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Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics

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ABSTRACT

The objective of this study was to investigate the prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in people, pets and the environment in households with a pet with a clinical MRSP-infection within the past year. Personnel and the environment at veterinary clinics were also screened. Nasal swabs (humans), nasal and perineal swabs (pets) and environmental wipes were examined using selective culturing.

Twenty households were enrolled; 10/20 index cases still had clinical signs of infection at the start of the study and all were MRSP-positive. Of the remaining 10 index cases five were MRSP-positive in nasal and/or perineal samples. Five of 14 (36%) contact dogs and four of 13 (31%) contact cats were found MRSP-positive. In the households with an index case with clinical signs of infection 6/7 (86%) contact animals were MRSP-positive. MRSP was cultured from 2/45 (4%) human nasal samples. Domestic contamination was widespread as positive samples were found in 70% of the households and 44% of all environmental samples were MRSP-positive. In all but one of these MRSP-positive households the index case was still MRSP positive.

Among the personnel in veterinary clinics 4/141 (3%) were MRSP-positive. MRSP was cultured from 31/200 environmental samples in 7/13 clinics at the first sampling and in 3/6 clinics the environment remained MRSP-positive after cleaning and disinfection indicating that current cleaning procedures often were unable to eliminate MRSP.

These results show that transmission of MRSP between infected or colonized dogs and cats and healthy people does occur but is relatively uncommon, while transmission to contact pets occurs frequently, especially when the index case still has clinical signs of MRSP-infection.

1. Introduction

Recently it was clarified that *Staphylococcus pseudintermedius* and not *Staphylococcus intermedius* is the species of the *S. intermedius* group (SIG) which colonizes and causes infections in dogs and cats (Perreten et al., 2010). In the present manuscript we use the term *S. (pseud)intermedius* when isolates formerly identified as *S. intermedius* are probably *S. pseudintermedius.*

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged as a significant pathogen in companion animals (Weese and van Duijkeren, 2010). At the Veterinary Microbiological Diagnostic Center (VMDC), the Netherlands, the number of methicillin-resistant *S. pseudintermedius* (MRSP) isolates found in clinical samples from dogs and cats increased from 1 in 2004 to 76 in 2009.

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Many infections with methicillin-resistant *S. pseudintermedius* (MRSP) are (surgical) wound infections, but a variety of other infections like pyoderma, otitis externa and urinary tract infections are also associated with MRSP (Perreten et al., 2010; Ruscher et al., 2010; Weese and van Duijkeren, 2010). MRSP can also be cultured from healthy dogs and cats. The percentage of MRSP positive animals varies between 1.5 and 4.5% in dogs in the community or upon admission to veterinary hospitals, 0–7% in dogs with skin disease in the United States and 30% in dogs at a veterinary clinic in Japan (Sasaki et al., 2007; Hanselman et al., 2009; Weese and van Duijkeren, 2010). Human infections with methicillin-resistant *S. (pseud)intermedius* are rare, although several cases have been described (Gerstadt et al., 1999; Campanile et al., 2007; Kempker et al., 2009; Stegemann et al., 2010). In two cases, contact with dogs or cats was absent or not investigated (Gerstadt et al., 1999; Campanile et al., 2007); in one case the patient owned a dog, but no samples were taken from the dog (Stegemann et al., 2010), and only in the case described by Kempker et al. (2009) indistinguishable isolates were cultured from the patient and the patient's pet dog.

Transmission of methicillin-resistant *S. (pseud)intermedius* between humans and animals in a veterinary clinic has also been reported (van Duijkeren et al., 2008). Information on interspecies transmission and environmental contamination with respect to MRSP is scarce. The objective of this study was to determine the MRSP contamination/colonization of humans, pets and the environment in 20 households in which a pet was proven to be infected with MRSP in the previous 12 months and to similarly investigate the environment and personnel at veterinary clinics in which one or more pets had been diagnosed with a MRSP infection. In addition, the environmental contamination at these clinics was investigated, in some cases before and after cleaning.

