The importance of between-farm transmission for the infection of broiler farms by *Campylobacter*

Report number WUR 1845092

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January 2019

This project was financed as a Public Private Partnership under the 'Topsectoren' programme of the Ministry of Agriculture, Nature and Food Quality. It is part of the research project TKI-AF-14203 'Beheersing van *Campylobacter* in de pluimveesector' (BO-33.04 AF8).

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Summary

Based on monitoring data of the Dutch Product Board for Poultry and Eggs (PPE) we analysed the importance of between-farm transmission for the infection of broiler farms in the Netherlands by *Campylobacter* using two approaches. Firstly, we evaluated the relationship between the density of broiler farms in the surroundings of a given broiler farm and the *Campylobacter* prevalence on this farm. Secondly, we performed a kernel analysis to determine the effect of the distance between a susceptible and an infectious broiler farm on the probability of the susceptible farm becoming infected by *Campylobacter*.

The results of the first analysis show that the relationship between the farm density and the *Campylobacter* prevalence was positive and significant in summer for farm densities based on circles with a radius ≥10 km. However, in the winter season, there was no evidence for such an effect, regardless of the radius of the circular area used to calculate the farm density.

The kernel analysis indicated that the transmission probability is much higher for farms within a distance of approximately 2 km compared to the distance-independent background infection probability in both the summer (+106%) and winter (+50%). The percentage of infected cycles across all farms that may have been avoided in absence of between-farm transmission amounted to approximately 31% in the summer and approximately 10.5% in the winter. Possible explanations for the increased risk of between-farm transmission within a 2 km radius of a broiler farm and for the seasonal variation in this transmission probability are given in the discussion.

Nederlandse samenvatting

Om meer inzicht te krijgen in de vraag of (met *Campylobacter* besmette) vleeskuikenbedrijven in de omgeving van een (onbesmet) vleeskuikenbedrijf een risico vormen voor *Campylobacter* besmetting op dat bedrijf is gebruik gemaakt van monitoringgegevens van het Nederlandse Productschap Pluimvee en Eieren (PPE). Met behulp van statistische analyses werden daarvoor twee benaderingen gevolgd; eerst is gekeken naar een eventuele relatie tussen de dichtheid van vleeskuikenbedrijven en de *Campylobacter*-prevalentie. Daarnaast is een zogenaamde 'kernel'-analyse uitgevoerd om het effect te bepalen van de afstand tussen een vatbaar (nog onbesmet) en een *Campylobacter* positief vleeskuikenbedrijf op de waarschijnlijkheid dat de vatbare boerderij besmet raakt.

De resultaten van de eerste analyse tonen aan dat er inderdaad een relatie bestaat tussen de dichtheid van boerderijen en de *Campylobacter*-prevalentie. Dit effect was significant in de zomer wanneer gerekend werd met het aantal vleeskuikenbedrijven in een omliggende gebied met een straal ≥ 10 km. In het winterseizoen was er echter geen aanwijzing voor een dergelijk effect, ongeacht de straal van de cirkel.

De kernelanalyse gaf aan dat binnen een afstand van ongeveer 2 km de transmissiekans voor bedrijven veel groter is in vergelijking met de afstands-onafhankelijke besmettingskans. Dit geldt zowel voor de zomer- (+ 106%) als de winterperiode (+ 50%). Wanneer er géén overdracht tussen bedrijven zou plaatsvinden, is de schatting dat in de zomer ongeveer 31% en in de winter zo'n 10,5% minder geïnfecteerde koppels zouden zijn gevonden. Mogelijke verklaringen voor het verhoogde risico van overdracht tussen bedrijven binnen een straal van 2 km van een vleeskuikenbedrijf en voor de seizoensgebonden variatie in deze transmissiekans worden gegeven in de discussie.

Introduction

In the Netherlands, nearly half of all produced broiler flocks are colonised with *Campylobacter* at time of slaughter. Factors that play a role in the infection of broiler farms by *Campylobacter* are however not understood very well. The aim of this short research project is to explore the importance of between-farm transmission for the infection of broiler farms in the Netherlands by *Campylobacter*.

Data

Between 1997 and 2014, a monitoring programme for *Campylobacter* colonization of Dutch broiler farms was carried out by the Product Board for Poultry and Eggs (PPE). According to guidelines from the PPE published in 2010 [1], broiler farms should be sampled at least once during the period from October to March and at least once during the period from April to September. Each sampling time, farmers were requested to take five faecal samples from at least one house on the farm. Data from this surveillance scheme were available for 616 broiler farms in the Netherlands for the period from 1 October 2010 to 31 March 2014 (Fig. 1). Each row in the database contained information about the overall test result for a specific farm house at a given sampling time (see Table 1 for an overview of the available fields in the database). We used the address information to determine the geodetic 'Rijksdriehoeks' coordinates (RD-coordinates) for each farm.

Table 1. The fields extracted from the database with information on the *Campylobacter* surveillance in broiler farms in the Netherlands.

Name company
Postal code
Street
City
Sample type
House number
Start date cycle
Sampling date
Name laboratory

