Abstract
Identical *Clostridium difficile* ribotype 078 isolates were cultured from piglets with diarrhea and from humans with CDI. This led to the hypothesis that interspecies transmission could occur. The aim of this study was to investigate whether people who have occupational contact with pigs, and their family members, have higher colonization rates with *C. difficile* and to determine if similar *C. difficile* ribotypes could be found in farmers, employees, family members, and pigs on the same farm. Therefore, fecal samples from pig famers and their employees, family members and pigs on the same farms were analyzed for the presence of *C. difficile*. In total 125 of the 400 pig samples were found positive for *Clostridium difficile*. Ribotype 078 was the most common ribotype, found in fecal samples from pigs at 37 of the 40 farms. Beside this ribotype also type 001, 126, 045 and an unknown ribotype were found in the pigs. At 16 farms humans were found positive for the bacterium. At all these farms corresponding ribotypes were found in the humans and the pigs. At 15 farms type 078 was predominant in the pigs and found in the humans, and at 1 farm only type 045 was found in the pigs and the humans. In total 13 (24%) farmers and employees, and 5 (16%) partners carried *Clostridium difficile* in their feces. All the partners that were positive for the bacterium reported a regular contact with the pigs. None of the children was positive for the bacterium. The results of this study indicate that occupational contact with pigs may lead to a higher risk of colonization with *C. difficile*, since higher colonization rates in people in direct contact with pigs, then in the general population were found.

Cross sectional study on the prevalence of *Clostridium difficile* in pig farmers, their family members and the pigs on the farm; an indication for interspecies transmission?

Introduction
Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacterium. It spreads indirectly through the fecal-oral route or through spores left on surfaces. The bacteria produce toxins which bind to receptors in the gut, where it may lead to inflammation and diarrhea (Hookman and Barkin, 2009). *C. difficile* is the most common pathogen implicated in hospital-aquired diarrhea (Indra et al., 2009). One of the most severe complications of infection with this bacterium is pseudomenbranous colitis.
Recent studies demonstrate that the disease associated with *C.* difficile infection (CDI), is rising in incidence and severity (Jhung et al., 2008; Indra et al., 2009). The cause of this growing incidence and severity is due to a hypervirulent strain of *C. difficile* 027 (Goorhuis et al., 2008a; Debast et al., 2009; Indra et al., 2009). This ribotype causes more serious disease and a higher rate of deaths. Other ribotypes are increasing in the UK and the Netherlands, respectively 106 and 078 (Jhung et al., 2008; Indra et al., 2009). Ribotype 078 is also considered hypervirulent (Goorhuis et al., 2008b).
Besides in humans *C. difficile* is present in food production animals (Keel et al., 2007; Jhung et al., 2008). Ribotype 078 is the most common ribotype in pigs and calves in the United States (Keel et al., 2007). The same ribotype was also found in neonatal pigs the Netherlands (Debast et al., 2009).
The finding of identical *Clostridium difficile* ribotype 078 isolates in piglets with diarrhea and in humans with CDI led to the suggestion that interspecies transmission could occur (Goorhuis et al., 2008b; Debast et al., 2009). Subsequently a high prevalence of this type 078 was isolated from healthy pigs, surfaces and air samples inside and outside a pig farm (Keessen et al., 2011). It is unclear whether occupational activities of pig farmers and their employees lead to a higher risk of colonization with *C. difficile* and if transmission to family members occurs. The goal of this study was to investigate fecal samples from farmers and employees, family members and pigs on the farm to determine whether this can indicate interspecies transmission.

Material and methods

1.1 Sampling

1.2 Human samples

Stool samples were taken from farmers and employees (57), partners (28), and children (47) living on 35 pig farms participated in the study. Furthermore, 3 veterinarians working on these farms submitted their own fecal samples.

1.3 Pig samples

Pooled fecal samples were collected from the floor of 10 different animal departments per farm.

1.4 Culturing

C. difficile detection was performed using standard protocols for samples from human and animal origin. In short, 1 gram of faeces was mixed with 9 ml of the CDMN broth and incubated anaerobically at 37°C for 7 days. The broth was alcohol-shocked for spore selection by mixing 2 ml homogenized broth with 96% ethanol in a sterile tube and left it for 1 hour at room temperature. After centrifuging this mixture (4000 x g for 10 minutes) the supernatant was discarded, and the pellet was streaked onto a CLO agar plate and incubated anaerobically for 48 hours at 37°C.

