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Prevalence and risk factors for colonization of *Clostridium difficile* among adults living near livestock farms in the Netherlands

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ABSTRACT

A cross-sectional study was performed among 2494 adults not living or working on a farm to assess prevalence of *Clostridium difficile* (CD) colonization and risk factors in a livestock dense area. CD prevalence was 1.2%. Twenty-one persons were colonized with a toxigenic strain and nine with a non-toxigenic strain. CD-positive persons did not live closer to livestock farms than individuals negative for CD. Antibiotic exposure in the preceding 3 months was a risk factor for CD colonization (odds ratio 3.70; 95% confidence interval 1.25–10.95).

Clostridium difficile infection (CDI) is an enteric disease that is mainly hospital-associated, but also increasing in the community [1, 2]. In the Netherlands, in about one-quarter of all diagnosed patients CDI is acquired in the community [3]. Over the past decade, a new strain, *C. difficile* (CD) ribotype 078, has emerged in patients with CDI especially acquired outside the hospital, but has also been found in high numbers in piglets, veal calves, and their immediate environment [3, 4]. To explain the emergence of community-acquired CDI, the following transmission routes have been suggested: environment-to-person, contact with infected or colonized individuals or animals, and foodborne transmission [5]. A previous study demonstrated that 25% (12/48) of farmers who had daily contact with pigs tested

positive for CD, and ribotype 078 was found on 15 of 32 pig farms in both humans and pigs suggesting animal-to-human transmission [6]. Highest rates were found in pig-breeding farms in piglets, 1–7 days old [6]. Data on the prevalence of CD in the Dutch general population (i.e. persons not living or working on farms), however, are scarce. In the Netherlands, the locations of pig farms and the area with high occurrence of human CD ribotype 078 infections overlap and CD can be detected in the immediate environment of farms [7], but a direct association between exposure to pigs and high occurrence has not been observed. Our study objective was to assess the prevalence of and risk factors for CD colonization in persons not living or working on a farm in areas with high livestock densities.

This cross-sectional study was part of a larger population-based study on health of residents living in a highly populated rural area with a high density of livestock farms in the Netherlands: the Livestock Farming and Neighbouring Residents' Health study (Dutch acronym: VGO). The methodology of the VGO study is described in detail by Borlée *et al.* [8]. In 2012, a questionnaire survey was conducted among the general population recruited via general practitioners [9]. Participants were included if they were aged 18–70 years, living in the province of Noord-Brabant or Limburg, and living in municipalities with <30 000 inhabitants. One eligible participant per household was invited. Questionnaire participants who gave consent for contact for a follow-up study, and who were not working or living on livestock farms, were eligible to participate in our cross-sectional population-based study, which was conducted from March 2014 to February 2015. Fecal samples to determine CD colonization (with or without symptoms) were taken once and participants were asked to complete a questionnaire to study risk factors. The distance between participants' residential address and the nearest farm was calculated [10]. The electronic medical records maintained by general practitioners were used to collect data on comorbidity and exposure to antibiotics [11]. CD was cultured by incubating approximately 1 g of feces in 9 ml CDBMN, CD enrichment modified broth (Mediaproduits, 46·1380) with CDMN Selective Supplement (Oxoid, SR0173E), and incubated 10–15 days at 37 °C under anaerobic conditions [6]. Subcultures were made onto chromID CD agar using direct plating and culture following alcohol shock (Biomérieux, 43871) and suspected isolates were identified as CD by polymerase chain reaction (PCR) to detect glutamate dehydrogenase [7]. Further characterization of CD was performed by determining the presence of toxin genes and by PCR ribotyping [12, 13].

Data were analyzed using SPSS version 22.0 (SPSS Inc., Chicago, IL). The Mann–Whitney *U*-test was used to compare the median distance to farms, the median number of farms within 500 and 1000 m, and the median number of farm animals within 1000 m. Using univariate logistic regression, odds ratios (ORs) with 95% confidence intervals (CIs) were obtained for risk factors. The VGO study was approved by the medical ethical committee of the University Medical Center Utrecht (number 13/533); all participants signed informed consent.