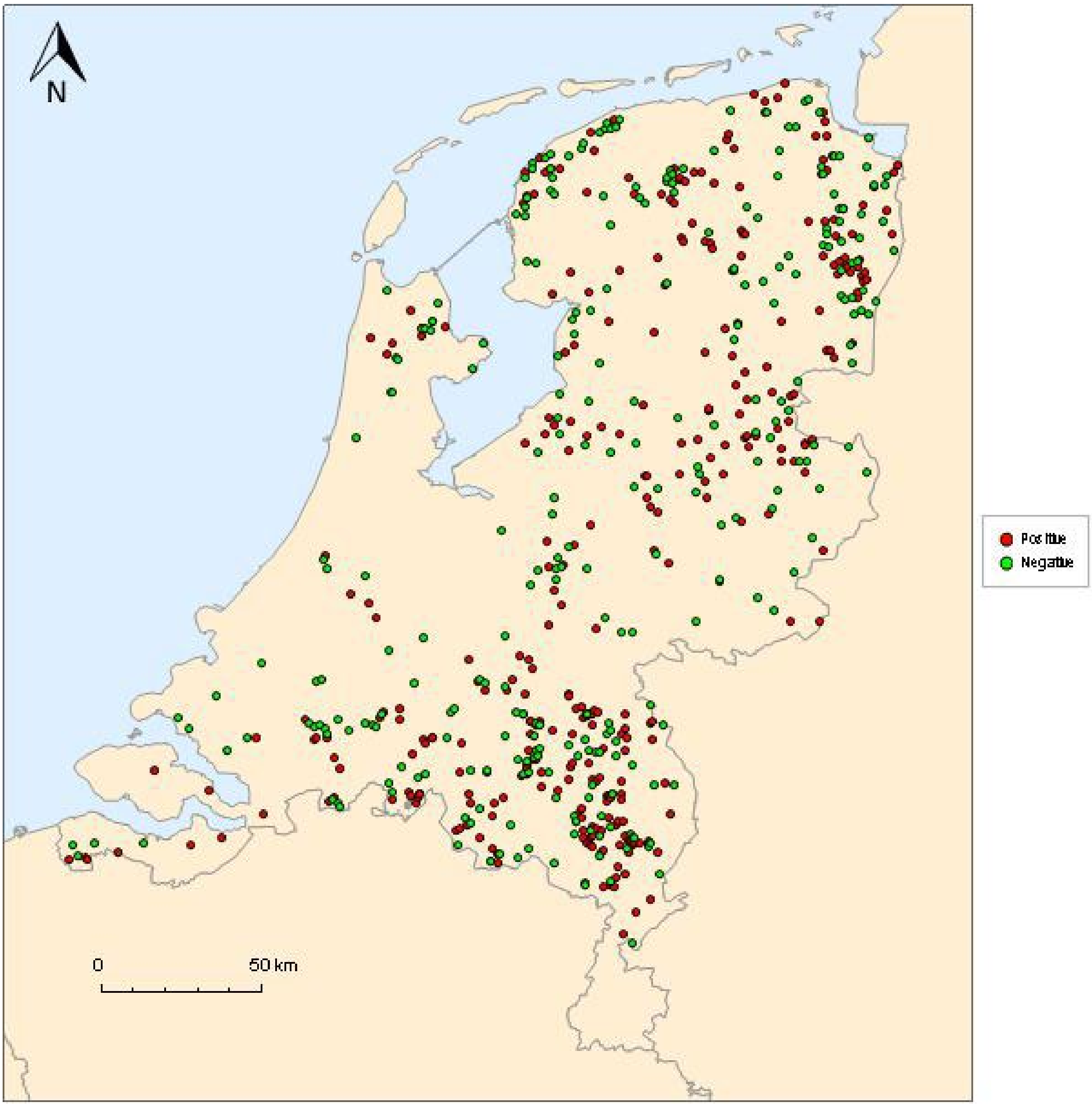


Figure 1. The location of the 616 broiler farms in the Netherlands that were tested for *Campylobacter* during the period from October 2010 until April 2014. Green dots represent farms that were always negative for *Campylobacter*, while red dots represent farms that were tested positive for *Campylobacter* at least once.

The assignment of samples to the summer or winter season

The prevalence of *Campylobacter* fluctuates seasonally and is higher in summer than in winter. To be able to account for this, we divided the year into a summer period consisting of months April to September and a winter period consisting of months October to March. By default, we assigned a production cycle to the summer or winter period based on the sampling date. However, we used a different set of rules for unusual cycles with a very short or long interval between the start of the growing period and sampling date and/or a very short or long interval between the sampling and test result date. Cycles were classified as unusual if the length of at least one of these periods was outside the 95% confidence interval based on all production cycles (Figs. 2 and 3). Unusual cycles therefore had an interval from the start of the growing period to sampling time <21 days or >60 days and/or an interval from sampling time to test result <3 days or > 12 days. If the start of the growing period or test result date of such a cycle fell into a different season as the sampling date, it was assigned to the season preceding or following the season containing the sampling date according to the set of rules listed in Annex 1.

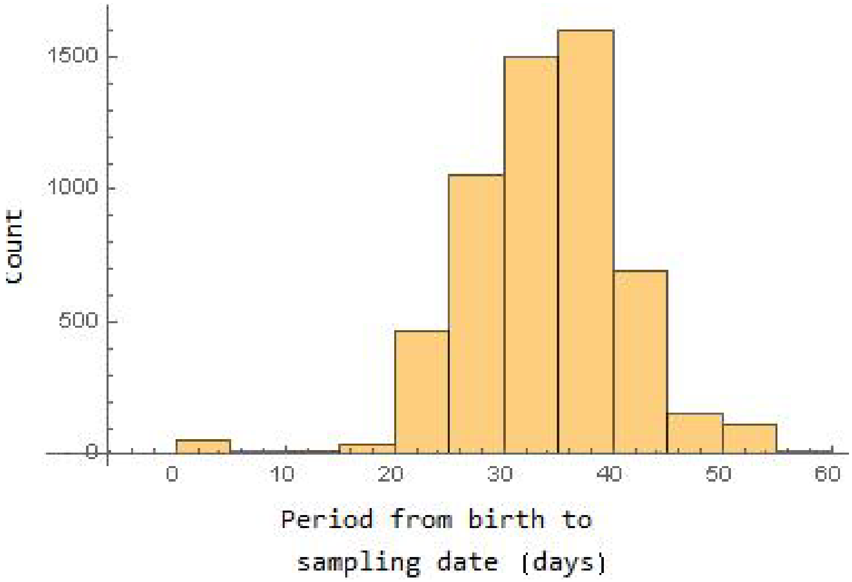


Figure 2. The distribution of the time period from the birth date to the sampling date of a production cycle for broiler farms in the Netherlands during the period from October 2010 to March 2014 based on data from 5782 production cycles.

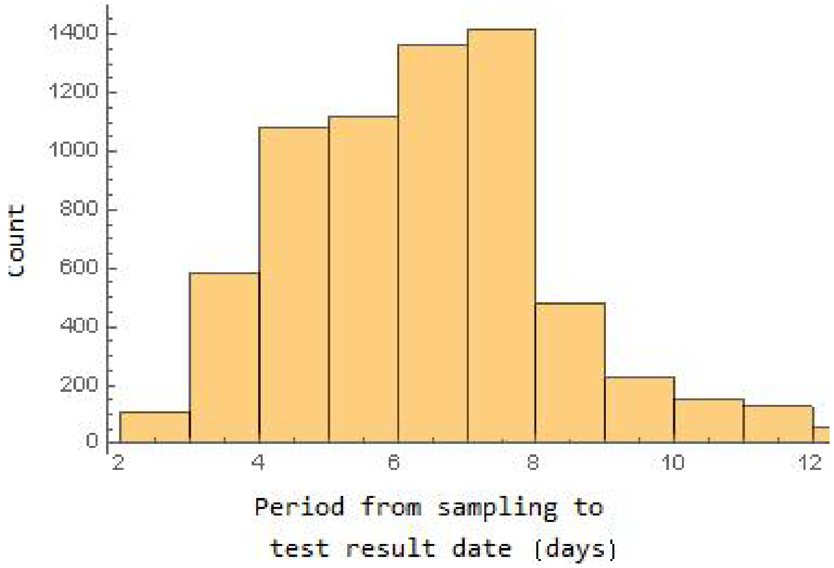


Figure 3. The distribution of the time period from the sampling date to the test result date of a production cycle for broiler farms in the Netherlands during the period from October 2010 to March 2014 based on data from 6857 production cycles.

