# Epidemiology of Clostridium difficile infections in the Netherlands and Europe: implications for surveillance and control

Dorp, S.M. van

*Chapter 6*

# Spatial clustering and livestock exposure as risk factor for community-acquired *Clostridium difficile* infection

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**Objectives.** *Clostridium difficile* infections (CDI) account for 1.5% of diarrhoeic episodes in patients attending a general practitioner in the Netherlands, but its sources are unknown. We searched for community clusters to recognise localised point sources of CDI.

**Methods.** Between October 2010 and February 2012, a community-based prospective nested case-control study was performed in three laboratories in the Netherlands with a study population of 2,810,830 patients. Bernoulli spatial scan and space-time permutation models were used to detect spatial and/or temporal clusters of CDI. In addition, a multivariate conditional logistic regression model was constructed to test livestock exposure as a supposed risk factor in CDI patients without hospital admission within the previous 12 weeks ('community-acquired CDI').

**Results.** In laboratory A, B and C, 1.3%, 1.8%, and 2.1% of patients with diarrhoea tested positive for CDI respectively. The mean age of community-acquired CDI patients (n=124) was 49 years (SD, 22.6); 64.5% were female. No spatial or temporal clusters of CDI cases were detected compared to *C. difficile* negative diarrhoeic controls. Except for one false-positive signal, no spatio-temporal interaction amongst CDI cases was found. Livestock exposure was not related to community-acquired CDI (OR, 0.99; 95% confidence interval, 0.44-2.24). Ten percent of community-acquired CDIs was caused by PCR ribotype 078, spatially dispersed throughout the study area.

**Conclusions.** The absence of clusters of CDI cases in a community cohort of diarrhoeic patients suggests a lack of localised point sources of CDI in the living environment of these patients.

# Introduction

*Clostridium difficile* infection (CDI) occurs when its spores germinate in the intestinal tract, and bacterial growth and toxin production surpass the host resistance. *C. difficile* toxins damage the intestinal epithelium causing symptoms ranging from diarrhoea to life-threatening colitis [1]. Although hospitalised patients have the greatest risk for CDI, the infection has been increasingly recognised in patients living outside healthcare facilities [2, 3].

The risk profile and transmission of community-acquired CDI (CA-CDI) are not fully understood. Fourteen to 17% of patients with CA-CDI have no evident risk factors that predispose for CDI, such as medication use, prior hospitalisation and underlying diseases [4, 5]. Transmission from infants, asymptomatic carriers, household members and pets has been suggested, but never thoroughly investigated [6 – 8]. Livestock animals can acquire *C. difficile* as well, and may contribute to transmission of certain subtypes of *C. difficile* in the community [9]. In the Netherlands, piglets are typically infected by *C. difficile* ribotype 078 [10] and its spores contaminate the farm environment [11]. In-depth genomic studies indicate that *C. difficile* transmission between pigs and humans is likely [12, 13]. A publication from North Carolina, one of the largest pig producing states in the United States, suggested that environmental exposure to livestock farms increases the risk for CA-CDI, but calls for further spatial analysis that includes data of molecular strain typing [14].

The main objectives of the present study were to investigate i.) the spatial and/or temporal clustering of patients with CDI compared to *difficile* negative diarrhoeic controls in the community, ii.) the association of community-acquired CDI with livestock exposure, and iii.) the *C. difficile* PCR ribotypes and risk factors associated with spatial clustering of CDI in the community.

# Methods

## STUDY DESIGN

We used data from a prospective community-based prospective nested case-control study on CDI, performed between October 2010 - February 2012 in the Netherlands. Details on the study design have been published previously [4]. In summary, three medical microbiological laboratories (A, B and C) tested all unformed stool samples of patients >2 yrs submitted by 832 general practitioners (with a population of 2,810,830 patients) for the presence of free *C. difficile* toxins in the faeces. Diagnostics of other enteropathogens were performed on request of the physician. The study area encompassed areas of varying levels of pig farming. Questionnaire data (e.g. on CDI risk factors and several environmental exposures, e.g. contact with livestock) were requested from all positive patients and a matched control group (on age, sex, and calendar time). PCR ribotyping was used to characterize all *C. difficile* isolates [15]. The study was approved by the LUMC Medical Review Ethics Committee.