A total of 7180 persons were invited to participate, which resulted in 2494 participants (response rate 34·7%). Of 2494 participants (median age 58 years (range 20–72), 45·3% male), 2432 mailed a fecal sample. The prevalence of CD colonization was 1·23% (30/2432; 95% CI 0·85–1·73). Four CD-positive individuals had a strain belonging to ribotype 078, resulting in a prevalence of 0·16% (95% CI 0·05–0·40). Other toxin producing ribotypes were 014 ($n = 7$), 002 ($n = 2$), 265

($n = 2$), 001 ($n = 1$), 005 ($n = 1$), 012 ($n = 1$), 013 ($n = 1$), 017 ($n = 1$), and 024 ($n = 1$). Of 30 CD isolates, nine (30%) did not have toxin genes and belonged to PCR ribotypes 039 ($n = 4$), 010 ($n = 1$), 085 ($n = 1$), 071 ($n = 1$), and an unknown ribotype ($n = 2$). Of 30 positive samples, 28 were positive both with and without alcohol shock, while two samples were only positive by direct plating without alcohol shock. Descriptive analyses of the questionnaire data demonstrated that 45% of CD positives had gastroenteritis in the month preceding the study, compared with 20% of negatives ($P < 0.01$). When including only the toxin-producing CD positives, this difference was respectively 50% vs. 20% ($P < 0.01$). The frequency of nausea, diarrhea and fever was not significantly different between CD positives compared with negatives (14 vs. 9%, 24 vs. 17%, and 10 vs. 4%, respectively).

The results of risk factor analyses are shown in Table 1. There was no association between CD colonization and distance to the nearest farm, the number of farms within 500 and 1000 m, and the number of farms animals within 1000 m (Table 1). However, CD positives were more likely to have used antibiotics 3 and 6 months before the study compared with negatives (3 months: OR 3.70; 95% CI 1.25–10.95) (6 months: OR 2.64; 95% CI 1.05–6.64) (Table 1). In addition, 11.5% of CD positives who used antibiotics 3 months before the study received penicillins compared with 1.9% of CD negatives (OR 6.76; 95% CI 1.95–23.47). Visiting farms was significantly negative associated with CD colonization (OR 0.42; 95% CI 0.20–0.88).

[TABLE 1]

To our knowledge, this is the first study on the prevalence of CD colonization among persons not living or working on farms in areas with a high density of livestock farms. There is limited information on the prevalence of CD in general healthy populations, since most of the studies focused on patients in healthcare facilities [14]. The prevalence of 1.2% in our study is considerably lower than the 4.7% that was reported among patients at time of admission to three different hospitals in the Netherlands [15]. However, the investigated groups differed considerable with higher age and more comorbidity in the latter study. Including persons living or working on farms, would probably also have resulted in a higher CD prevalence.

The most frequently found toxigenic PCR ribotypes in our study were ribotypes 014 ($n = 7$; 23.3%) and 078 ($n = 4$; 13.3%). Those PCR ribotypes, together with 020, are also the most frequently isolated types in the Netherlands amongst hospitalized patients with CDI [16]. Ribotype 078 has been found in pigs and humans who have direct contact with pigs (i.e. pig farmers) [6]. No association was observed between CD colonization and living near (pig) farms, suggesting that there is no significant transmission from the environment around farms to humans. However, it should be noted that farmers were excluded from this study. A limitation of our study is the lack of information on the presence of CD at the farms in the study area, since no microbiological investigations on the farms and their surroundings were performed. An ecological association between the occurrence of human CD type 078 and the location of pig farms in the Netherlands has been reported [7]. Moreover, a previous study reported CD to be present in both humans and animals in about half of pig farms (15/32) [6]. However, it is unknown whether there are trends in time and what this prevalence is in farms with other animal species than pigs. In our study, we

found a significant negative association between CD and visiting farms, for which we have no explanation.

Antibiotic usage is an established risk factor for CDI [17]. We also found an association between CD colonization and exposure to antibiotics, especially penicillins. Penicillins have previously been identified as a risk factor for community-associated CDI [17]. We did not find an association between CD colonization and other well-known risk factors, such as previous hospitalization, increasing age, and comorbidity [18]. We also did not find an association between proton pump inhibitors and community-acquired CDI. This could be due to the small number of cases and characteristics of this study population.

In conclusion, the prevalence of CD colonization among adults living near livestock farms was 1.2%. In a livestock dense area, CD positive individuals did not live closer to livestock farms than CD negative individuals, indicating that the effect of exposure through the farm environment is limited. Antibiotic exposure was a risk factor for CD colonization.