The effect of farm density on *Campylobacter* prevalence

Methods

To analyse the relationship between farm density and *Campylobacter* prevalence, we performed a logistic regression analysis of the data using the logit link function to transform the prevalence data. We assumed a binomial distribution for the dependent variable. This generalized linear model is given by the equation

$$logit(Y) = \alpha + \beta X$$

with X representing the farm density within a certain radius of a farm and Y representing the *Campylobacter* prevalence at this farm. Parameter α represents the predicted *Campylobacter* prevalence (after logit transformation) when there are no other broiler farms present within a certain radius of a farm. Parameter β is the slope of the regression line and determines how sensitive the *Campylobacter* prevalence (after logit transformation) is to a change in the farm density. A higher value of parameters α and β corresponds to a higher *Campylobacter* prevalence for a given farm density X. We performed separate analyses for the summer and winter season.

The prevalence for a specific farm in the summer or winter season was calculated as the number of production cycles that was positive for *Campylobacter* divided by the total number of cycles for which test data were available across all summer or winter seasons during the period from October 2010 to March 2014. For the calculation of the disease prevalence, production cycles in different houses on the same farm with the same sampling date were counted as one 'compound' cycle. This compound cycle was assumed to be positive if at least one of the houses was positive for *Campylobacter*.

The radius used to calculate the farm density was varied from 1 to 5 km in steps of 1 km and then up to 30 km in steps of 5 km. We repeated the logistic regression analysis for each of these radii.

In order to visually check the fit of the model to the data, we created a number of farm density categories and calculated the mean disease prevalence for each category based on all farms within a given category.

Results

Results for the fit of the logistic regression model to the data on *Campylobacter* prevalence and farm density are given in Table 3 for the summer season and in Table 4 for the winter season. These tables show the intercept and slope of the regression lines that could best describe the effect of farm density on *Campylobacter* prevalence for farm densities based on a range of circle sizes. They also show a number of measures indicating the goodness of fit. Regression models with a P-value < 0.05 describe the data significantly better than a simple horizontal line through the data (null model). The fit of the models to the data is shown in Figures 4 and 5 for the summer season and in Figures 6 and 7 for the winter season. The effect of farm density on the *Campylobacter* prevalence clearly differs between the summer and winter seasons. In the winter season, there was no evidence for such an effect when the radius of the circular area used to calculate the farm density was varied from 1 to 30 km. However, in the summer season the positive relationship between farm density and *Campylobacter* prevalence became stronger when the radius of the circular area used to calculate the farm density increased. This relationship was significant for farm densities based on circles with a radius ≥10 km.

Table 3. The output of the logistic regression analysis of the effect of farm density on the *Campylobacter* prevalence on broiler farms in the Netherlands during the summers (April to September) of the years 2011 to 2013.

Radius (km)	deviance null model[a]	deviance proposed model[a]	df[b] null model	df[b] proposed model	fitted intercept	fitted slope	P-value intercept	P-value slope
1	390.4015	390.2028	569	568	-1.20991	0.233439	2.59E-07	0.653286
2	390.4015	389.3612	569	568	-1.26218	0.994211	5.23E-13	0.30161
3	390.4015	388.3927	569	568	-1.3043	2.007118	5.44E-15	0.151754
4	390.4015	386.8228	569	568	-1.36673	3.465867	3.05E-16	0.055825
5	390.4015	387.2466	569	568	-1.35754	4.07285	1.55E-15	0.07328
10	390.4015	386.1089	569	568	-1.42113	8.269587	3E-15	0.036839
15	390.4015	384.9368	569	568	-1.47002	11.80024	1.32E-15	0.018434
20	390.4015	384.5363	569	568	-1.50776	14.78399	5.81E-15	0.014774
25	390.4015	386.3231	569	568	-1.45713	14.56599	2.23E-13	0.042124
30	390.4015	385.8495	569	568	-1.4965	17.51253	5.04E-13	0.031869

[a]) The null model describes the effect of farm density *Campylobacter* prevalence with only one parameter. The proposed model is the logistic regression model that we described in the methods section. A smaller deviance indicates a better fit to the data. The proposed model is significantly better than the null model if it reduces the deviance by an amount that is given by the χ^2 square distribution and the difference in the degrees of freedom between both models.

[b]) df = degrees of freedom

Table 4. The output of the logistic regression analysis of the effect of farm density on *the Campylobacter* prevalence on broiler farms in the Netherlands during the winters (October to March) 2010/2011 to 2013/2014.

Radius (km)	deviance null model[a]	deviance proposed model[a]	df[b] null model	df[b] proposed model	fitted intercept	fitted slope	P-value intercept	P-value slope
1	350.4943	349.0968	563	562	-1.83088	0.679627	2.26E-12	0.225044
2	350.4943	350.4093	563	562	-1.59893	0.334695	3.39E-15	0.769211
3	350.4943	350.494	563	562	-1.5521	0.029378	2.75E-16	0.985712
4	350.4943	350.4345	563	562	-1.58675	0.523324	5.18E-17	0.806235
5	350.4943	350.4942	563	562	-1.54758	-0.0302	6.67E-16	0.99102
10	350.4943	350.4838	563	562	-1.56669	0.476303	1.04E-14	0.918319
15	350.4943	349.9143	563	562	-1.68214	4.463199	6.21E-16	0.44307
20	350.4943	349.6938	563	562	-1.71634	6.352701	4.5E-15	0.367527
25	350.4943	350.1269	563	562	-1.66772	5.096252	1.5E-13	0.542372
30	350.4943	349.531	563	562	-1.75221	9.373659	1.32E-13	0.323005

[a]) The null model describes the effect of farm density *Campylobacter* prevalence with only one parameter. The proposed model is the logistic regression model that we described in the methods section. A smaller deviance indicates a better fit to the data. The proposed model is significantly better than the null model if it reduces the deviance by an amount that is given by the χ^2 square distribution and the difference in the degrees of freedom between both models.

