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Short communication

Vermin on pig farms are vectors for *Clostridium difficile* PCR ribotypes 078 and 045

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ABSTRACT

Clostridium difficile is a gram positive, spore forming, toxin producing, anaerobic bacteria and an opportunistic pathogen for Man and many animal species, causing diarrhea in young piglets. Piglets probably become colonized from the environment. To investigate the possible spread and transmission of *C. difficile* by vermin, vermin samples were collected on a pig farm in the Netherlands and investigated for the presence of *C. difficile*. Samples of house mice ($n = 53$), drain flies ($n = 39$), lesser houseflies ($n = 95$), and yellow mealworms ($n = 11$) were found positive for *C. difficile* in 66%, 97%, 56% and 100% of cases respectively. *C. difficile* PCR ribotype 078 was found in all categories of vermin and ribotype 045 was found in two samples from the skeletal muscle of mice. House sparrows found dead on the premises ($n = 35$) and bird droppings ($n = 26$) were also investigated and carried *C. difficile* in 66% and 4% of cases respectively. PCR ribotype 078 was identified in bird and droppings samples but ribotype 045 was not. We conclude that vermin can play a role in the spread and transmission of *C. difficile* types 078 and 045 within pig farms and to other locations.

1. Introduction

Clostridium difficile is a gram positive anaerobic spore forming opportunistic enteropathogen for humans and animals, causing diarrhea under certain conditions, such as the use of antibiotics (Freeman et al., 2010; Keessen et al., 2010). *C. difficile* is found in the intestinal tract of many animal species, in soil, in water and on meat (Al Saïf and Brazier, 1996; Hopman et al., 2011). Toxins produced include TcdA (enterotoxin), TcdB (enterotoxin and cytotoxin) (Kuehne et al., 2010) and binary toxin (Keessen et al., 2010).

In the Netherlands PCR ribotype 078 is the most frequently found ribotype in neonatal piglets and the second most occurring ribotype in humans with CDI (12.7%) (Debast et al., 2009; Hensgens et al., 2011; Keel et al., 2007; Keessen et al., 2010; Koene et al., 2011). In view of genetic similarities between the human and pig ribotype 078, transmission between pigs and humans directly, via the air, or via meat seems possible but has not yet been established (Bakker et al., 2010; Keessen et al., 2011). It is known that vermin on pig farms play a role in the transmission of *Campylobacter* spp. (Meerburg et al., 2006), *Salmonella* spp. (Wang et al., 2011), *Mycobacterium* (Fischer et al., 2001), viruses (Blunt et al., 2011; Pitkin et al., 2009) and parasites (Förster et al., 2009). However, vermin have not yet been confirmed as potential sources of *C. difficile* infection.

The aim of this study was to survey vermin on a pig farm for the presence of *C. difficile*.

2. Materials and methods

2.1. Sample collection

Vermin samples were collected from May to September 2011, on a commercial pig farm The Tolakker at the Faculty

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Table 1
Frequency and location of animal species collected in the pig unit for *C. difficile* investigation.

Species found	Frequency and place
Rattus norvegicus B. (brown rat)	None found
Mus musculus L. (house mouse)	51 mice caught in the behavioral research unit where pigs were being housed for experiments and 2 caught in the feed store of the main building 6 samples of droppings
Psychoda alternate (drain fly)	39 samples containing hundreds of flies collected throughout the pig unit
Fannia canicularis L. (lesser housefly) (predominately) and *Drosophilidae* (fruitfly)	95 samples containing hundreds of flies collected throughout the pig unit
Tenebrio molitor L. (yellow mealworm beetle)	11 specimens found only in the hallway between the farrowing pens
Passer domesticus L. (house sparrow)	35 found in the pregnant sow ward and the behavioral research unit (both open to the air) 26 samples of droppings

of Veterinary Medicine at Utrecht University. Pigs on this farm were recently shown to carry *C. difficile* (Hopman et al., 2011). Only animals which are considered as vermin according to Dutch law (Flora-en faunawet, Article 4) were collected e.g. the house mouse (*Mus musculus* Linnaeus), the brown rat (*Rattus norvegicus* Berkenhout) and the black rat (*Rattus rattus* L.). Other species which are not desirable in the pig unit but which are protected by law, such as birds, were only collected if found dead inside the building (Table 1). Fresh droppings from birds and vermin were collected as well. Sample collection was performed using sterile plastic bags and plastic gloves. Care was taken to exclude the inclusion of dust from the floor as far as possible. Samples were stored at 4 °C or in a freezer (−18 °C) until analysis.

