

Aerial dissemination of *Clostridium difficile* spores inside and outside a pig farm

E.C. Keessen*¹

C.J. Donswijk¹, C. Hermanus², E.J. Kuijper², L.J.A. Lipman¹

¹ Utrecht University, Institute for Risk Assessment Sciences, PO Box 80175, 3508 TD Utrecht, The Netherlands. ² Leiden University Medical Center, Department of Medical Microbiology, PO Box 9600, 2300 RC, Leiden, The Netherlands

*Yalelaan 2, 3508 TD, Utrecht, The Netherlands. E-mail: eckeessen@uu.nl, fax: 0031-2532365

Abstract

In both human and veterinary medicine *Clostridium difficile* infections are increasingly reported. The observation that aerial dissemination occurs in a hospital environment and can play a role in the transmission of human *C. difficile* infection, resulted in the present study to the occurrence of airborne *C. difficile* in, and nearby a pig farm with a high prevalence of *C. difficile*. Airborne *C. difficile* was detected in all farrowing and weaned piglets' wards, and up to 20 m distant from the farm. Personnel activity in the farrowing pens was significantly associated with peaks in airborne *C. difficile* colony counts.

Introduction

Clostridium difficile has been described as a pathogen for piglets since the beginning of the 21st century (Songer, 2004). In North America, *C. difficile* infection (CDI) is now considered the most important cause of neonatal diarrhea in piglets (Songer & Uzal, 2005). In humans, the bacterium is known since decades as a major cause of nosocomial infections. Recently, CDI is increasingly reported as a community acquired infection (Bauer et al, 2009, Indra et al., 2009). The majority of the community acquired infections are caused by *C. difficile* PCR Ribotype 078 (Kuijper et al., 2006; Wilcox et al., 2008; Bauer et al., 2009). The 078 strain is also predominantly present in piglets with CDI (Keessen et al., 2010). The genetical similarity of the human and pig strains gave rise to the concern that zoonotic transmission of this strain is likely to occur (Debast et al., 2009; Rupnik & Songer, 2010; Weese, 2010). Nonetheless, until now there is no evidence of a zoonotic transmission (Weese 2010) and there is still little knowledge on possible transmission routes from animals to humans (Jhung et al., 2008).

Aerial dissemination of *C. difficile* was suggested to play a role in the transmission of human CDI in hospitals (Best et al., 2010). This prompted us to study whether aerial dissemination of *C. difficile* also occurs in pig farms and whether personnel activity is related to increases in spore counts. An additional goal of this study was to determine whether *C. difficile* could be detected in the air in the close vicinity of the farm.

Material and Methods

2.1. Farm

Air sampling was done at a pig-breeding farm with a known high prevalence of *Clostridium difficile* in the pigs. The ventilation in the pens is based on a negative pressure system. Fresh air enters the pens from the hallway through slotted air inlets in the doors. The air leaves the pens through a fan, at a height of four meter, which directs the air into an airshaft or directly into the outside environment.

2.2. Sampling procedure

A MB1 MICROBIO Air Sampler (Parrett Technical Developments) was used for collection of airborne *Clostridium difficile*. The air was directed on commercially prepared *C. difficile* agar plates (CLO-agar, BioMérieux). Following sampling the agar plates were kept in a refrigerated box, until the laboratory was reached.

2.3. Sampling strategy

2.3.1. Inside air sampling

Sampling of the farrowing wards was performed in the ventilation shaft of the building. The numbers of pigs and piglets of each farrowing ward and pen were registered at the beginning of the experiments. Subsequent parturitions and

changing number of piglets in the farrowing ward were registered as well. The air of two farrowing wards was continuously sampled combined with the registration of personnel activity. Comparison between the activity data and the colony count was possible as both were taken as a function of time.

2.3.2. Continuous sampling during movement of weaned piglets

On three occasions sampling was performed prior, during and after movement of weaned piglets from their farrowing pen to the weaned piglets ward. Air coming from these farrowing pens was sampled continuously with a sampling time of 5 minutes.