2. Materials and methods

2.1. Part 1: Intrahousehold transmission and domestic contamination

In 2007 and the beginning of 2008, 20 patients with culture confirmed MRSP infections were selected at the VMDC of Utrecht University. Patients from 10 different veterinary clinics were included. The 20 households of these pets were visited. The inclusion criterion for this part of the study was the presence of a household pet (any age, gender or breed) in which a MRSP-infection had been diagnosed at the VMDC in the previous 12 months. The veterinary clinics involved permitted us to approach the owners. Households were recruited on a voluntary basis and written informed consent was obtained from all pet owners and the other household members. Nasal swab specimens were collected from consenting humans. Humans collected the samples themselves according to instructions that were provided. Nasal and perineal swab specimens were collected from all pets present in the households. If the index case still had signs of clinical MRSP infection a swab specimen from the infected area (wound, ear) was also taken. Samples from the animals were taken by veterinary students. Animals were regarded as MRSP-positive when at least one sample from the infection site, the perineum or the nose was MRSP-positive.

In each household, 5–8 environmental wipes were taken depending on the size of the household. They were taken from the sleeping place of the index case, the feeding site, the floor underneath the sofa, the door mat, the window-sill, the cupboard and sometimes from other additional sites like the staircase, the computer or the hall. The environmental wipes were taken by rubbing sterile gauzes (Cutisoft®, BSN Medical BV, Almere, the Netherlands) over a surface of approximately 10 cm × 30 cm using both sides of the gauze. Each wipe was taken wearing new sterile gloves to prevent cross-contamination, put into sterile containers and immediately transported to the laboratory.

2.2. Culturing

Samples were processed the same day. Swabs were plated directly onto sheep blood agar (Biotrading, Mijdrecht, The Netherlands) and thereafter put into a tube with 5 ml tryptone soya broth (TSB) containing 4% NaCl, 1% mannitol, 16 μg/ml phenol red, 50 μg/ml aztreonam and 5 μg/ml ceftizoxime. The containers with wipes were filled with 15 ml of this broth. After incubation for 48 h at 37 °C, 10 μl was plated onto sheep blood agar (Biotrading, Mijdrecht, The Netherlands). Suspect colonies were identified as members of the *S. intermedius* group (SIG) using standard techniques: colony morphology, Gram stain, catalase and coagulase and API ID 32 Staph (BioMérieux, Marcy l'Etoile, France). *S. pseudintermedius* isolates were identified using PCR-restriction fragment length polymorphism (RFLP) assay based on the *Mbo*I-digestion pattern of a PCR-amplified internal fragment of the *pta* gene as described (Perreten et al., 2010). Colonies suspect of being MRSP based on their resistance pattern (data not shown) were tested by PCR for the *mecA* gene (De Neeling et al., 1998).

2.3. PFGE

If MRSP isolates were found from different sources in the same household (e.g. from the index case, the contact animal and/or the environment) PFGE analysis using *Sma*I or *Cfr*9I as restriction enzyme was performed as described (Perreten et al., 2010). One isolate per source (index case, environment, contact animal) was included. PFGE patterns were analyzed using the BioNumerics software.

2.4. Part 2: Transmission and environmental contamination in private veterinary clinics

Thirteen private veterinary clinics in which at least one patient had been diagnosed with a MRSP infection in the past 12 months were enrolled in the study. Nasal swab specimens from all consenting personnel were taken by themselves.

To assess the environmental contamination, surface wipes of the environment (n = 9–27, depending on the size of the clinic) were taken as described in part 1. Samples

were taken from the doormat, the counter, the waiting room, the consultation room, the radiography department, the operating theatre, the balance, the laboratory and the hospitalization area. If two or more environmental samples were positive, the clinic was sampled a second time within one month after the first sampling and within 4 h after it had been cleaned and disinfected according their current protocol. Culturing and identification of the bacteria was done as described under part 1.

This study has been approved by the Institutional Ethics Committee on Animal Experiments DEC (2007.II.11.227).