1.5 Typing of isolates

Suspicious colonies based on gram stain appearance, colony morphology (swarming, non-haemolytic, greyish, rough) and characteristic odour were PCR ribotyping according to the protocol of Paltansing et al., 2007(Paltansing et al., 2007).

Results

C. difficile was cultured from porcine fecal samples on 39 of the 40 farms.

The presence of *C. difficile* in the pooled fecal samples from pigs on the farm varied per farm between 10% and 80%. In total 125 of the 400 samples from the pigs were positive for *C. difficile.* Most positive samples originated from the farrowing ward (49 / 87, 56.3%), the weaned piglets ward (31/98, 31.6%), the juvenile sow ward (16 / 68, 23.5%), and the pregnant sow ward (15/59, 25.4%). Ribotype 078 was the predominant ribotype and present at 37 of the 40 farms. At 5 farms another ribotype, besides type 078, was present in the feces from pigs. These ribotypes were type 126 (1 farm), type 001 (1 farm), and type 045 (3 farms). At 1 farm only type 045 was present and at 1 farm the presence of *C. difficile* was confirmed by detection of the GluD gene, but the ribotype of the isolates could not be identified. Humans, positive for *C. difficile* were identified at 15 farms. In total 13 (24%) farmers and employees, and 5 (16%) partners carried the bacterium in their feces. All partners reported that they had regular contact with the pigs. None of the children was positive for the bacterium.

Corresponding ribotypes were found in farmers, employees, their partners and the pigs at all the farms. At 14 farms ribotype 078 was present in the humans and the pigs. At 1 of these farms type 045 was cultured from the porcine samples, besides type 078. At 1 farm type 045 was present in the farmer and the pigs. An overview of the data is given in table 1.

Ribotype	No. farms	Humans
78	37	17
078 + 126	1	
078 + 1	1	
078 + 45	2	
45	1	1
GluD	1	

Table 1: Number of ribotypes found on the farms and in human samples.

Discussion

The prevalence of *C. difficile* in the general population varies between 3 and 17,5% (Barbut and Petit, 2001; Kato et al., 2001; Bartlett. 2002; Ozaki et al., 2004; Kuijper et al., 2006; Sunenshine and McDonald, 2006; McNamara et al., 2011). The results from researches of the western part of the world are significantly lower than those from the eastern part. This may be the result of different sampling methods or a geographical difference.

Compared with the results from the studies conducted in the Western part of the world, the carriage rate found in the farmers and their partners is higher than expected. However, it is difficult to compare the data from our study with data on the prevalence of *C. difficile* with data from other studies, because different methods to culture *C. difficile* were used, with as a consequence different sensitivities to detect the presence of the bacterium.

All the partners that were positive for *C. difficile* reported frequent contact with pigs. To determine risk factors for colonization with *C. difficile* for partners more information on lifestyle factors is necessary. Furthermore, the total number of sampled partners (n=28) might be too small for determination of risk factors.

The finding of the same bacterium in humans and animals does not necessarily indicate zoonotic transmission. In this study corresponding ribotypes were found in humans and animals. Nonetheless, comparing ribotypes from isolates cultured from human and porcine feces is not discriminatory enough to determine genetic relatedness of strains. To obtain more insight in the genetic relatedness of the same animal and human C. difficile ribotypes, Multiple-Locus Variable number tandem repeat Analysis (MLVA) is necessary.

A limitation of this study is the fact that the human fecal samples were sent to the laboratory by post, thus without cooling. The effect of these storage conditions on the recovery of *C. difficile* from the samples is unknown, but it could have led to overgrowth of *C. difficile* and false-negative results. Another limitation of this study is that the samples from the pigs were pooled samples from the floor, and therefore the individual contribution from pigs is unknown.

Conclusion

C. difficile was frequently found at pig farms, with type 078 as the predominant ribotype and present at 37 of the 40 farms. Most positive samples from pigs originated from the farrowing ward (49 / 87, 56.3%), the weaned piglets ward (31/98, 31.6%), the juvenile sow ward (16 / 68, 23.5%), and the pregnant sow ward (15/59, 25.4%). Humans, positive for *C. difficile* were identified at 16 farms. The carriage rate in the population of people with occupational contact with pigs (24%) is higher than the carriage rates observed in the general population. Corresponding ribotypes were found in farmers, employees, their partners and the pigs at all the farms. However, finding similar ribotypes does not necessarily indicate that interspecies transmission occurs. Further research such as MLVA must reveal whether interspecies transmission is likely to occur.

References

Barbut, F., Petit, J.C., 2001. Epidemiology of Clostridium Difficile-Associated Infections. Clin. Microbiol. Infect. 7, 405-410.