2.3.3. Outside air sampling

Outside air sampling was performed above roof exhausts and at distances 20, 40, 80 and 140 meter downwind from these exhausts at a height of 1.5 m. Sampling time was set on 5 minutes. Data of the Dutch Meteorological Institute was used to determine wind speed and temperature. Control sampling was performed at an upwind point 20 meter distant from the nearest exhaust to exclude any other sources of airborne *Clostridium difficile*.

2.4. Analysis procedure

Samples were incubated on the CLO-agar plates at 37 °C for 48 h under anaerobic conditions. Using Gram staining the isolates with morphology typical of *C. difficile* were identified. Per ward and experiment two isolates were randomly chosen, both to be ribotyped according to the method described by Paltansing (2007). All Isolates from the outside samples were ribotyped as well. Colony counts were calculated per m³.

2.5. Statistical analysis

Data from the continuous sampling experiments were analyzed using the t-test to investigate the correlation between personnel activity and colony count.

Results

3.1. Inside air sampling

Personnel activity was significantly associated with an increase in airborne colony count in both farrowing wards ($P = 0.043$, $P = 0.034$). Highest colony counts up to 575 colonies per m³ were found during or shortly after feeding, ear tagging and entrance of the farmer.

Most peaks in colony counts corresponded with activity prior to sampling. One of the two highest peaks (575 colonies per m³) was not preceded by registered activity. On the other hand, activity was not always related to a peak; e.g. medical care by students did not result in an increasing number of airborne colonies.

Movement of the weaned piglets from their farrowing to the weaned piglets' ward correlated significantly to an increase in colony count ($P = 0.028$).

The numbers of colonies found during movement increased on average 7.7 times compared to the numbers of colonies found prior to the movement. The numbers of colonies of the three pens show a fast decline once the piglets have been moved. The air of one pen continued to have a high concentration of colonies, with the highest concentration found 20 minutes after movement of the pigs.

3.2. Outside air sampling

Air from all four exhausts on the top of the building (consisting of air coming from farrowing, boar and young sow ward) tested positive for *C. difficile*, the numbers ranged from 6 colonies/m³ to 120 colonies/m³. Outside air tested positive 2 out of 4 times at a distance of 20 m downwind from the building. No colonies were found 40, 80 and 140 meter distant of the building. Outside temperature ranged from 2 °C to 8 °C, airspeed ranged from 0.83 m/s to 5.3 m/s. Positive air samples were obtained with the highest airspeeds (5.3 and 3.2 m/s). All upwind air samples were negative for *C. difficile*.

3.3 PCR Ribotyping

In most air samples within the farm and at 20 m distance from the farm *C. difficile* was detected. A collection of this share was ribotyped. All 20 *C. difficile* isolates were identified as PCR ribotype 078.

Discussion

The aim of this study was to detect *C. difficile* in the air of a pig farm and to relate colony counts of *C. difficile* in air samples to personnel activity. The results demonstrate that personnel activity correlates significantly to an increase in colony count. One of the highest peaks was not linked to personnel activity. An explanation for this peak might be that this increase in colony count was caused by animal activity, though this was not registered.

In outside air, colonies were detected up to 20 m distant from the farm. The large decrease in colony count immediately outside the building is a logical consequence of the dilution by outside air, and generally applies to the total bacteria concentration (Homes et al., 1996). Limited dispersal of airborne *Clostridium difficile* to the outside environment could implicate a low risk of human exposure to airborne *Clostridium difficile*. We could not find previous studies on the potential and mechanisms of infection by airborne spread of *C. difficile* in animals. Other gastro-intestinal pathogens such as *Salmonella* species, *Campylobacter* species and *Clostridium botulinum* have been proven to be able to infect by airborne transmission (Sugiyama et al., 1986; Pillai & Ricke, 2002; Oliveira et al., 2006).

Conclusion

This study demonstrates a significant correlation between personnel activity and airborne *C. difficile* colony counts. The widespread aerial dissemination of *Clostridium difficile* in the farrowing wards may have implications for aerial transmission of *Clostridium difficile* between piglets. The finding of *C. difficile* in limited numbers at a 20m distance in the air needs further research to the spread of *C. difficile* in the environment of pig farms, to determine its significance for human health.

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