3. Results

3.1. Part 1

3.1.1. Animals

3.1.1.1. Index cases. Of the total of 20 index cases 18 were dogs and two were cats. The index dogs had post-operative infections ($n = 11$), otitis externa ($n = 2$), pyoderma ($n = 1$), non-surgical wounds ($n = 1$), sepsis ($n = 1$), balanopostitis ($n = 1$) and a fistula ($n = 1$). The index cats had cystitis ($n = 1$) and otitis externa ($n = 1$). Thirteen of 18 index dogs and both index cats were still MRSP-positive when the investigation started (15/20 households) and in 4 of these households the index case had no signs of clinical infection anymore and in one case the index case with sepsis had died after the household was selected, but a few hours before the household was visited. In this case samples from the organs (liver and spleen) and perineum were collected post-mortem at the Department of Veterinary Pathology of Utrecht University. So, ten index cases (8 dogs and 2 cats) still had clinical signs of MRSP infection when the investigation started and all tested MRSP-positive (Table 1). The median time from first diagnosis to enrolment was 87.5 days (range 2–355) for all 20 index cases, 33 days (range 9–252) for index cases which still had clinical signs of infection when the study started and 237 days (range 31–355) for index cases without clinical signs of infection. The time from first diagnosis to enrolment was shorter for index cases with clinical signs of infection than for those without (Wilcoxon rank sum test $p < 0.05$).

3.1.1.2. Animals total. At the start, a total of 47 animals (29 dogs and 18 cats including the index cases) were present in the 20 selected households. Twenty-four of these animals (18 dogs and 6 cats) were MRSP-positive at one or more sampling sites.

3.1.1.3. Contact animals. Twenty-seven contact animals (14 dogs and 13 cats), were present in 13 households, whereas no contact animals were present in the other 7 households. A total of 5/14 (36%) contact dogs and 4/13 (31%) contact cats were found MRSP-positive in six households. In 5 of these 6 households the index case still had clinical signs of infection. In the 10 households with an index case with clinical signs of infection 2/2 contact dogs (100%) and 4/5 (80%) contact cats were MRSP-positive; in four other households no contact animals were present; in one household the contact cat was MRSP-negative. In households were the index case had no clinical signs of MRSP-infection anymore, no contact animals were MRSP-positive except for one household where the index case, a pup, had died from MRSP septicemia and 3/5 contact dogs were MRSP-positive. This difference was

Table 1
Summary of the results of the 20 households investigated in part 1 of the study.

Index case	Animal species	Clinical condition	Index case MRSP+/− at enrolment	Days from first diagnosis to enrolment	Clinical infection	Number of contact animals MRSP+/total	Number of humans MRSP+/total	Number of environmental samples MRSP+/total
1	Dog	Otitis externa	Positive	31	Yes	1/1	0/4	4/6
2	Dog	Postoperative wound infection	Negative	293	No	0/4	0/2	0/5
3	Dog	Postoperative wound infection	Negative	268	No	0/1	0/1	0/6
4	Dog	Postoperative wound infection	Positive	252	Yes	1/1	0/1	4/6
5	Dog	Postoperative wound infection	Negative	237	No	0/3	0/4	0/7
6	Dog	Postoperative wound infection	Positive	180	Yes	No	0/1	0/8
7	Dog	Postoperative wound infection	Negative	63	No	No	0/2	0/6
8	Dog	Postoperative wound infection	Positive	92	No	0/2	0/2	1/6
9	Dog	Postoperative wound infection	Positive	124	Yes	2/2	0/3	7/7
10	Dog	Wound	Positive	10	Yes	1/1	0/2	7/7
11	Dog	Otitis externa	Positive	35	Yes	No	No	6/7
12	Cat	Cystitis	Positive	11	Yes	No	2/2	6/7
13	Dog	Postoperative wound infection	Positive	355	No	0/4	0/1	3/7
14	Dog	Postoperative wound infection	Positive	253	No	0/1	0/5	0/7
15	Dog	Postoperative wound infection	Positive	15	Yes	No	0/2	4/6
16	Cat	Otitis externa	Positive	9	Yes	0/1	0/3	4/7
17	Dog†	Sepsis	Positive	2	†	3/5	0/3	2/5
18	Dog	Fistula	Negative	31	No	No	0/2	1/6
19	Dog	Pyoderma	Positive	83	Yes	1/1	0/2	4/8
20	Dog	Balanopostitis	Positive	106	No	No	0/2	5/7
MRSP+/total			15/20 75%			9/27 33%	2/45 4.%	58/131 44%

†: Died.

statistically significant ($p < 0.01$, Pearson's chi-square 17.7). None of the eight contact animals in a household in which the index case was MRSP-negative was MRSP-positive.