## GEOCODING AND MAPPING

Full residential postal codes were requested from both *C. difficile* positives (n = 194) and *C. difficile* negative patients with diarrhoea (n = 12,520). If the exact residential postal code was unknown (all patients of laboratory A, four patients of laboratory B, and two patients of laboratory C), the location of the general practitioners' practice was used. Locations were obtained for 6,882 patients for laboratory A (83%), 3,009 patients for laboratory B (100%) and 1,367 patients for laboratory C (100%). Locations were geocoded to X- and Y-coordinates of the centroid of the full postal code. ArcGIS version 10.5 was used for mapping (Environmental Systems Research Institutes, Inc. Redlands CA).

## SPATIAL CLUSTERING ANALYSIS AND SPACE-TIME PERMUTATION MODEL

Scan statistics were used to detect clusters of CDI in the community in temporal, spatial and space-time settings [16 – 18]. Likelihood ratio tests were used to detect the most likely clusters, while Monte Carlo simulation was used to correct for multiple testing [16, 18]. Bernoulli models were applied to assess if CDI cases were non-randomly distributed in space and time compared to *C. difficile* negative diarrhoeic controls [18]. Subsequently, we searched for space-time interaction of CDI and non-CDI diarrhoeic events by space-time permutation models [17]. For both models, we aggregated the data per week, used a standard maximal temporal cluster size of 50% of the population at risk, and a maximal cluster size of 25km, and scanned for high rates. As the three participating laboratories had slightly different study periods, we performed separate space-time permutation analyses for three laboratories. SaTscan (version 9.4.4, M Kulldorf, Boston, MA, USA) was used to perform all geospatial analysis.

## LIVESTOCK EXPOSURE AS A RISK FACTOR FOR COMMUNITY-ACQUIRED CDI

We tested livestock exposure as a risk factor for CA-CDI by multivariate conditional logistic regression analysis. Patients were excluded if they were admitted to a hospital <12 weeks prior to onset of diarrhoea [19]. Livestock exposure included professional contact and/or recreational contact with farm animals (e.g. visiting a children's farm) <30 days before the onset of diarrhoea. Using data from the literature, putative confounders (other than matching variables) were antibiotic use [5, 20 – 23], hospital visits [22, 23], PPI use [24], CDI household contacts [8], and contact with infants [25] and comorbidities [23]. Antibiotic exposure was categorized into 4C antibiotics (cephalosporins, clindamycin, ciprofloxacin and amoxicillin/clavulanic acid), non-4C antibiotics and antibiotics of unknown type [26]. We created a comorbidity score adapted from the chronic disease score for infectious diseases (CDS-ID), but scored reported illnesses in <1 year before diarrhoea [27]. Putative confounders were visualized in a directed acyclic graph ( Supplementary Figure 1 ) [28] and incorporated in the multivariate model accordingly. For the total effect of animal exposure we adjusted for age and gender (matching variables), comorbidities, and contact with infants. Odd ratios (ORs) were presented with a 95% confidence interval (95% CI). We used STATA version 14.1 (StataCorp, College Station, TX, USA) for our analyses.

# Results

## *C. DIFFICILE* PREVALENCE

The study covered a population of 2,810,830 patients inhabiting 3,848 postal code areas. In total, 194 of 12,714 patients (1.5%) tested positive for *C. difficile* toxins. Laboratory A tested 111 of the 8,338 patients positive for *C. difficile* (1.3%) between October 4, 2010 and October 28, 2011. Laboratory B tested 54 of the 3,009 patients positive for *C. difficile* (1.8%) between November 16, 2010 and January 31, 2012. Laboratory C tested 29 out of 1,367 patients positive for *C. difficile* (2.1%) between September 30, 2010 and September 30, 2011. The distribution of *C. difficile* positive and negative patients of all three laboratories is depicted in Figure 1. Of the 194 *C. difficile* positive patients, 152 completed the questionnaire and 124 complied with the definition of CA-CDI. The mean age of CA-CDI patients was 49 years (SD, 22.6), and 64.5% were female.

## SPATIAL AND TEMPORAL CLUSTERS OF CDI

According to Bernoulli modelling, no significant clusters of CDI (n = 179) were detected compared to *C. difficile* negative diarrhoeic controls (n = 11,258) for laboratory A, B and C. Furthermore, testing for purely spatial or temporal clusters did not yield significant results. One non-significant temporal cluster of 45 CDI cases (RR, 2.06; $p = 0.051$) was found between the October 23, 2010 and April 1, 2011 for laboratory A, and one of 11 cases (RR, 2.10; $p = 0.89$) between the November 30, 2011 and January 24, 2012 for laboratory B.

