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ORIGINAL RESEARCH

Effect of topical antimicrobial therapy and household cleaning on meticillin-resistant *Staphylococcus pseudintermedius* carriage in dogs

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Abstract

Background: Meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a multidrug-resistant canine pathogen with a low zoonotic potential. This study investigated MRSP carriage and clearance through topical antimicrobial therapy and household cleaning in dogs recovered from MRSP infection.
Methods: Dogs were swabbed for MRSP carriage; household contamination was assessed using contact plates. Carrier dogs were allocated randomly to receive topical fusidic acid and chlorhexidine/miconazole treatment combined with owners implementing a household hygiene protocol (H&T) or implementation of hygiene alone (H) over three weeks. Carriage-negative dogs were monitored monthly. The relatedness of isolates over time was investigated by pulsed-field gel electrophoresis (PFGE).
Results: At inclusion, MRSP carriage was confirmed in 31/46 (67.4%) index dogs and 16/24 (66.7%) contact dogs, and contamination was found in 18/40 (45%) environments. In dogs completing all cycles, interventions cleared carriage in 5/9 (55.6%) dogs in group H&T and 2/6 (33.3%) in group H. Environmental contamination was infrequent but associated with carrier dogs ($p = 0.047$). Monthly monitoring of initially negative dogs showed intermittent carriage in 9/14 dogs. PFGE-concordance was found among all 34 MRSP isolated from eight index dogs over time.
Conclusion: MRSP carriage was common in dogs after recovery from infection. Topical antimicrobial therapy temporarily eliminated carriage but recurrence was frequent. Management efforts must include the prevention of recurrent infections and hygiene.

KEYWORDS
antimicrobial resistance, decolonisation, infection control, pyoderma, zoonosis

INTRODUCTION

Meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) is recognised in many countries as one of the major multidrug-resistant bacterial pathogens affecting dogs. Associated challenges for small animal practice include difficulties in treating some infections as isolates are typically resistant to most or all clinically relevant antimicrobial drugs authorised for systemic use. Furthermore, MRSP appears to follow a veterinary nosocomial epidemiology with easy spread within veterinary facilities, and last, its zoonotic potential, although low, requires comprehensive owner education.[1]

Staphylococcus pseudintermedius, irrespective of its drug resistance, belongs to the normal canine microbiota and colonises the skin and mucosae of most dogs. MRSP carriage (identified through single sampling events rather than repeatedly over time as required to determine true colonisation) has been reported in less than 10% of healthy dogs in various screening studies[2–6] but is thought to be higher when preceded by MRSP infection.[7,8] Asymptomatic carrier dogs will contribute to the spread of MRSP through direct transmission on contact with other dogs and humans and indirectly through contamination of environments. In addition, MRSP carriage poses a risk to hosts themselves as forthcoming infections

may be complicated by this multidrug-resistant bacterium. In humans, *S. aureus* nasal carriage has long been recognised as a major risk factor for subsequent *S. aureus* infection[9] and has become an area of extensive research in efforts to prevent meticillin-resistant *S. aureus* (MRSA) infections. A similar link between carriage and infection has been proposed for *S. pseudintermedius* from dogs where molecular analyses showed that up to 80% of isolates from superficial pyoderma were identical to those from carriage sites,[10] and MRSP carriage was a risk factor for the development of surgical site infections in a study of 549 dogs admitted for tibial plateau levelling osteotomy.[11]

Strategies for the decolonisation of carriers, which in the context of MRSA human carriage refers to the elimination of MRSA from carriage sites, could therefore be useful in the management of MRSP carriers in veterinary settings with potential benefits for clinical outcomes, infection control measures and for prevention of zoonotic infections with multidrug-resistant bacteria. Protocols for decolonisation in human medicine focus on topically applied antimicrobial agents and biocides, for example, mupirocin and chlorhexidine,[12] sometimes in combination with systemic therapy. However, despite substantial research efforts, the value of decolonisation measures for MRSA carriage in humans remains controversial and assessment is hampered by a lack of results from comparable studies and by insufficient information that takes into account the complexities of transmission, for example, contact people and environments.[13] Of particular concern is that even after successful clearance, nasal re-colonisation frequently occurs within weeks to months.[14] In one longitudinal study, 58% of 137 participants were initially successfully decolonised but only 32% had remained MRSA-negative 12 months later.[15] Also, interpretation of efficacy studies is complicated by the occurrence of spontaneous or natural resolution of carriage in untreated groups.[16] Natural decolonisation is likely related to a fitness cost from multidrug-resistance to staphylococci whereby MRSA (and potentially MRSP) might be displaced by less resistant, fitter counterparts provided no further selection pressure from antimicrobial therapy exists.[17]

In veterinary medicine, no studies on MRSP decolonisation strategies have been published to date. *S. pseudintermedius* could be eliminated from carriage sites of healthy beagles by twice daily application of a fusidic acid gel but skin populations increased again within a week of cessation of therapy while mucosal populations remained lower for longer[18]; the same gel combined with chlorhexidine washes was used to eliminate MRSA carriage in two dogs in an animal-shelter setting.[19] Whether dogs can lose MRSP carriage spontaneously remains unclear, but earlier studies have indicated that MRSP carriage in healthy dogs may be intermittent[8] and can also persist for over a year after the infection has resolved.[20]

The aim of this longitudinal study was to identify MRSP carriage in dogs recovered from MRSP infection and investigate the efficacy of topical antimicrobial therapy combined with environmental cleaning in eliminating MRSP from the skin and mucosal sites of carrier dogs.

MATERIALS AND METHODS

Ethics and enrolment

The study was approved by the Royal Veterinary College Ethics and Welfare Committee (URN 2012 1166). Written informed consent was obtained from owners at enrolment.