Plastic rat and mouse traps (Luxan traps, DCM Netherlands B.V. Katwijk, The Netherlands) were placed throughout the pig unit. The traps were placed with the baited end toward the walls, along the presumed route used by rats or mice and baited with banana, peanut butter or fried bacon. All types of bait were offered in all sections of the pig unit and traps were visited daily to collect cadavers and replace the bait. Flies insects were collected throughout the main pig production unit by means of adhesive fly papers (Roxasect, Vemedia B.V., Diemen, The Netherlands) and electric fly traps (Eurom) that were already present. Crawling insects were collected from the floor, avoiding the inclusion of dust as far as possible. Identification of vermin was carried out using a vermin classification handbook (Stichting Vakopleiding Ongediertebestrijding, 2001).

2.2. Investigating samples for the presence of C. difficile

After trapping, the mice were aseptically dissected into four different parts: the fur; the feet with tale and snout; gut and gut contents, and muscle tissue. Using a *C. difficile*-free pestle and mortar approximately one gram of material was ground. Detection of *C. difficile* in the samples was carried out using the method described by Hopman et al. (2011) with one modification. After transferral of a 2 ml portion of sample in enrichment broth to a sterile tube for the ethanol shock, the remainder of the sample was incubated a further 5 d. Samples were classed as negative for *C. difficile* if both portions gave a negative result. Colonies of Gram-positive rods with characteristic horse manure odor and typical morphology were identified as *C. difficile*. A selection of colonies were further identified and characterized at the National Reference Laboratory at Leiden, The Netherlands (Paltansing et al., 2007).

3. Results

Species collected in the pig unit were: *Psychoda alternate* (drain flies), *M. musculus* L. (house mouse), *Passer domesticus* L. (house sparrow), *Fannia canicularis* L. (lesser housefly), *Drosophilidae* (fruit fly), *Tenebrio molitor* L. (yellow mealworm beetle). No rats were caught. The frequency and location of the species found are presented in Table 1. In addition to these, six samples of mouse droppings were collected in the building and 26 samples of wild bird droppings were collected in the open area for sows.

Table 2
Results of *C. difficile* analysis of animals and droppings collected in the pig unit.

Species	Sample	Number of samples collected	Number (and percentage) of samples positive for *C. difficile*	*C. difficile* ribotype identified
M. musculus	Fur	53	27 (51%)	078: 13/13
	Paws, tail and snout	53	35 (66%)	078: 14/14
	Gut and gut contents	53	4 (8%)	078: 1/1
	Muscle tissue	53	17 (32%)	078: 2/4 045: 2/4
	Droppings	6	2 (33%)	078: 1/1
P. alternate		39	38 (97%)	078: 34/35
F. canicularis, *M. domestica*, *D. melanogaster*		95	53 (56%)	078: 34/34
T. molitor		11	11 (100%)	078: 9/9
P. domesticus	Specimens found dead	35	23 (66%)	078: 5/5
Wild bird	Droppings	26	1 (4%)	078: 1/1

All samples were checked for presence of *C. difficile* and the ribotype of a representative number of the positive samples was determined by PCR. The results are described in Table 2. Ribotype 078 predominated and was found in all categories of samples; mice (including muscle tissue), mouse droppings, all species of insects, dead birds and bird droppings. Ribotype 045 was found in two samples of skeletal muscle from mice.

4. Discussion and conclusion

The results presented in this study show for the first time that vermin are contaminated with *C. difficile*. Whether the vermin picked up *C. difficile* from the environment or directly from the pigs remains unclear. In any case it is clear that rodents, insects and wild birds found here represent a risk factor for colonizing later populations of pigs and other buildings, people and animals nearby. In plans to prevent colonization of piglets or pig farms by *C. difficile*, transmission via vermin has to be taken into account. It has recently been proposed that vermin on farms may function as an indicator of parasitic load in the production animals (Meerburg et al., 2012). Although it would be preferable to eradicate all vermin from pig production units, pests caught in traps in farm buildings may function as an indicator of *C. difficile* carriage by farm animals.

Previously it has been shown that dust in pig units can contain *C. difficile* (Keessen et al., 2011). It is therefore possible that dust from the floor contaminated samples and gave a false positive result for *C. difficile*, although samples were taken with utmost care to avoid this. Dust contamination cannot explain the *C. difficile* found in the gut contents and skeletal muscles of mice. Particularly the muscle infection seems surprising. Weese et al. (2011) has suggested that infection of muscle tissue may play a role in the infection of humans through positive meat products. Examining meat for contamination with *C. difficile* should therefore probably not only be aimed at *C. difficile* spores and bacteria on meat but also in meat.

C. difficile was found in all the different sample categories; therefore prevention of contamination of piglets via vermin is important. The most effective method of achieving this is to prevent vermin entering the pig unit by keeping doors and windows shut and to seal any openings in walls, doors and window sills. Fly screens or fly curtains can cover entrances if pigs have access to outdoor runs. If vermin are already present it should be eradicated. Furthermore, good hygienic practices should be implemented including an effective cleaning and disinfection schedule and preventing easy access to feed by vermin (Meerburg et al., 2007).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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