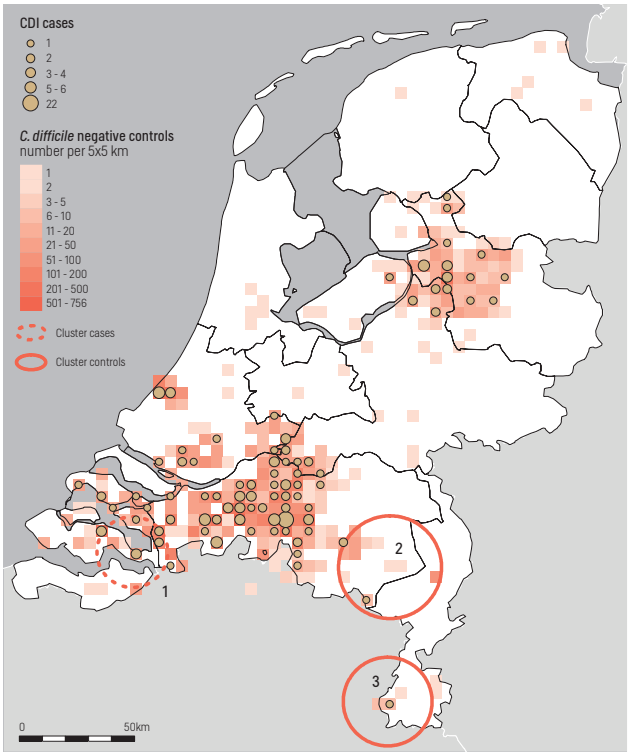
## SPACE-TIME INTERACTION OF CDI

One significant spatio-temporal cluster of six CDI cases (not caused by one ribotype) between December 11, 2010 - February 4, 2011 was found (15.31 km radius; $p = 0.0066$; Figure 1). However, this cluster was located in an area surrounded by large water surfaces ( Figure 1 ), not found by Bernoulli modelling, and interpreted as false positive. CDI patients were not clustered according to a space-time permutation model for laboratory B and C.

## SPACE-TIME INTERACTION OF *C. DIFFICILE* NEGATIVE PATIENTS WITH DIARRHOEA

Two clusters of *C. difficile* negative diarrhoeic controls were detected for laboratory A ( Figure 1 ). The first consisted of 159 patients diagnosed between October 16, 2010 – April 22, 2011 (22.25 km radius, $p < 0.0001$) of whom 9% were tested positive for a combination of infectious pathogens causing diarrhoea. The second cluster included 15 diarrhoeal patients without infectious pathogens detected between November 20, 2010 and January 21, 2011 (19.19 km radius, $p = 0.0012$; Figure 1). For laboratory B and C, *C. difficile* negative diarrhoeic controls were not clustered according to a space-time permutation modelling.

**Figure 1** Distribution of 179 community CDI cases (yellow circles) in contrast to density of *C. difficile* negative diarrhoeic controls (number per 5x5 km; blue) as detected by three medical microbiology laboratories in the Netherlands. Red circles indicate spatial-temporal clusters of community CDI cases (dotted line; false-positive) and *C. difficile* negative diarrhoeic controls (solid line in three regions of the Netherlands.




## RISK FACTORS FOR CA-CDI

Table 1 shows the putative risk factors for community-acquired CDI cases (n = 124) and controls (n = 232). Comorbidities and antibiotic exposure were associated with CA-CDI, while there was no apparent association with environmental exposures to livestock. In multivariate analysis, livestock exposure was not related to CA-CDI (OR, 0.99; 95% confidence interval, 0.44-2.24).

## PCR RIBOTYPES OF CA-CDI

*C. difficile* isolates were available for PCR ribotyping of 120 patients with community-acquired CDI. Of 25 different PCR ribotypes found, ribotypes 002 (11.4%), 015 (10.1%), and 078 (10.1%) were most common. Since no CDI clustering was found, associations to specific PCR ribotypes could not be investigated. PCR ribotype 078 cases and those caused by the highly related ribotype 126 were spatially dispersed throughout the study area.