[b]) df = degrees of freedom

Figure 4. The fit of the logistic regression model describing the relation between the density of broiler farms and the *Campylobacter* prevalence during the summer season (April-September) in the period from 2011 to 2013 in the Netherlands. The radius of the circular area for which the farm density was calculated varied from 1 to 5 km.

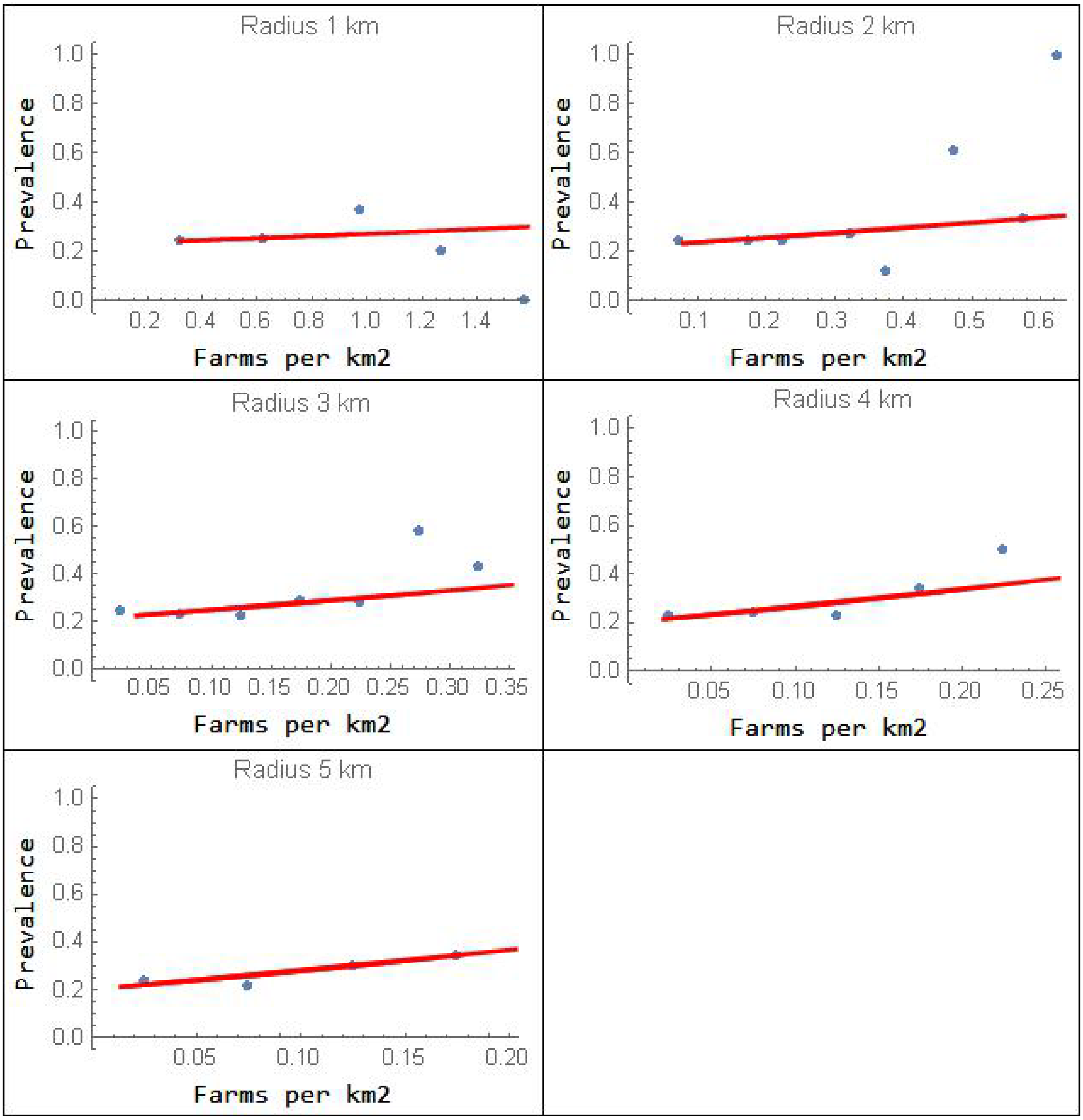


Figure 5. The fit of the logistic regression model describing the relation between the density of broiler farms and the *Campylobacter* prevalence during the summer season (April-September) in the period from 2011 to 2013 in the Netherlands. The radius of the circular area for which the farm density was calculated varied from 10 to 30 km.

