Cleaning and disinfection and epidemiology of Clostridium difficile on a pig farm

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Abstract

Background: piglets become infected with *Clostridium difficile* (CD) within 48 hours after birth through spores from the environment (Hopman et al. 2011). The aim of this study was to investigate which materials used on a pig farm can act as vector for CD spores and how cleaning and disinfection can play a role in reduction of the spores in the farrowing pens.
Methods: In this study 3 farrowing pens were sampled on 3 different times; dirty, after the normal cleaning procedure and after disinfection. In total, 72 samples were taken from the floor of the farrowing pens. All floor samples were quantitatively analyzed, 24 floor samples were qualitatively analyzed. 5 pairs of boots and 4 overalls were tested, just as inaccessible places in the pens; the frame of the sow, bars of the manure storage and walls. These samples were qualitative and quantitative analyzed.
Quantitative analysis: Samples were immersed with 36 ml of PFZ and put in the stomacher for 180 seconds. 1 ml of the mix was taken out and mixed with 1ml 96% ethanol to be alcohol shocked for 60 minutes. 0.1 ml was taken to be plated on CLO-plates and incubated for 48 hours under anaerobic conditions at 37°C.
Qualitative analysis: CDMN broth was used instead of PFZ. The immersion was incubated for 7 days under anaerobic conditions. After that, 2 ml was alcohol shocked and centrifuged. The sediment was plated out and incubated for 48 hours under anaerobic conditions at 37°C.
Results: Quantitative analysis: samples from the dirty pens were positive 17 times (70.8%). All samples taken from the pens after cleaning and disinfection were negative. 2 (50%) overalls were tested positive and 1 (33.3%) pair of boots was positive. Inaccessible places were negative.
Qualitative analysis: 20 floor samples (83.3%) were positive. 3 (75%) overalls were positive, 4 (80%) pairs of boots were positive. Inaccessible places were all positive (100%).
Conclusion: Based on the gained results, the cleaning procedure shows some effectiveness against CD. However, not all spores are washed away as shown after 7 days of incubation. This means that piglets can still get infected with the remaining spores. The positive tested overalls and boots can be a vector for CD spores.

Introduction

Clostridium difficile (CD) is an obligate anaerobic, Gram-positive and spore forming bacterium. The spores of CD survive for many years on surfaces and are very resistant

against drying, heat and different types of disinfectants like alcohol-based products, which are often used in standard hand hygiene protocols (Best et al. 2010, Vonberg et al. 2008, Kuijper et al. 2006). The spores of CD are very difficult to eradicate and are widely distributed in the environment; they can be found in soil and water and in intestinal tracts of different animals, including pigs (Kuijper et al. 2006).

In humans, CD causes *Clostridium difficile*-induced colitis (CDIC) and also more severe pseudo-membranous colitis which can be fatal. CDIC is often associated with hospital-acquired diarrhea and the use of antibiotics (Kuijper et al. 2006, Bartlett, Perl 2005). However, the disease can also develop under different conditions, e.g. outside the hospital in patients with no previous use of antibiotics (Kuijper et al. 2006). CDIC in humans is an upcoming disease with an increasing incidence rate. The importance of different ribotypes changed during the last couple of years. In humans, ribotype 078 is one of the most important in Europe, besides ribotype 001 and ribotype 014 (Hensgens et al. 2010, Bauer et al. 2011). Ribotype 078 is the most predominant in piglets and is comparable to the ribotype 078 in humans (Keessen et al. 2010). This suggests interspecies transmission.