**Table 1.** Putative risk-factors of community-acquired CDI, and multivariate conditional logistic regression analysis of livestock exposure as a risk factor for community-acquired CDI. aAdjusted for age and gender, bmatching variables. CA-CDI; community-acquired CDI; OR: Odds ratio; CI: confidence interval; MVA: multivariate analysis; sd: standard deviation.

| | CA-CDI (N=124) | | | Controls (N=232) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | N | % | n | N | % | OR[a] | (95% CI) | | MVA | OR | (95% CI) |
| **Age, mean (±sd)[b]** | **49.0** | **22.6** | | **48.4** | **22.4** | | | | | | | |
| **Female[b]** | **80 / 124** | **64.5** | | **151 / 232** | **65.1** | | | | | | | |
| **Comorbidities <1yr before diagnosis** | | | | | | | | | | | | |
| Diabetes | 12 / 124 | 9.7 | | 18 / 230 | 7.8 | | 1.34 | 0.62 | 2.91 | | | |
| Respiratory illness | 20 / 124 | 16.1 | | 20 / 230 | 8.7 | | 2.03 | 1.06 | 3.87 | | | |
| Kidney disease | 5 / 124 | 4.0 | | 4 / 230 | 1.7 | | 2.49 | 0.58 | 10.70 | | | |
| Transplant | 1 / 124 | 0.8 | | 0 / 230 | 0.0 | | | | | | | |
| Cancer | 3 / 124 | 2.4 | | 3 / 230 | 1.3 | | 1.76 | 0.24 | 12.73 | | | |
| Gastro-intestinal illness | 12 / 124 | 9.7 | | 17 / 230 | 7.4 | | 1.35 | 0.60 | 3.03 | | | |
| Comorbidity score, mean (±sd) | 0.78 | 1.17 | | 0.49 | 1.04 | | 1.29 | 1.05 | 1.58 | 1.27 | 1.03 | 1.56 |
| **Antibiotic use <90 days before diarrhoea** | | | | | | | | | | | | |
| 4C antibiotics | 19 / 122 | 15.6 | | 3 / 228 | 1.3 | | 43.42 | 8.43 | 223.64 | | | |
| Non-4C antibiotics | 27 / 122 | 22.1 | | 6 / 228 | 2.6 | | 24.33 | 7.08 | 83.55 | | | |
| Unknown type of antibiotics | 18 / 122 | 14.8 | | 23 / 228 | 10.1 | | 4.32 | 1.78 | 10.48 | | | |
| Overall antibiotic use | 64 / 122 | 52.5 | | 32 / 228 | 14.0 | | 10.87 | 5.14 | 22.96 | | | |
| **Hospital visits <30 days** | 39 / 119 | 32.8 | | 64 / 216 | 29.6 | | 1.16 | 0.71 | 1.89 | | | |
| **CDI household contacts** | 1 / 121 | 0.8 | | 2 / 221 | 0.9 | | 1.01 | 0.09 | 11.20 | | | |
| **Diarrhoeic household contacts** | 6 / 121 | 5.0 | | 18 / 222 | 8.1 | | 0.62 | 0.24 | 1.56 | | | |
| **Contact with infants <2 year old** | 35 / 121 | 28.9 | | 89 / 246 | 36.2 | | 0.76 | 0.46 | 1.28 | 0.87 | 0.51 | 1.49 |
| **Animal, manure, and meat exposure** | | | | | | | | | | | | |
| Livestock | 10 / 121 | 8.3 | | 22 / 231 | 9.5 | | 0.94 | 0.42 | 2.07 | 0.99 | 0.44 | 2.24 |
| Pet(s) | 79 / 119 | 66.4 | | 137 / 228 | 60.1 | | 1.36 | 0.82 | 2.23 | | | |
| Gardening | 34 / 119 | 28.6 | | 85 / 219 | 38.8 | | 0.59 | 0.35 | 0.99 | | | |
| Working in food and beverage | 4 / 123 | 3.3 | | 4 / 229 | 1.7 | | 1.84 | 0.46 | 7.40 | | | |
| Eating meat | | | | | | | | | | | | |
| never | 2 / 121 | 1.7 | | 4 / 231 | 1.7 | | ref. | | | | | |
| 1-2 times a week | 16 / 121 | 13.2 | | 35 / 231 | 15.2 | | 0.88 | 0.15 | 5.17 | | | |
| 3-6 times a week | 54 / 121 | 44.6 | | 113 / 231 | 48.9 | | 0.94 | 0.17 | 5.24 | | | |
| daily | 49 / 121 | 40.5 | | 79 / 231 | 34.2 | | 1.32 | 0.23 | 7.49 | | | |