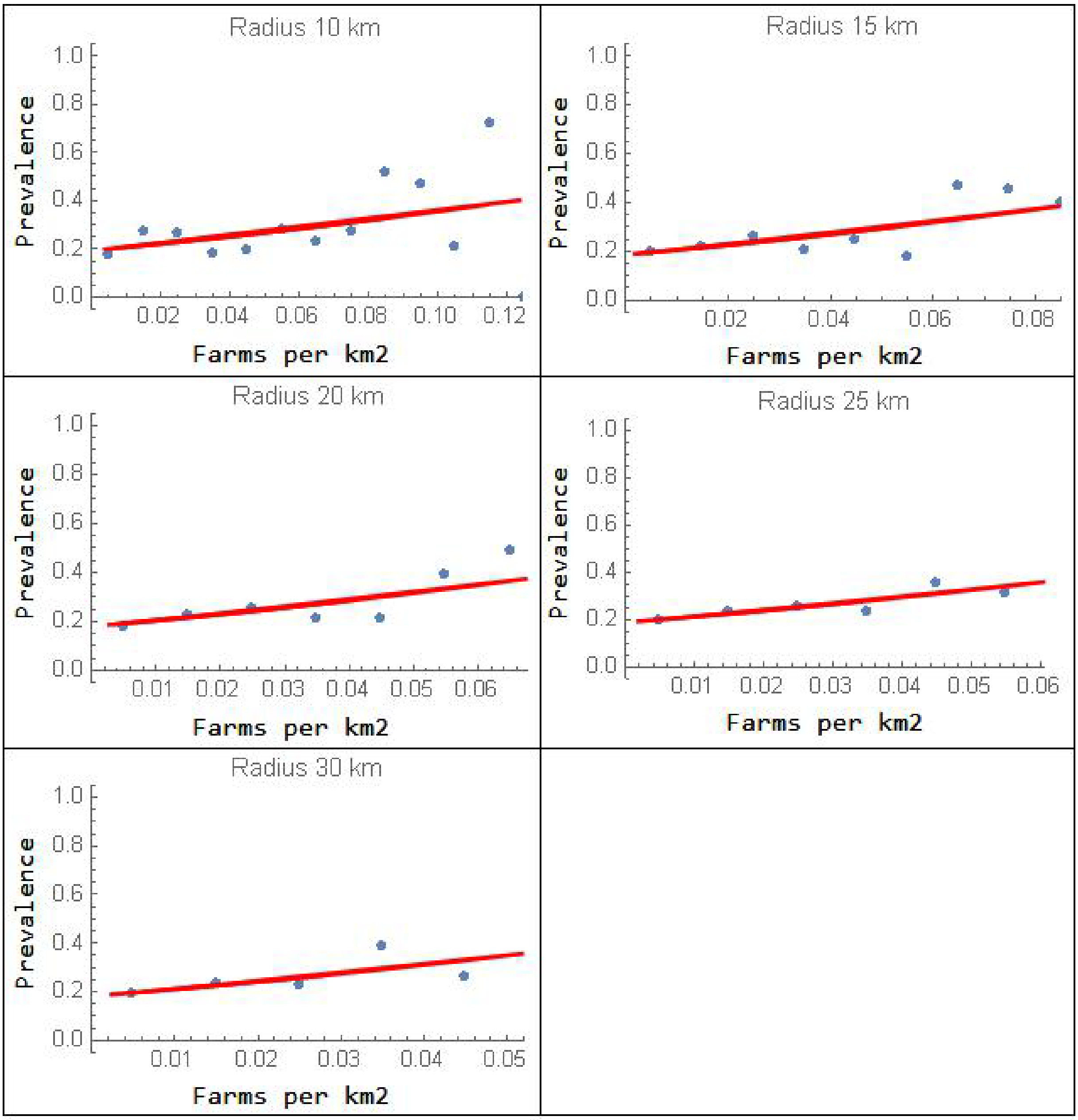


Figure 6. The fit of the logistic regression model describing the relation between the density of broiler farms and the *Campylobacter* prevalence during the winter season (October-March) in the period from 2010/2011 to 2013/2014 in the Netherlands. The radius of the circular area for which the farm density was calculated varied from 1 to 5 km.

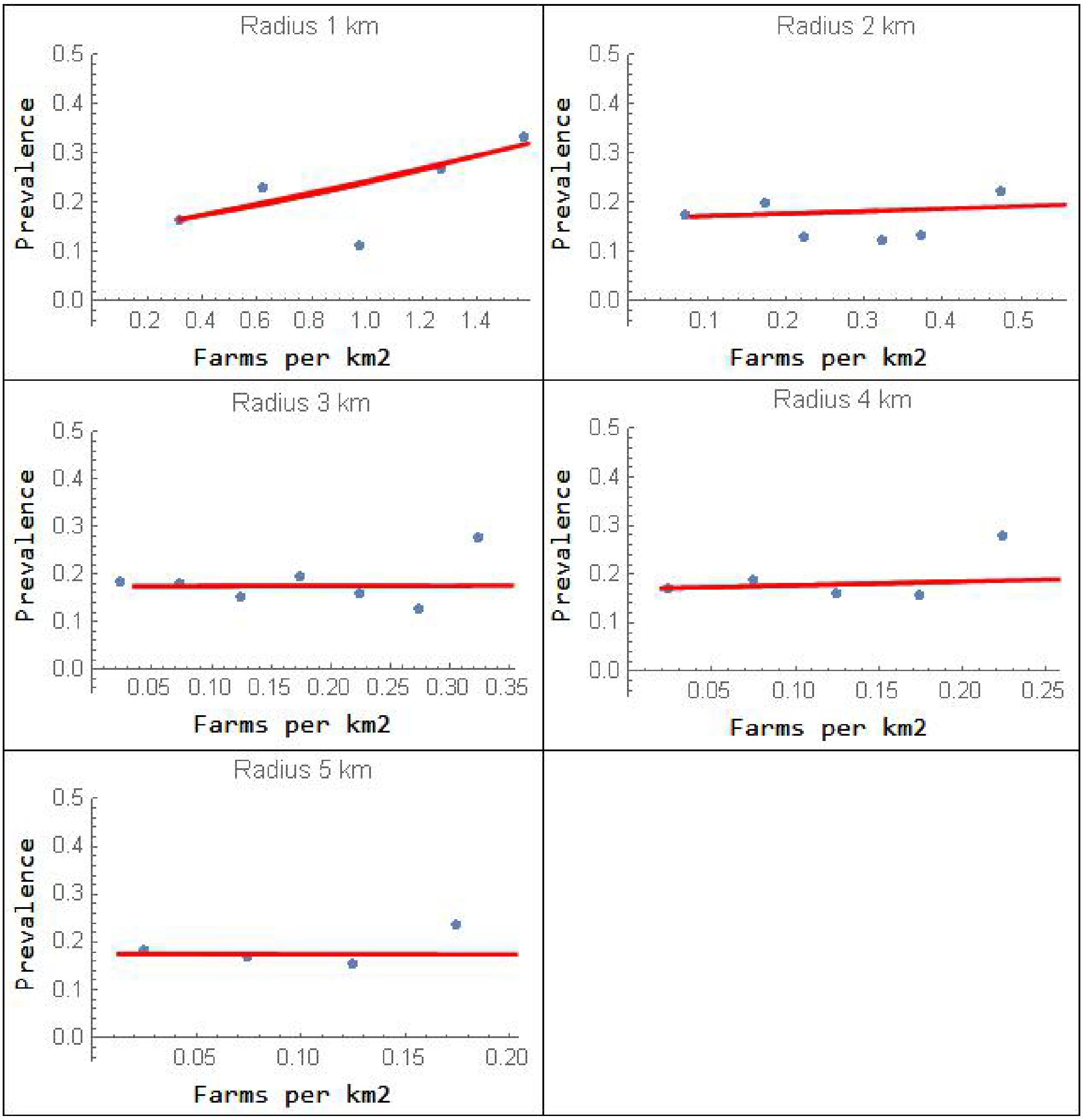
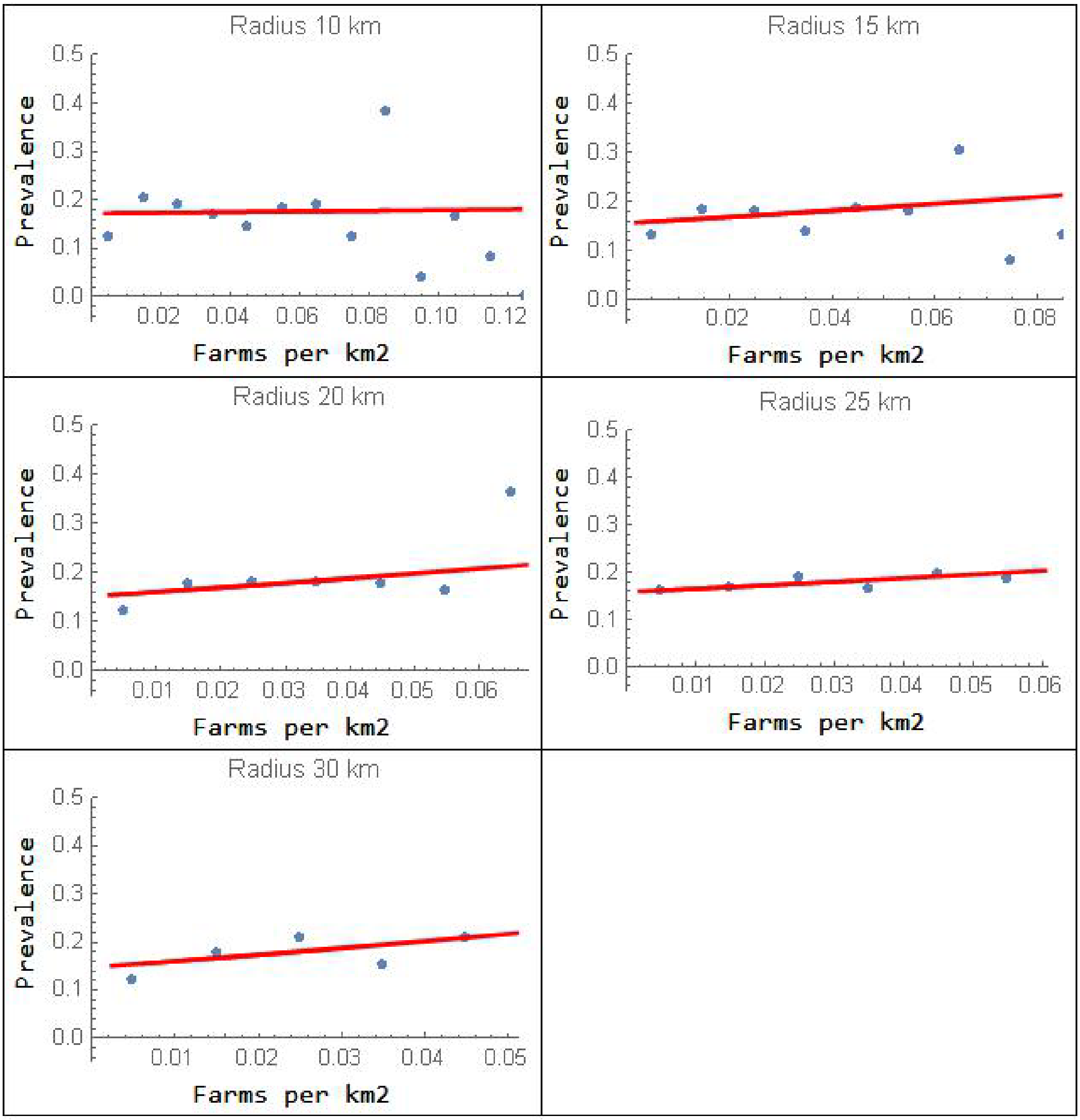