3.1.1.4. Humans. 2/45 (4%) of the human nasal samples were MRSP-positive. These two persons lived in the same household and their cat still had clinical signs of cystitis.

3.1.1.5. Domestic contamination. Positive environmental wipes were found in 14 (70%) households; in 13/15 (87%) households in which the index case was still MRSP-positive at least one wipe was MRSP-positive; in 9/10 (90%) households in which the index case still had a clinical infection at least one wipe was MRSP-positive. Only one wipe of 30 tested was MRSP-positive in households in which the index case was MRSP-negative.

A total of 58/131 (44%) environmental samples were MRSP-positive. The feeding site was found MRSP-positive in 11 households, the sleeping place in 9, the floor underneath the sofa and the doormat in 7, the window-sill in 4 and the cupboard in 2.

PFGE analysis showed that MRSP isolates from sources within the same household were highly similar (one or two bands difference) or indistinguishable. Four isolates from two households were non-typeable with *Sma*I, but showed indistinguishable *Cfr*9I patterns.

3.2. Part 2

A total of 141 personnel consented to participate in the study. Only four persons (3%) working in two private clinics (both two positive persons) were MRSP-positive.

Thirty-one of 200 (16%) of the environmental samples in 7/13 (54%) private clinics were MRSP-positive at the first sampling. Between 7% and 71% of the samples taken at the contaminated clinics were positive. After cleaning and disinfection according to their current protocols, 14 of 101 (14%) environmental samples at 3 of 6 (50%) clinics were still MRSP positive (Table 2).

All isolates described in parts 1 and 2 of the study were *S. pseudintermedius* as confirmed by RLFP-PCR and were *mecA* positive.

4. Discussion

The most important finding of this study was that humans living in a household with a pet with an ongoing or past MRSP infection and persons working in a veterinary clinic where MRSP-infected pets were treated were infrequently found MRSP-positive (6/188). The contact animals as well as the environmental samples, however, were frequently MRSP positive indicating that the household and clinic environments were contaminated and thus the exposure considerable. MRSP-positivity can be the result of contamination directly through physical contact with the index case or indirectly via the environment, or of true MRSP colonization. The current study set up did not discriminate between contamination and colonization. Thus, the MRSP-positive humans in this study may have been colonized but they can also be cases of nasal contamination through MRSP carrying dust particles as described for livestock-associated MRSA (Heederik et al., 2010) although the exposure to dust in barns is larger than in households or clinics. Our data suggest that the risk for humans of becoming colonized in an MRSP-contaminated environment is relatively small or that they eradicate MRSP colonization more quickly than dogs or cats. This corroborates with the scarcity of reports on human clinical *S. (pseud)intermedius* infections (Mahoudeau et al., 1997; Van Hoovels et al., 2006; Weese and van Duijkeren, 2010). It must be noted, however, that the number of persons owning a pet is very high in Europe and the United States of America and the number of MRSP positive animals is increasing, so therefore the total number of persons a risk is considerable. In addition, five dogs were MRSP-negative when the study started, and therefore the risk of transmission of MRSP to humans in the same household might have been underestimated. The high MRSP positivity in the contact pets can partly be explained by the high

Table 2
Summary of the results of the 13 private clinics investigated in part 2 of the study.

Private clinic Nr.	Number of MRSP+ patients within last 12 months	MRSP first sampling of the environment	Number of MRSP+ samples/ total number of samples	Second sampling of the environment	Number of MRSP+ samples/ total number of samples	Number of MRSP-positive personnel/ total number of personnel tested
1	21	+	4/21	–	0/10	2/10
2	3	+	4/15	–	0/17	0/12
3	1	–	0/9	NT		0/8
4	2	–	0/17	NT		0/6
5	5	–	0/27	NT		0/14
6	3	+	2/19	+	2/17	0/24
7	1	–	0/14	NT		0/8
8	1	–	0/10	NT		0/6
9	1	+	1/15	NT		0/17
10	4	+	12/17	–	0/17	2/9
11	6	+	2/10	+	7/25	0/6
12	1	+	6/14	+	5/15	0/16
13	1	–	0/12	NT		0/7
MRSP+/total		7/13 (54%)	31/200 (16%)	3/6 (50%)	14/101 (14%)	4/143 (3%)