# Discussion

The incidence of CDI in the community in the Netherlands was estimated at 0.67 per 10,000 persons per years (95% confidence interval, 0.58-0.78), comparable to Salmonella infections [4]. Our multicentre study of community CDI is the first to assess both spatial clustering and environmental risk factors for CA-CDI in combination with molecular typing data. We did not find spatial clusters of CDI in a large community cohort of diarrhoeic patients. Correspondingly, there was no space-time interaction indicative for unusual increases of CDI in the community except for one false-positive signal. Our results support the hypothesis that CDI transmission in the community derives from widespread sources and not from localised environmental point sources, such as livestock farms [11].

Concerns that livestock farms –piglet and pig farms in particular– contribute to transmission of CDI to humans occurred for several reasons in the Netherlands [9]. High rates of *C. difficile* ribotype 078 have been found among piglets, farmers and the farm environments [10, 11, 29]. One out of four persons with daily contact with pigs was positive for intestinal carriage with *C. difficile* of which virtually all were ribotype 078 [29]. Application of whole-genome sequencing confirmed the presence of 100% identical ribotype 078 strains in pigs and humans [13]. In the current study, ribotype 078 cases accounted for 10% of CA-CDI, but its occurrence was not spatially clustered in areas of livestock farming. Other studies indicated that livestock farming does not lead to regional increases of CDI associated with PCR ribotype 078, or to a higher risk of *C. difficile* colonization in neighbouring residents [30, 31].

Our study incorporated spatial scan statistics. To our knowledge, two other studies assessed spatial clustering of CDI in the community in Australia [32] and North Carolina [14] respectively. In Australia, no spatial clusters were found among 1,792 *C. difficile* cases deriving from 392 postal code areas in Queensland [32]. In contrast, clustering was found in 21% of the 1,895 CA-CDI cases analysed in 10 counties of North Carolina comprising an area of approximately 1.94 million residents [14]. CA-CDI was associated with living in proximity to a livestock farm, farming raw materials service and nursing home [14]. The latter finding may result from the fact that long term care facility residents were eligible for inclusion as CA-CDI patients. Both studies included several demographic and environmental factors in the constructed spatial models, but molecular typing data and detailed patient information on CDI risk factors and animal exposure was lacking. A comparison between these findings and our case-control data is not straightforward. Interestingly, *C. difficile* PCR ribotype 014 was the most prevalent type in pigs in Australia, whereas ribotype 078/Toxinotype V was most prevalent in pigs in North Carolina [33] and the Netherlands [10]. In Australia, transmission of genetically identical ribotype 014 isolates was demonstrated between pigs and humans [12], similarly as for ribotype 078 in the Netherlands [13].

Our study has several limitations. We were not able to use exact residential data for all patients and experienced overall 10% missing location data. In the spatial analyses of laboratory A, we used locations of the general practitioners' clinics which may have caused a bias towards more clustering in this region. Second, our control group consisted of patients with diarrhoea due to other causes than *C. difficile* and not the total population-at-risk. Therefore we might have overlooked CDI clusters that occurred at the same time and place as non-CDI diarrhoeic clusters. We assumed that CDI result in a different pattern of clustering than other (also non-infectious) causes of diarrhoea, but there is no literature to support this assumption. We compensated by use of a second spatial model (space-time permutation model), not requiring population-at-risk data. However, further exploration of the scale and spatial patterns to be incorporated in spatial models for CDI are needed. Finally, we have not included cases (and controls) that did not visit their general practitioner or did not submit a stool sample [34].

## CONCLUSIONS

Our study using spatial scan statistics did not find clusters of CDI in the community. The lack of geographical and temporal clustering in the present study in combination with a lack of environmental risk factors (e.g. livestock exposure) suggest that widespread sources most likely are key in CDI infection and transmission in the community.