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REFERENCES

1. MPM Hensgens , et al. Diarrhoea in general practice: when should a *Clostridium difficile* infection be considered? Results of a nested case-control study. *Clinical Microbiology and Infection* 2014; 20: O1067–O1074.
2. FC Lessa , et al. Burden of *Clostridium difficile* infection in the United States. *New England Journal of Medicine* 2015; 372: 825–834.
3. MPM Hensgens , et al. *Clostridium difficile* infection in the community: a zoonotic disease? *Clinical Microbiology and Infection* 2012; 18: 635–645.
4. K Keel , et al. Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves, and other species. *Journal of Clinical Microbiology* 2007; 45: 1963–1964.
5. AM Otten , et al. Disease transmission model for community-associated *Clostridium difficile* infection. *Epidemiology & Infection* 2010; 138: 907–914.
6. EC Keessen , C Harmanus , W Dohmen . *Clostridium difficile* infection associated with pig farms. *Emerging Infectious Diseases* 2013; 19: 1032–1034.
7. A Goorhuis , et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clinical Infectious Diseases* 2008; 47: 1162–1170.
8. F Borlée , et al. Spirometry, questionnaire and electronic medical record based COPD in a population survey: comparing prevalence, level of agreement and associations with potential risk factors. *PLoS ONE* 2017; 12: e0171494.
9. F Borlée , et al. Increased respiratory symptoms in COPD patients living in the vicinity of livestock farms. *The European Respiratory Journal* 2015; 46: 1605–1614.
10. LAM Smit , et al. Q fever and pneumonia in an area with a high livestock density: a large population-based study. *PLoS ONE* 2012; 7: e38843.
11. M Prins , R Verheij . NIVEL Zorgregistraties eerste lijn: Huisarts (Netherlands Primary Care Database: General Practitioners). Utrecht, The Netherlands: NIVEL, 2015 (<http://www.nivel.nl/node/4346>).
12. WN Fawley , et al. Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *Clostridium difficile* . *PLoS ONE* 2015; 10: e0118150.

13. S Paltansing , et al. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. *Clinical Microbiology and Infection* 2007; 13: 1058–1064.
14. L Furuya-Kanamori , et al. Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications. *BMC Infectious Diseases* 2015; 15: 516.
15. EM Terveer . Poster 0942 presented at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, 9–12 April 2016.
16. MJT Crobach . Tenth annual report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance May 2015-May 2016. RIVM report, Bilthoven, The Netherlands (<http://www.rivm.nl/dsresource?objectId=5fc6f4bc-ba13-45d9-a5a5-098f1b740e9b&type=org&disposition=inline>).
17. A Deshpande , et al. Community-associated *Clostridium difficile* infection and antibiotics: a meta-analysis. *Journal of Antimicrobial Chemotherapy* 2013; 68: 1951–1961.
18. EC Keessen , W Gaastra , LJA Lipman . *Clostridium difficile* infection in humans and animals, differences and similarities. *Veterinary Microbiology* 2011; 153: 205–217.

TABLE 1

Table 1. Risk factors for *Clostridium difficile* (CD) among adults living near livestock farms in the Netherlands

| Risk factor | CD positive median (range min–max) (n = 30) | CD negative median (range min–max) (n = 2402) | P-value ^a |
|--|--|--|----------------------|
| Distance in meters from patient to nearest | | | |
| Farm | 486 (22–1709) | 400 (3–1546) | 0.35 |
| Pig farm | 726 (283–2127) | 689 (11–2500) | 0.28 |
| Poultry farm | 990 (125–3945) | 930 (39–4145) | 0.50 |
| Cattle farm | 504 (38–1709) | 482 (3–1566) | 0.67 |
| Horse farm | 815 (22–3385) | 760 (16–3432) | 0.41 |
| Goat farm | 2683 (460–9867) | 2494 (99–11 477) | 0.73 |
| Sheep farm | 1204 (268–3598) | 1291 (13–5047) | 0.61 |
| Number of farms | | | |
| Within 500 m | 1 (0–6) | 1 (0–12) | 0.30 |
| Within 1000 m | 7 (0–24) | 9 (0–32) | 0.62 |
| Number of farm animals within 1000 m | | | |
| Pigs | 2867 (0–21 280) | 2652 (0–65 059) | 0.64 |
| Chickens | 218 (0–497 295) | 1052 (0–911 052) | 0.25 |
| Cows | 419 (0–3944) | 564 (0–6804) | 0.50 |
| Horses | 34 (0–436) | 28 (0–708) | 0.68 |
| Goats | 0 (0–1659) | 0 (0–5015) | 0.27 |
| Sheep | 7 (0–450) | 10 (0–1410) | 0.71 |