In piglets, CD can cause severe diarrhea, due to gross lesions and mesocolonic edema (Songer, Anderson 2006). Neonatal piglets, 1-7 days old, can be affected and often show diarrhea. Some piglets become obstipated (Songer et al, 2006). Newborn piglets become infected with CD within 48 hours after birth (Hopman et al. 2011). Hopman et al. (2011) tested sows for presence of CD078 in their feces. No positive sows were found before they were placed in the farrowing pens (Hopman et al. 2011). The floors of the farrowing pens were tested positive. After birth, piglets became positive and after that, also the sows tested positive for CD (Hopman et al. 2011). It is likely that piglets become infected through the spores from the environment. This suggests that sows are not likely to be the primary source of *C. difficile*, but cannot be excluded completely, due to the knowledge that CD can be found in the intestinal tract of pigs (Kuijper et al. 2006, Keessen et al. 2010).

Therefore, the efficacy of cleaning and disinfection protocols on a pig farm and the possibilities to introduce spores to a farrowing crate by means of vectors, e.g. boots and overalls plays a crucial role in transmission of CD spores. No research after all these possible vectors has been done.

Besides illness in piglets caused by CD, pigs are a possible reservoir for CD in humans (Hopman et al. 2011, Bakker et al. 2010). Because this suggested interspecies transmission, knowledge of transmission of CD is needed. This knowledge of epidemiology is necessary to advise farmers about measurements to diminish the presence of CD in the pig stable.

The objective for this study was to determine how cleaning and disinfection can play a role in reduction of the spores in the farrowing pens and which materials used on the pig farm can act as vectors for CD. Therefore, the floors, walls and corners of farrowing pens were sampled, as well as overalls and boots.

Material and Methods

The research had been done on a Dutch pig farm which is known for the presence of *Clostridium difficile* ribotype 078.

Sampling procedure

Floors:

The floors of 4 different farrowing pens were sampled at three subsequent moments: after the relocation of the sows and the piglets, after cleaning and after disinfection. Samples from the dirty floors were taken directly after the sows and piglets were relocated in other departments in the farm. After sampling, the regular cleaning procedure of the pig farm was executed by an employee of the farm. An alkali foam detergent, BIO-CID-S, was used in the cleaning procedure. After cleaning, the floor was dried with and air-blower and directly afterwards floor samples were taken. These samples were classified as clean. After cleaning and sampling, 3 different disinfection products were used in 3 different farrowing pens: Halamid, MEGA-DES and OXY-DES. The active substances of the disinfectants are for Halamid a chloride-T ion, for MEGA-DES a quaternary ammonium, and for OXY-DES a hydrogen peroxide. The floor was rinsed with water after the exposure with the disinfectants which were used as recommended by the manufacturer. A control farrowing pen was only rinsed with water. All the pens were dried with an air-blower and thereupon floors samples were taken. These samples were classified as disinfected. The sampled surface was 1.074m^2 for dirty samples and 0.537m^2 for the clean and disinfected samples. All samples were taken with a Swiffer® cloth and were both qualitatively and quantitatively analyzed.

Inaccessible areas

Besides sampling the floor, other parts of the farrowing pens were sampled; the frame of the sow, bars of the manure storage and the walls. These places were sampled with 1 electrostatic cloth per farrowing crate. These places were sampled because the areas are more difficult to clean and were therefore phrased “inaccessible”. It is expected that bacteria and spores can survive and accumulate in these places. The areas were sampled when the surfaces were considered “dirty’ namely after the removal of the sows and piglets and when the surfaces were considered “clean” after the normal cleaning procedures were followed. Samples were taken with a Swiffer® cloth and the sampled surface was noted. The samples were analyzed both quantitatively and qualitatively.