Figure 7. The fit of the logistic regression model describing the relation between the density of broiler farms and the *Campylobacter* prevalence during the winter season (October-March) in the period from 2010/2011 to 2013/2014 in the Netherlands. The radius of the circular area for which the farm density was calculated varied from 10 to 30 km.



The effect of the distance to infectious farms on the infection probability

Methods

Boender et al. [2] developed a method to describe the relationship between the infection probability of a susceptible farm during a certain time step dt and the distance to infectious farms using a kernel function. To be able to estimate the parameters of this kernel function, information is required on the disease status of farms in time. Broiler farms were usually only sampled once during the summer and once during the winter period and data on the absence or presence of *Campylobacter* is missing for many production cycles. It is therefore not possible to determine a precise function for the infection probability per unit of time as a function of the distance to infectious farms. However, we can explore the shape of the kernel by assuming that 1) farms may have been infected by any other farm that was positive for *Campylobacter* during the same season and that 2) infected farms were infectious during the whole summer or winter period. Hereafter, for simplicity, we will denote the value of the kernel function as the infection probability. We explored a range of kernel functions varying from exponentially decreasing and inverse sigmoid curves to block-shaped curves, which assume a stepwise reduction in the infection probability as a function of distance. We focused on the block-shaped kernel functions, because these could describe the data best. We studied kernel functions with one step (single block) and two steps (double block) downwards. The single block function (f_1) is defined as

$$f_1(r) = \begin{cases} y_b + y_1, & r \le r_1 \\ y_b, & r > r_1 \end{cases}$$

and assumes that the infection probability is constant at level $y_b + y_1$ for distances (r) up to r_1 km and drops to the distance-independent background level y_b for larger distances. The double block function (f_2) is defined as

$$f_2(r) = \begin{cases} y_b + y_2 + y_1, & r \le r_1 \\ y_b + y_2, & r_1 < r \le r_2 \\ y_b, & r > r_2 \end{cases}$$

and assumes that the infection probability is constant at level $y_b + y_1 + y_2$ for distances up to r_1 km, then drops to $y_b + y_1$ for larger distances larger up to r_2 km and finally reduces to the distance-independent background level y_b for distances larger than r_2 km. In these equations, $y_b \ge 0$, $y_1 \ge 0$ and $y_2 \ge 0$. The background value y_b represents the probability of infection from sources other than between-farm transmission.

We fitted separate kernel functions for the summer and winter season using the maximum likelihood approach described in Boender et al. [2]. The two simplifying assumptions described above can be accounted for by setting time step dt in the likelihood function to one whole summer or winter season. We used Akaike's Information Criterion (AIC) to determine the most parsimonious kernel shape.

To quantify the contribution of between-farm transmission to the total infection probability, we calculated the percentage by which the infection probability increases relative to the background infection probability due to the presence of a single infectious farm within a certain distance. We also estimated the Population Attributable Risk (PAR) as the percentage of infected cycles across all farms that may have been avoided in absence of between-farm transmission. The mathematical equations for these two variables are described elsewhere [3] and were performed for both the summer and winter season.