Bartlett, J.G., 2002. Clostridium Difficile-Associated Enteric Disease. Curr. Infect. Dis. Rep. 4, 477-483.

Debast, S.B., van Leengoed, L.A., Goorhuis, A., Harmanus, C., Kuijper, E.J., Bergwerff, A.A., 2009. Clostridium Difficile PCR Ribotype 078 Toxinotype V found in Diarrhoeal Pigs Identical to Isolates from Affected Humans. Environ. Microbiol. 11, 505-511.

Goorhuis, A., Bakker, D., Corver, J., Debast, S.B., Harmanus, C., Notermans, D.W., Bergwerff, A.A., Dekker, F.W., Kuijper, E.J., 2008a. Emergence of Clostridium Difficile Infection due to a New Hypervirulent Strain, Polymerase Chain Reaction Ribotype 078. Clin. Infect. Dis. 47, 1162-1170.

Goorhuis, A., Debast, S.B., van Leengoed, L.A., Harmanus, C., Notermans, D.W., Bergwerff, A.A., Kuijper, E.J., 2008b. Clostridium Difficile PCR Ribotype 078: An Emerging Strain in Humans and in Pigs? J. Clin. Microbiol. 46, 1157; author reply 1158.

Hookman, P., Barkin, J.S., 2009. Clostridium Difficile Associated Infection, Diarrhea and Colitis. World J. Gastroenterol. 15, 1554-1580.

Indra, A., Lassnig, H., Baliko, N., Much, P., Fiedler, A., Huhulescu, S., Allerberger, F., 2009. Clostridium Difficile: A New Zoonotic Agent? Wien. Klin. Wochenschr. 121, 91-95.

Jhung, M.A., Thompson, A.D., Killgore, G.E., Zukowski, W.E., Songer, G., Warny, M., Johnson, S., Gerding, D.N., McDonald, L.C., Limbago, B.M., 2008. Toxinotype V Clostridium Difficile in Humans and Food Animals. Emerg. Infect. Dis. 14, 1039-1045.

Kato, H., Kita, H., Karasawa, T., Maegawa, T., Koino, Y., Takakuwa, H., Saikai, T., Kobayashi, K., Yamagishi, T., Nakamura, S., 2001. Colonisation and Transmission of Clostridium Difficile in Healthy Individuals Examined by PCR Ribotyping and Pulsed-Field Gel Electrophoresis. J. Med. Microbiol. 50, 720-727.

Keel, K., Brazier, J.S., Post, K.W., Weese, S., Songer, J.G., 2007. Prevalence of PCR Ribotypes among Clostridium Difficile Isolates from Pigs, Calves, and Other Species. J. Clin. Microbiol. 45, 1963-1964.

Keessen, E.C., Donswijk, C.J., Hol, S.P., Hermanus, C., Kuijper, E.J., Lipman, L.J., 2011. Aerial Dissemination of Clostridium Difficile on a Pig Farm and its Environment. Environ. Res. 111, 1027-1032.

Kuijper, E.J., Coignard, B., Tull, P., ESCMID Study Group for Clostridium difficile, EU Member States, European Centre for Disease Prevention and Control, 2006. Emergence of Clostridium Difficile-Associated Disease in North America and Europe. Clin. Microbiol. Infect. 12 Suppl 6, 2-18.

McNamara, S.E., Abdujamilova, N., Somsel, P., Gordoncillo, M.J., DeDecker, J.M., Bartlett, P.C., 2011. Carriage of Clostridium Difficile and Other Enteric Pathogens among a 4-H Avocational Cohort. Zoonoses Public. Health. 58, 192-199.

Ozaki, E., Kato, H., Kita, H., Karasawa, T., Maegawa, T., Koino, Y., Matsumoto, K., Takada, T., Nomoto, K., Tanaka, R., Nakamura, S., 2004. Clostridium Difficile Colonization in Healthy Adults: Transient Colonization and Correlation with Enterococcal Colonization. J. Med. Microbiol. 53, 167-172.

Paltansing, S., van den Berg, R.J., Guseinova, R.A., Visser, C.E., van der Vorm, E.R., Kuijper, E.J., 2007. Characteristics and Incidence of Clostridium Difficile-Associated Disease in the Netherlands, 2005. Clin. Microbiol. Infect. 13, 1058-1064.

Sunenshine, R.H., McDonald, L.C., 2006. Clostridium Difficile-Associated Disease: New Challenges from an Established Pathogen. Cleve. Clin. J. Med. 73, 187-197.