Overalls and boots

The overalls and boots of the employees in the farm can be possible vectors for transmission of spores and bacteria. The overalls and boots that were sampled hung at the wall in the changing area of the pig farm. The changing area is divided in two areas: ‘dirty’ and ‘clean’. The dirty area is the area where all visitors and employees enter the pig farm. Shoes and clothes have to be replaced by clothes and disposable shoes of the pig farm. There is also a possibility for a shower. After changing, all visitors and employees can enter the clean room, were boots and overalls hang at the wall. This is classified as a clean area and the overalls

and boots that were sampled in this area were classified as clean, although there was visible dirt on the overalls as well as the boots. The sleeves and legs of the overalls were sampled. The sampled surface was 0.67 m^2. The soles of a pair of boots were sampled. The sampled surface was 0.0525m^2. The sampled boots were sampled under different conditions. Samples were taken after a short walk through the corridor and were classified as dirty. Samples were also taken from boots as they hung at the wall in the 'clean' area and were classified as clean. These samples were also taken with Swiffer® cloths.
All cloths were transported to the lab concealed in a stomacher bag in a cooler.

Culturing *Clostriudium difficile*
Quantitative analysis: samples were separately immersed into 36 ml PFZ. This immersion was homogenized for 180 seconds in the stomacher. 1 ml of the homogenized broth was transferred in a sterile tube and alcohol shocked for 60 minutes, at room temperature, using 1 ml 96% ethanol. Afterwards 0,1 ml was taken out and plated out on CLO agar (BioMérieux, Marcie l'Etoile, France) which is selective for CD. After incubation for 48 hours at 37 °C, identification has taken place by morphology, the characteristic horse manure odor and Gram-staining.

Qualitative analysis: samples were separately immersed into 36 ml *Clostridium difficile* moxalactam norfloxacin (CDMN) broth with sodium taurocholate, which is a specific grow medium for C. difficile (produced by Mediaproducts, The Netherlands). This immersion was homogenized for 180 seconds in the stomacher. The gained solution was incubated at 37°C under anaerobic conditions for 7 days. After 7 days, 2 ml broth was transferred to a sterile tube and alcohol shocked for 60 seconds with 2 ml of 96% ethanol. After 60 minutes, this mixture was centrifuged for 10 minutes at 4000 x g, the supernatant was discharged. The pallet was plated out on a CLO agar and incubated for 48 hours at 37°C under anaerobic conditions. Identification has taken place as described earlier.

Results

Quantitative analysis
The results of the quantitative analysis are presented in table 1. In total, 87 samples were taken, of which 20 (23%) samples were tested positive. 72 floor samples have been quantitatively analysed. 17 (23,6%) samples were positive. All the positive samples were taken under dirty conditions. Not all dirty samples tested positive; 70.8% were positive. Inaccessible places were tested negative in all conditions. The tested overalls (n=4) were tested positive in 50% of the samples. The dirty boots (n=3) were positive in 1 of the 3 samples (33.3%). The clean boots were tested negative.

Sample type	Sample condition	Number of samples	Positive samples (%)	Spores/m^2 (mean)
Floor	Dirty	24	17 (70.8)	2327
	Clean	24	0 (0)	0
	Disinfected	24	0 (0)	0
Inaccessible places	Dirty	3	0 (0)	0
	Clean	3	0 (0)	0
Overall	Dirty	4	2 (50)	2956
Boots	Dirty	3	1 (33.3)	8000
	Clean	2	0 (0)	0
Total		87	20 (23)	

Table 1: results of quantitative analysis

Qualitative analysis

The results of qualitative analysis are presented in table 2. In total, 39 samples were qualitative analysed, of which 33 (84.6%) were tested positive. 24 floor samples were taken: 20 (83.3%) were tested positive. All samples taken after cleaning and after disinfection were positive. Remarkably, not all dirty samples were tested positive; 4 of those remained negative. The samples taken at inaccessible places (n=6) all (100%) tested positive. The samples overalls (n=4) were positive in 3 of the 4 (75%) samples. The sampled dirty boots were positive in 2 of the 3 cases (66.6%). The clean boots were tested positive in both cases (100%).

Sample type	Sample condition	Number of samples	Positive samples (%)
Floor	Dirty	8	4 (50)
	Clean	8	8 (100)
	Disinfected	8	8 (100)
Inaccessible places	Dirty	3	3 (100)
	Clean	3	3 (100)
Overall	Dirty	4	3 (75)
Boots	Dirty	3	2 (66.6)
	Clean	2	2 (100)
Total		39	33 (84.6)

Table 2: results of qualitative analysis.