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# References


**1.** Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nat Rev Dis Primers. 2016; 2: 16020

**2.** Eyre DW, Cule ML, Wilson DJ, et al. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med. 2013; 369(13): 1195-205

**3.** Bloomfield LE, Riley TV. Epidemiology and Risk Factors for Community-Associated *Clostridium difficile* Infection: A Narrative Review. Infect Dis Ther. 2016; 5(3): 231-51

**4.** Hensgens MP, Dekkers OM, Demeulemeester A, et al. Diarrhoea in general practice: when should a *Clostridium difficile* infection be considered? Results of a nested case-control study. Clin Microbiol Infect. 2014; 20(12): O1067-74

**5.** Kuntz JL, Chrischilles EA, Pendergast JF, Herwaldt LA, Polgreen PM. Incidence of and risk factors for community-associated *Clostridium difficile* infection: a nested case-control study. BMC Infect Dis. 2011; 11: 194

**6.** Rousseau C, Lemee L, Le Monnier A, Poilane I, Pons JL, Collignon A. Prevalence and diversity of *Clostridium difficile* strains in infants. J Med Microbiol. 2011; 60(Pt 8): 1112-8

**7.** Durham DP, Olsen MA, Dubberke ER, Galvani AP, Townsend JP. Quantifying Transmission of *Clostridium difficile* within and outside Healthcare Settings. Emerg Infect Dis 2016; 22(4): 608-16

**8.** Loo VG, Brassard P, Miller MA. Household Transmission of *Clostridium difficile* to Family Members and Domestic Pets. Infect Control Hosp Epidemiol. 2016; 37(11): 1342-8

**9.** Hensgens MP, Keessen EC, Squire MM, et al. *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012; 18(7): 635-45

**10.** Hopman NE, Keessen EC, Harmanus C, et al. Acquisition of *Clostridium difficile* by piglets. Vet Microbiol. 2011; 149(1-2): 186-92

**11.** Keessen EC, Donswijk CJ, Hol SP, Harmanus C, Kuijper EJ, Lipman LJ. Aerial dissemination of *Clostridium difficile* on a pig farm and its environment. Environ Res. 2011; 111(8): 1027-32

**12.** Knight DR, Squire MM, Collins DA, Riley TV. Genome Analysis of *Clostridium difficile* PCR Ribotype 014 Lineage in Australian Pigs and Humans Reveals a Diverse Genetic Repertoire and Signatures of Long-Range Interspecies Transmission. Front Microbiol. 2016; 7: 2138

**13.** Knetsch C, Connor T, Mutreja A, et al. Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill. 2014; 19(45)

**14.** Anderson DJ, Rojas LF, Watson S, et al. Identification of novel risk factors for community-acquired *Clostridium difficile* infection using spatial statistics and geographic information system analyses. PLoS One. 2017; 12(5): e0176285

**15.** Bidet P, Lalande V, Salauze B, et al. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing *Clostridium difficile*. J Clin Microbiol. 2000; 38(7): 2484-7

**16.** Kulldorff M. A spatial scan statistic. Commun Statist Theory Methods. 1997; 26: 1481–96

**17.** Kulldorff M, Heffernan R, Hartman J, Assuncao R, Mostashari F. A space-time permutation scan statistic for disease outbreak detection. PLoS Med. 2005; 2(3): e59

**18.** Kulldorff M, Nagarwalla N. Spatial disease clusters: detection and inference. Stat Med. 1995; 14(8): 799-810

**19.** Kuijper EJ, Coignard B, Tüll P, ESCMID Study Group for *Clostridium difficile*, EU Member States, European Centre for Disease Prevention and Control. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect. 2006; 12 Suppl 6: 2-18

**20.** Kavanagh K, Pan J, Marwick C, et al. Cumulative and temporal associations between antimicrobial prescribing and community-associated *Clostridium difficile* infection: population-based case-control study using administrative data. J Antimicrob Chemother. 2017; 72(4): 1193-1201

**21.** Deshpande A, Pasupuleti V, Thota P, et al. Community-associated *Clostridium difficile* infection and antibiotics: a meta-analysis. J Antimicrob Chemother. 2013; 68(9): 1951-61

**22.** Søes LM, Holt HM, Bottiger B, et al. Risk factors for *Clostridium difficile* infection in the community: a case-control study in patients in general practice, Denmark, 2009-2011. Epidemiol Infect. 2014; 142(7): 1437-48