NT: not tested. +: MRSP-positive; – MRSP-negative.

level of contamination of their environment. At the same time, it is likely that part of the contact animals were actually colonized because perineal samples from the dogs were frequently found positive. The perineum is thought to be the primary colonization site for *S. pseudintermedius* (Devriese and De Pelsmaecker, 1987; Fazakerley et al., 2009). It should be noted that dogs and cats are natural hosts for *S. pseudintermedius* while humans are not (Talan et al., 1989) and this may in part explain the relative ease with which contact animals become colonized with MRSP compared to humans. In addition, the owners of the dogs knew that there pet was infected with MRSP at the start of the study and might have taken precautions, like washing their hands after contact with the index cases, to prevent transmission.

Contact dogs and cats living in a household with an index case that still had clinical signs of MRSP infection were more often MRSP-positive than contact animals in households with an index case without clinical signs of infection ($p < 0.01$) and both MRSP-positive humans lived in a household with a cat with cystitis. As expected, time from first diagnosis to enrolment was shorter for animals which still had clinical sigs of infection during the study compared to animals which had no clinical sign of infection. Hanselman et al. (2009) investigated the prevalence of MRSP in humans and their household pets and found only 1/242 humans (0.4%) to be MRSP-positive, but it must be noted that the pets in this study had no history of MRSP infection or colonization and only a small percentage of these pets were found to be MRSP-positive. A higher percentage of MRSP-positive humans (14%) was found in a study investigating owners of dogs with recurrent pyoderma (Frank et al., 2009). Guardabassi et al. (2004) who found that the occurrence of *S. (pseud)intermedius* in dog-owners of dogs with deep pyoderma was significantly higher compared to humans without dog contact. Together these data indicate that persons in contact with dogs with clinical methicillin resistant (and methicillin susceptible) *S. pseudintermedius* infections are at higher risk of getting colonized or contaminated than persons in contact with healthy dogs or persons without dog contact.

Finding MRSP in sites where there is little or no physical contact with the index case or contact pets (e.g. the floor underneath the sofa) indicates that dust particles (e.g. hairs, epithelial cells) carry MRSP to those sites. Notably, pets produce a lot of dust particle like hairs, especially during the shedding season. The presence of MRSP isolates with similar or indistinguishable PFGE patterns in index cases, contact animals and environmental wipes in the same household suggests that transmission and contamination occurs within the household. All isolates from two households could not be digested by *Sma*I but were digested by its neoshizomeer *Cfr*9I, which cuts at the same recognition site GGGCCC, but at a different position. Whether this is the result of DNA-methylation as described for MRSA ST398 by Bens et al. (2006) remains to be investigated.

In households with MRSP-negative animals only 1/30 (3%) environmental samples yielded MRSP which shows that most MRSP-contamination disappears over time, probably as a result of repeated cleaning, but also as a result of natural decline.

Persons working at a clinic in which at least one pet had been diagnosed with a MRSP infection infrequently tested MRSP-positive (3%). This is in accordance with data of Morris et al. (2010), who found 9/171 (5%) veterinary dermatology clinic staff MRSP-positive and those of Ishihara et al. (2010) who found 13/219 (6%) veterinarians, students and staff members at a Japanese veterinary teaching hospital to be MRSP-positive. Boost et al. (2009) investigated 150 veterinary personnel at 22 veterinary practice in Hong Kong and found only one person (0.6%) MRSP-positive. These differences can be explained by local difference in MRSP prevalence in companion animals, difference in the human populations studied (e.g. staff of teaching hospital versus private clinics; clinics with known MRSP history compared to those selected at random), and differences in the sampling and culture methods used.

In conclusion, our results show that humans under exposure infrequently become MRSP-positive. However, it must be noted that persons colonized with MRSP may have a higher risk of developing MRSP infections in case of surgical or non-surgical wounds. Whether MRSP-positive humans are really colonized or merely contaminated remains to be investigated. Pets exposed to MRSP become colonized more easily, especially when they are in contact with pets with clinical MRSP infections; whether this is long-term colonization remains to be investigated.

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