| Risk factor | CD positive % (n) | CD negative % (n) | OR (95% CI) |
|---|----------------------|----------------------|-------------------|
| Gender (male) | 36.7 (11) | 45.3 (1089) | 0.70 (0.34–1.47) |
| Age | | | |
| 20–29 | 3.3 (1) | 2.0 (49) | 1.75 (0.22–13.61) |
| 30–39 | 10.0 (3) | 6.5 (157) | 1.63 (0.46–5.80) |
| 40–49 | 13.3 (4) | 17.8 (427) | 0.80 (0.26–2.47) |
| 50–59 | 30.0 (9) | 27.4 (657) | 1.17 (0.50–2.76) |
| ≥60 | 43.3 (13) | 46.3 (1112) | Ref. |
| Household with partner | 75.9 (22) | 80.4 (1921) | 0.77 (0.33–1.80) |
| Household with children <4 years | 6.9 (2) | 3.7 (89) | 1.91 (0.45–8.18) |
| Level of accomplished education | | | |
| Low | 36.7 (11) | 25.5 (612) | 2.15 (0.79–5.84) |
| Medium | 43.3 (13) | 44.7 (1073) | 1.45 (0.55–3.83) |
| High | 20.0 (6) | 29.9 (717) | Ref. |
| Specific diet (without meat or fish or animal products) | 3.4 (1) | 5.6 (134) | 0.60 (0.08–4.45) |
| Hospitalized last 12 months ^b | 0 | 0.3 (7) | NA |
| Antibiotics last 6 months ^c | 23.1 (6) | 10.2 (205) | 2.64 (1.05–6.64) |
| Antibiotics last 3 months ^c | 15.4 (4) | 4.7 (94) | 3.70 (1.25–10.95) |
| Proton pump inhibitors | 20.0 (6) | 11.8 (282) | 1.86 (0.76–4.60) |
| Comorbidity ^d | 15.4 (4) | 20.3 (408) | 0.71 (0.24–2.08) |
| During work/study contact with | | | |
| Patients | 15.4 (4) | 12.2 (278) | 1.31 (0.45–3.83) |
| Residents of nursing homes | 23.1 (6) | 15.6 (356) | 1.62 (0.65–4.07) |
| Children | 7.7 (2) | 12.7 (291) | 0.57 (0.13–2.43) |
| Animals | 3.8 (1) | 6.2 (142) | 0.60 (0.08–4.48) |
| Travelled last 12 months to | | | |
| South/East Europe | 35.7 (10) | 29.5 (702) | 1.19 (0.54–2.63) |
| Africa, Asia, Latin-America | 7.1 (2) | 14.4 (342) | 0.49 (0.11–2.13) |
| No travel or travel to West/North Europe, Australia, New-Zealand, North-America | 57.1 (16) | 56.1 (1332) | Ref. |
| During childhood lived on a farm | 20.7 (6) | 34.3 (815) | 0.50 (0.20–1.23) |
| During childhood performed jobs on a farm | 39.3 (11) | 53.0 (1215) | 0.58 (0.27–1.23) |

Table 1 (cont.)

| Risk factor | CD positive % (n) | CD negative % (n) | OR (95% CI) |
|--|----------------------|----------------------|------------------|
| During childhood had holiday on a farm | 35.7 (10) | 48.8 (1141) | 0.58 (0.27–1.27) |
| Kept pets now or last 5 years ^c | 65.5 (19) | 51.8 (1236) | 1.77 (0.82–3.82) |
| Visited a farm last 12 months | 41.4 (12) | 62.8 (1500) | 0.42 (0.20–0.88) |
| Contact at home or during farm visit with farm animals | 25.0 (7) | 41.7 (981) | 0.47 (0.20–1.10) |
| Contact at home or during farm visit with | | | |
| Horses | 17.9 (5) | 20.7 (484) | 0.83 (0.32–2.20) |
| Pigs | 0 | 7.8 (182) | NA |
| Poultry | 14.8 (4) | 20.0 (468) | 0.70 (0.24–2.02) |
| Cows | 7.1 (2) | 15.7 (367) | 0.41 (0.10–1.75) |
| Goats | 11.1 (3) | 12.0 (281) | 0.91 (0.27–3.05) |
| Sheep | 3.7 (1) | 9.9 (231) | 0.35 (0.05–2.59) |

^aMann–Whitney *U*-test.

^bHospitalized in the Netherlands and/or abroad.

^cFor 415 persons data on antibiotics and comorbidity was missing.

^dIncluded cerebrovascular disease, chronic cardiovascular disease, liver disease, chronic lung disease, chronic renal disease, auto-immune disease, neurological comorbidity, diabetes, and malignancy.

^eDog, cat, bird, rabbit, guinea pig, hamster, mouse, rat, fish, turtle.