Results

Results for the fit of the summer and winter kernel functions to the data are given in Table 5. This table shows the estimated values of the parameters for the single and double block kernel functions. For both seasons, the double block kernel function fitted the data best (lowest negative log likelihood) and were most parsimonious (lowest AIC). The best fitting kernel functions are shown in Figure 8 for the summer and in Figure 9 for the winter. Interestingly, for both seasons, the first step of the double block kernel function occurred around a distance of 2 km and the second step much further away around a distance of 30 km. Also, for both seasons, the decrease in the value of the double block kernel function was much higher for the first step than it was for the second step. The distance-independent background infection probability was similar for both the summer and winter kernels. This background infection probability represents the risk of infection due to all causes other than between-farm transmission and amounted to around 0.17 over the entire 6 months summer period and around 0.16 over the entire 6 months winter period. However, for between-farm distances smaller than approximately 2 km (before first step downwards), the probability of between-farm transmission was much higher in the summer than in the winter. In the summer, this probability was 106% higher than the background transmission probability compared to 50.3% in the winter. The percentage of infected cycles across all farms that may have been avoided in absence of between-farm transmission amounted to 31% in the summer and 10.5% in the winter.

Table 5. Parameter estimates for the single and double block functions that were used to describe the correlation between the infection probability of susceptible broiler farms and the distance to infectious farms for the summer and winter seasons in the Netherlands during the period from October 2010 to March 2014.

Kernel type	Parameter estimates for the double block kernel function					Negative log likelihood	AIC[a]
	y_1	y_2	y_b	r_1 (km)	r_2 (km)		
Summer							
constant	-	-	0.3072	-	-	698.99	1399.97
single block	0.0122	-	0.1817	28.84	-	683.21	1372.42
double block	0.2316	0.0091	0.1847	1.84	28.94	672.29	1354.58
Winter							
constant	-	-	0.2218	-	-	556.78	1115.56
single block	0.0061	-	0.1735	31.47	-	552.62	1111.23
double block	0.0963	0.0051	0.1755	2.24	27.53	549.66	1109.31

[a] Akaike's Information Criterion

Figure 8. The double block kernel function that best describes the influence of the distance to infectious broiler farms on the infection probability of a susceptible broiler farm (see text) during the summer seasons in the period from 2011 to 2013 in the Netherlands.

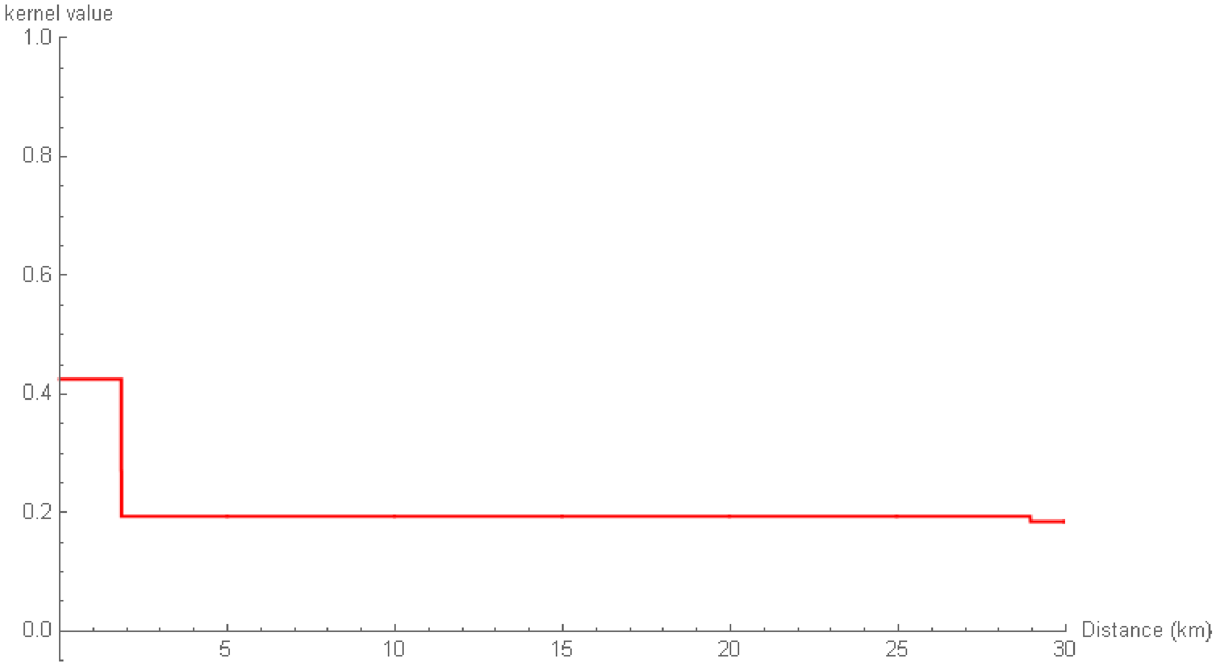
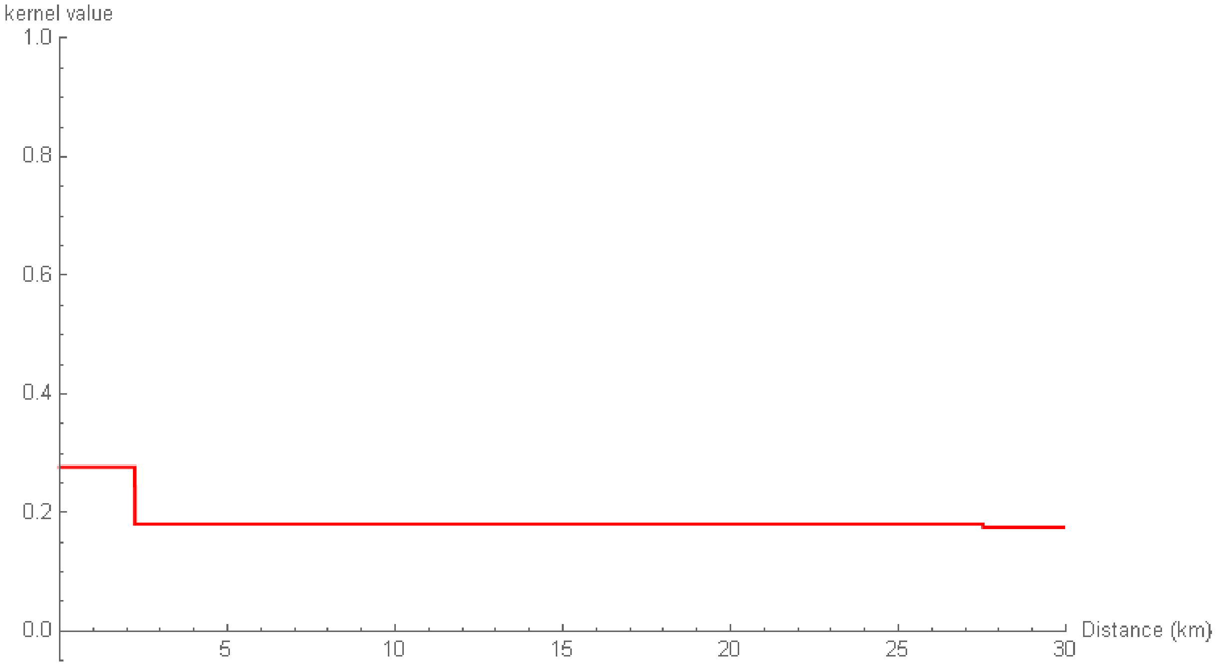