**23.** Kutty PK, Woods CW, Sena AC, et al. Risk factors for and estimated incidence of community-associated *Clostridium difficile* infection, North Carolina, USA. Emerg Infect Dis. 2010; 16(2): 197-204

**24.** Gupta A, Khanna S. Community-acquired *Clostridium difficile* infection: an increasing public health threat. Infect Drug Resist. 2014; 7: 63-72

**25.** Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-associated *Clostridium difficile* infection. J Antimicrob Chemother. 2008; 62(2): 388-96

**26.** Marwick CA, Yu N, Lockhart MC, et al. Community-associated *Clostridium difficile* infection among older people in Tayside, Scotland, is associated with antibiotic exposure and care home residence: cohort study with nested case-control. J Antimicrob Chemother. 2013; 68(12): 2927-33

**27.** Stevens V, Concannon C, van Wijngaarden E, McGregor J. Validation of the chronic disease score-infectious disease (CDS-ID) for the prediction of hospital-associated *clostridium difficile* infection (CDI) within a retrospective cohort. BMC Infect Dis. 2013; 13: 150

**28.** Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology. 2011; 22(5): 745

**29.** Keessen EC, Harmanus C, Dohmen W, Kuijper EJ, Lipman LJ. *Clostridium difficile* infection associated with pig farms. Emerg Infect Dis. 2013; 19(6): 1032-4

**30.** Zomer TP, van Duijkeren E, Wielders CCH, et al. Prevalence and risk factors for colonization of *Clostridium difficile* among adults living near livestock farms in the Netherlands. Epidemiol Infect. 2017: 1-5

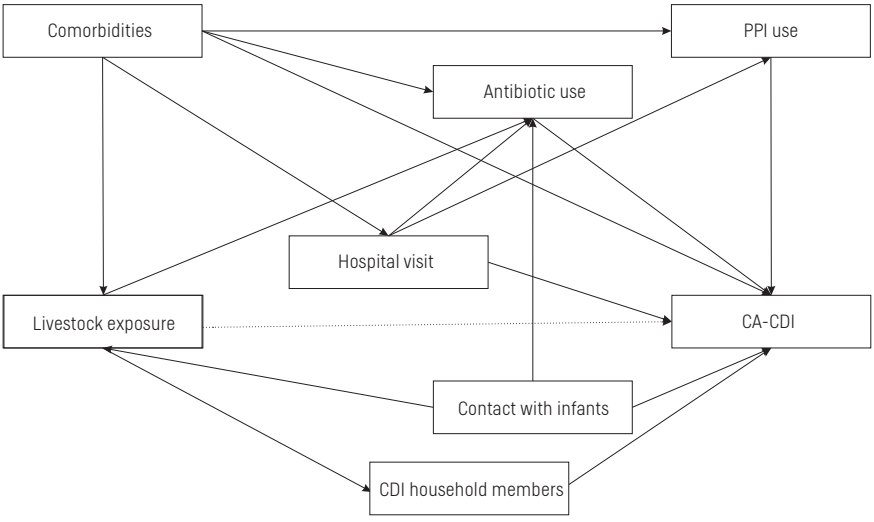
**31.** van Dorp SM, de Greeff SC, Harmanus C, et al. Ribotype 078 *Clostridium difficile* infection incidence in Dutch hospitals is not associated with provincial pig farming: Results from a national sentinel surveillance, 2009-2015. PLoS One. 2017; 12(12): e0189183

**32.** Furuya-Kanamori L, Robson J, Soares Magalhaes RJ, et al. A population-based spatio-temporal analysis of *Clostridium difficile* infection in Queensland, Australia over a 10-year period. J Infect. 2014; 69(5): 447-55

**33.** Fry PR, Thakur S, Abley M, Gebreyes WA. Antimicrobial resistance, toxinotype, and genotypic profiling of *Clostridium difficile* isolates of swine origin. J Clin Microbiol. 2012; 50(7): 2366-72

**34.** Bouwknegt M, van Dorp SM, Kuijper EJ. Burden of *Clostridium difficile* infection in the United States. N Engl J Med. 2015; 372(24): 2368

# Supplementary figures




**Supplementary Figure 1** Directed acyclic graph of the supposed relation of livestock exposure on evelopment of community-acquired CDI (CA-CDI).