Discussion

The aim of this study was to determine which materials used on a pig farm could be a possible vector for CD spores and how cleaning and disinfection can play a role to diminish spores in farrowing pens. None of the quantitative clean and dirty samples were tested positive. As a result, nothing can be concluded about the different disinfectants. It seems that the cleaning procedure is partially effective against CD. However, there is no 100% eradication, as can be seen in the qualitative results, which shows that there are still spores detectable. The amount of spores which can be found in qualitative analysis probably lies under the level of detection of the quantitative method. This can mean that the used method for quantitative analysis is not sensitive enough for the amount of spores in farrowing pens. It is possible that this sampling method gives better results on a pig farm with a higher amount of neonatal diarrhea caused by *C. difficile*, because of the higher amount of spores. Based in the gained results it seems that the used materials can act as possible vectors of CD. The tested overalls (75%) and boots (80%) were sometimes positive. The pig farm is used for educational purposes, which means that many different people come and go and handle piglets. Overalls are an important vector, due to the average amount of 2956 spores/m^2. Piglets rest on the arms of visitors and can get spores on their bodies and possibly pick them up. The collected spores can be transmitted through the farrowing pen, which can cause disease.
The inaccessible places were positive in qualitative analysis. The tested places are contact places for piglets. During the sampling, it was particularly striking that the frame of the sow was, even after cleaning, covered with feces. It is recommended that more attention is paid to this, because it can cause accumulation of spores, which plays a role in transmission of spores.

There are some remarkable results in the qualitative analysis. One negative dirty floor sample was tested positive in quantitative analysis. Also, the negative tested overall in qualitative analysis was tested positive in quantitative analysis. This seems counterintuitive, because the enrichment method used in qualitative analysis is more sensitive than the direct plating in quantitative analysis (Weese et al. 2009). Weese et al (2009) reported similar results in retail meat which was both quantitative and qualitative analysed. The positive testing in quantitative analysis could be due to non-homogenous distribution of CD in the mixture. However, this cannot be proven and more research for more reliable research methods is recommended (Weese et al. 2009).

There is a possibility that the spores are wiped away as a result of the sampling method. Due to the limited surface in the farrowing pens, it was impossible to sample another surface every time. Mechanical wiping can make a difference in log-reduction after cleaning, as Alfa et al (2009) demonstrated (Alfa et al. 2009). They compared the results of log-reduction between only cleaning and disinfection and mechanical cleaning. They reported a higher log reduction in the cleaning and wiping method. Not all disinfectants showed comparable

results. Hydrogen peroxide showed significant differences between only cleaning and disinfection and after wiping. This implicates that hydrogen peroxide should be more effective after wiping (Alfa et al. 2009). It could mean that in our experiment bacteria and spores are wiped off when the dirty samples were taken and the remaining spores were under level of detection for the quantitative analysis. Floors were separated in different surfaces when samples were taken after cleaning and after disinfection. This was done to prevent that the low amount of spores were wiped off during the 'clean' sampling. Due to the limited surface, overlapping cannot be excluded. The effect of mechanical cleaning should be further investigated.

Conclusion

Clostridium difficile is very difficult to eradicate completely. Cleaning washes some bacteria and spores away, but not all spores are eradicated. As a result, piglets can become infected from the remaining spores on the floor, walls and corners. The inaccessible places of farrowing pens need special attention in the cleaning process.
Vectors like boots and overalls are easily forgotten, but very important. Through the handling of piglets, they can pick up spores from overalls and spread these through the farrowing pens. Therefore, good hygiene is necessary; washing the overalls on often and regular basis and dirty overalls, like when they are covered with feces, have to be taken out. Special attention is needed for the overalls used in farrowing pens with neonatal diarrhea.

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