Figure 9. The double block kernel function that best describes the influence of the distance to infectious broiler farms on the infection probability of a susceptible broiler farm (see text) during the winter seasons in the period from 2010/2011 to 2013/2014 in the Netherlands.



Discussion and conclusions

The results show that there was a clear difference between summer and winter seasons regarding the effect of farm density on the *Campylobacter* prevalence. In the summer season, the relationship between farm density and *Campylobacter* was positive and significant for farm densities based on circles with a radius ≥10 km. However, in the winter season, there was no evidence for such an effect, regardless of the radius of the circular area used to calculate the farm density.

The kernel analysis showed that the transmission probability is much higher for farms within a distance of approximately 2 km compared to the distance-independent background infection probability in both the summer (+106%) and winter (+50%). The percentage of infected cycles across all farms that may have been avoided in absence of between-farm transmission amounted to 31% in the summer and 10.5% in the winter. It should be kept in mind that these are rough estimates, since data on the presence of *Campylobacter* were missing for many production cycles. Interestingly, the best fitting kernels predict a stepwise reduction in the transmission probability, while kernels usually have a more fluent sigmoid shape (e.g. avian influenza [2] and classical swine fever [4]) with the transmission probability gradually decreasing as a function of distance.

A possible mechanism explaining the sharp rise in transmission over smaller distances may be that farms have more social/business contacts with neighbouring farms than with farms that are further away. Neighbouring farms also share the same local roads. Independent of the contact structure of farms, the decay of *Campylobacter* in the environment will also limit the distance over which it can spread around an infected farm. The probability of transmission between farms that are further away may be less, simply because *Campylobacter* cannot survive long enough to travel that far. Modelling analyses of small-scale transmission experiments have shown that random diffusion of particles containing *Campylobacter* around an infection source in combination with decay of *Campylobacter* in the environment may result in a sharp decrease of the amount of viable *Campylobacter* in the environment at a certain distance from an infected farm [5]. One may speculate in what way *Campylobacter* diffuses in the environment. Perhaps transmission through randomly moving insects, and (wild) animals is a possibility [6, 7]. A second much smaller step in the kernel functions for the summer and winter occurs at a between-farm distance of around 30 km. Based on this relatively large distance one may speculate that human-mediated contact is involved. A possible explanation may be the contacts that broiler farms have with suppliers or processing businesses, although slaughter houses and feed suppliers in the Netherlands seem to serve larger areas.

The higher prevalence of *Campylobacter* in broiler farms in the summer may be caused by an increased probability of between-farm transmission, since the distance-independent background infection probability was very similar in the summer and winter season. The higher between-farm transmission probability in the summer may be due to higher densities and a higher activity of e.g. insects and wild animals. An increased frequency of between-farm contacts due to a higher activity of people in the summer may also contribute.

A recent study in Norway found that the presence of livestock farms within a 2 km radius of a broiler farm increases the probability of *Campylobacter* infection [8]. This is in agreement with the higher transmission probability for between-farm distances smaller than approximately 2 km found in our analysis. Although, a block-shaped kernel with sharp changes in the transmission probability over very short distances is unusual, a recent study on the transmission of Highly Pathogenic Avian Influenza subtype H5N2 between poultry farms in Minnesota in 2015 also found that a block-shaped kernel could describe the transmission patterns best [9]. In this case, the infection probability dropped sharply around a distance of approximately 30 km.

One limitation of this study is that we only analysed data on the *Campylobacter* prevalence in broiler farms, since *Campylobacter* surveillance is limited to this poultry sector. A recent study investigated the *Campylobacter* prevalence on a small number of Dutch layer farms and all five farms were found positive [10]. There are approximately twice as many layer as broilers farms in the Netherlands and, in addition, the production cycle of layers is much longer (approximately 1.5 years) than for broilers. Transmission of *Campylobacter* between layer and broiler farms may therefore be an important cause of new *Campylobacter* infections on broiler farms, depending on the frequency/intensity of contacts and transmission pathways between these two poultry sectors (or farms with other animal species). Another limitation of this study is that data on the absence or presence of *Campylobacter* on broiler farms is missing for many production cycles, because farms were usually only sampled once during the summer and once during the winter period. It was therefore necessary to make simplifying model assumptions in order to determine the farms that may have been responsible for new *Campylobacter* infections.

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Annex 1. Rules for the assignment of unusual production cycles to the summer or winter season.

Season to which an unusual cycle is assigned	*Rules determining the assignment of unusual cycles to a season*
Default season (containing the sampling date)	1A) the start, sampling and test result dates all fell within the same season OR 1B) the start date fell in an earlier season than the sampling date, while the test result date fell within a later season than the sampling date OR 1C) no other test result was available for the same farm in the season containing the sampling date
Preceding season	2A) none of the above was true and the start date fell in the season preceding the season containing the sampling date AND 2B) the farm was not yet sampled in the season containing the start date AND 2C) in case there were several unusual cycles meeting conditions 2A and 2B, only the cycle with the sampling date closest to the preceding season was assigned to this preceding season
Following season	3A) none of the above was true and the test result date fell in the season following the season containing the sampling date AND 3B) the farm was not yet sampled in the season containing the test result date AND 3C) in case there were several unusual cycles meeting conditions 3A and 3B, only the cycle with the sampling date closest to the following season was assigned to this following season