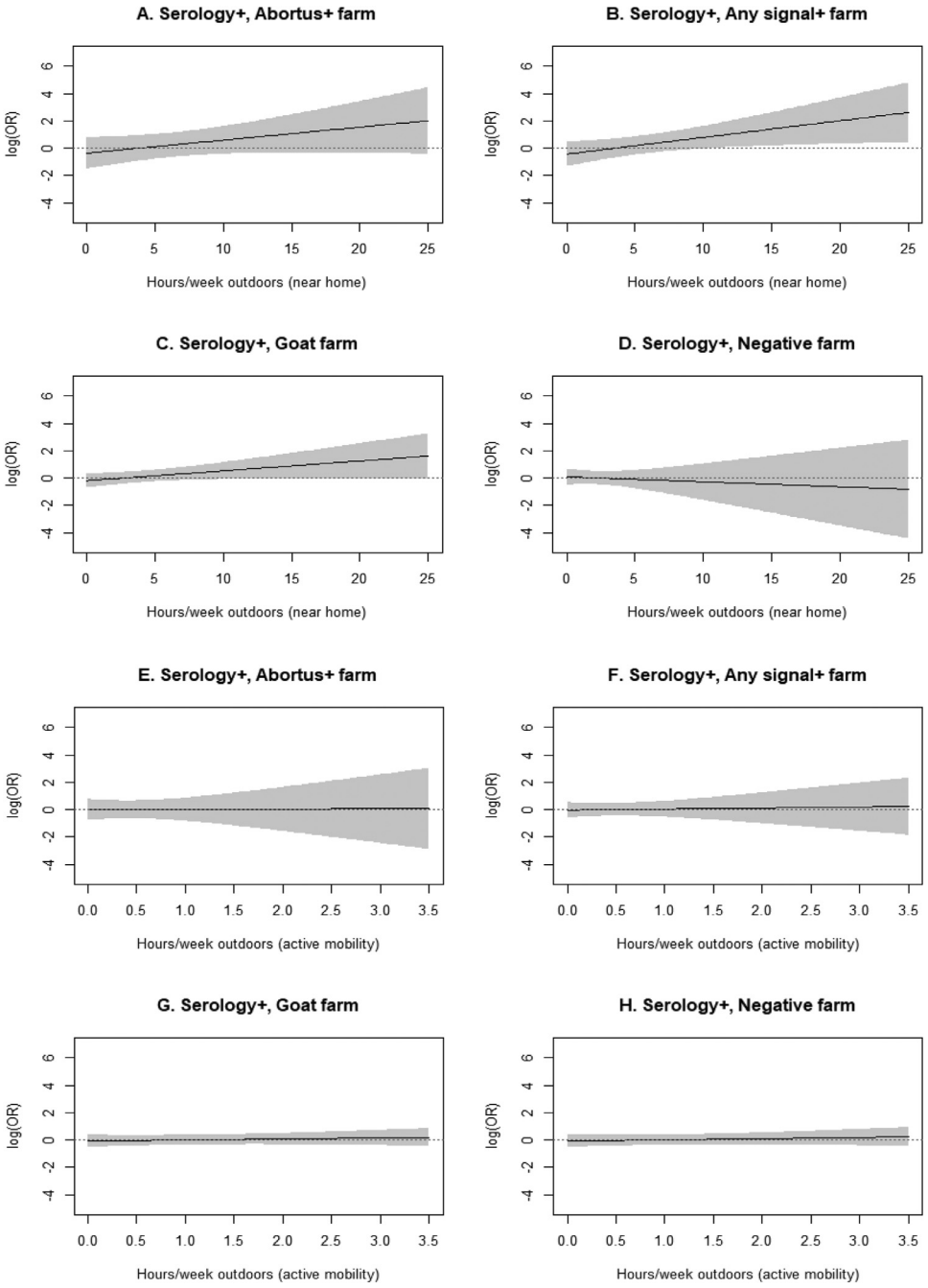

Fig. 3. Spline analysis for the risk of positive serology for *C. burnetii* antibodies (log (OR)) associated with hours/week spent outdoors near the home (A-D) or routine hours/week of active mobility (E-H) within a buffer of 2000m around a goat farm. A. hours/week spent outdoors near the home within 2000m of an 'abortus-wave' positive goat farm. B. hours/week spent outdoors near the home within 2000m of an 'any *C. burnetii* signal' positive goat farm. C. hours/week spent outdoors near the home within 2000m of a goat farm. D. hours/week spent outdoors near the home within 2000m of a 'negative' goat farm. E. routine hours/week of active mobility within 2000m of 'abortus-wave' positive goat farms. F. routine hours/week of active mobility within 2000m of 'any *C. burnetii* signal' positive goat farms. G. routine hours/week of active mobility within 2000m of 'goat farms' and H. routine hours/week of active mobility within 2000m 'negative' goat farms. Note, the differences in the scaling of the x-axis, hours/week spent outdoors near the home (A-D) have a maximum X of 25 hours and the hours/week spent on active mobility (E-H) have a maximum X of 3.5 hours.

Data collection for the VGO study occurred between March 2014 and February 2015[19] and GPS measurements were performed between September 2014 and January 2016[7,21]. These periods did not coincide with the Q fever outbreak in the Netherlands[1] therefore, our study is based on the assumptions that residential address and activity patterns measured between 2014 and 2016 reflect those during the outbreak period. Daily routines of people have been reported not to change much over time and if they change this is mainly age and life-stage related (e.g. puberty, having children, retirement)[33,34], factors that may not have changed to a large extent within our population (Supp. Fig. 8). If outdoor activities changed independently of *C. burnetii* serology status, then this would imply that non-differential misclassification may have attenuated our risk estimates. In this case, our risk estimates may have been biased towards unity. The true effect of time spent outdoors near *C. burnetii* positive farms on *C. burnetii* serology turnover therefore, may be even stronger than the effect we observed in our study.

Conclusions

We observed that outdoor exposure may have contributed to the risk of becoming *C. burnetii* serology positive. These associations were stronger if people lived closer to *C. burnetii* positive farms.

Depending on the causal pathogen in the event of a future livestock related outbreak of a zoonotic disease[35], if feasible, hours/week spent outdoors or being actively mobile close to infected farms should be included to outbreak management approaches.

Declaration of Competing Interest

All authors declare they have no conflicts of interest.

Acknowledgements

We like to thank all the participants, Lützen Portengen for statistical input and Daisy de Vries for textual input. The VGO GPS Study is funded by UMC Utrecht, publications fees for this article were available from IRAS. The Livestock Farming and Neighboring Residents' Health (VGO) study was funded by the Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs of the Netherlands, and supported by a grant from the Lung Foundation Netherlands (Grant number: 3.2.11.022).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.04.013.

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