Enrolment criteria for 'index dogs' were that (a) MRSP had been isolated by a veterinary diagnostic laboratory (based on a characteristic susceptibility pattern and speciation from either MALDI-TOF (matrix-assisted laser desorption ionization time of flight) or phenotypic identification) from a clinical sample submitted for diagnostic investigation (any infection type), (b) clinical signs of bacterial infection had resolved, (c) antimicrobial therapy had been discontinued (systemic: at least one week; topical: at least two days before inclusion sampling). Other dogs living in the same household were enrolled as 'contact dogs', irrespective of their health conditions.

Veterinarians were invited to initially discuss the study aim and requirements with owners and assess if owners were willing to present their dog(s) to the practice for multiple sampling visits for an up to six-month period, to follow a household-sampling and a household-cleaning protocol and to apply topical medication to their dog(s). For the purpose of this study, 'household' refers to environmental surfaces only and does not include humans or other non-canine species living in the house.

All study material including treatments, sampling materials, stamped and addressed return packages for samples, and paperwork was supplied free of charge to practices; study medication was prescribed and labelled by the dog's veterinary surgeon. Time for sampling provided by the veterinary staff was accepted as 'good will' and in support of infection control.

Study design and group allocation

Two interventions were tested prospectively in a non-blinded design for their ability to eliminate MRSP from dogs' carriage sites. One consisted of topical antibacterial therapy for carrier dogs combined with a household hygiene protocol (group H&T), the other tested the household hygiene protocol alone (group H).

Index dogs, if their inclusion carriage sampling had yielded MRSP, were allocated randomly (www.random.org), together with their contact dogs and household, to one of the two intervention groups. Index dogs were also allocated to an intervention group if they had sampled negative for MRSP themselves but if one or more of their contact dog(s) were positive. MRSP carriage-negative index dogs (provided their contact

dogs were also negative) were monitored by sampling as close to monthly as possible (group MM) for six months (or longer if compliance was good). At the end of an intervention, owners were offered for their dogs to cross over into either the other intervention group if a dog (index or contact) still sampled positive for MRSP or to the MM group if the last carriage sample had been negative.

After group allocation, subsequent sampling material and relevant medication were posted to the practice fresh to reduce the risk of degradation of contact plates. Up to two telephone or email reminders to the practice were scheduled if samples were not returned after two weeks.

Interventions

In both groups, interventions were prescribed as three seven-day cycles, separated by six treatment-free days according to a study calendar (Supplement 1).

In group H&T, index and contact dogs received topical antibacterial therapy using medication authorised for use in dogs in the United Kingdom, commonly prescribed for staphylococcal infections and containing active ingredients with proven efficacy against *S. pseudintermedius* including meticillin-resistant staphylococci in vivo.[19,21] Fusidic acid (Isathal, Dechra Veterinary Products Ltd. [DVP]) was applied twice daily to mucosal sites (both nostrils, eyes, prepuce or vulva, anus); dogs were washed with a 2% chlorhexidine/2% miconazole shampoo (Malaseb, DVP) on Days 1 and 4 of the cycle with a 10-min contact time (Supplement 2). In addition, owners were advised to follow a household hygiene protocol (Supplement 3) that included both daily and weekly procedures aiming for: (1) mechanical removal of debris through vacuuming and detergent cleaning and (2) use of bleach, where appropriate, at a recommended concentration of 0.25% (2.5 ml bleach/1L water) adapted from protocols on dermatophytosis in animal shelters and on MRSA in bathwater[22,23]; owners were also asked to (3) reduce physical contact with their dog and (4) practice good hand hygiene during the study period.[24,25]

For dogs in group H, owners were asked to follow the household hygiene protocol only.

Sampling

Carriage swabbing was performed at or outside veterinary practices by veterinary surgeons or nurses using the material provided by post. Six skin and mucosal sites (Supplement 4) were sampled for 5 s each using separate dry Amies charcoal transport cotton swabs (SLS). The household environment was sampled by owners guided by an instructional poster (Supplement 5) to apply paired contact plates (55 mm; Fisher Scientific), containing either mannitol salt agar (MSA; CM0085, Fisher Scientific) or MSA with 6 mg/L oxacillin (MSA+; oxacillin sodium salt, Sigma-Aldrich Ltd.) to each of five sites for 5 s. Swabs and plates were returned to the investigators in the postal box provided.

Microbiology

On arrival at the RVC, swab tips were immediately suspended and incubated in separate vials of 5 ml tryptone soya broth (CM0129, Fisher Scientific) containing 10% sodium chloride (99.5%; Sigma-Aldrich Ltd.) at 37°C for 48 h before streaking onto MSA and MSA+. Contact plates were incubated at 37°C for 48 h. Presumptive MRSP were phenotypically identified based on initial morphology on MSA+, subsequent haemolysis, coagulation ability, DNase and Vogues-Proskauer test results from a growth on 5% sheep blood agar (Oxoid; TCS Bioscience). Owners and practices were informed of the result at this stage. Isolates were subsequently confirmed genotypically through the presence of *mecA* and *S. intermedius*-group-specific *nuc* using PCR as previously described.[26]

To investigate the genetic relatedness of MRSP over time, isolates from index dogs, collected at least three months apart from a dog, were compared using pulsed-field gel electrophoresis (PFGE) following the Harmony protocol [27] with few modifications. Briefly, bacterial DNA embedded in agarose plugs was digested with *Sma*I or its neoschizomer *Cfr*9I. Plug slices were run using a Chef DR III system (Bio-Rad, Feldkirchen) to separate, visualise and compare patterns of bacterial DNA digests between isolates. The run program consisted of a switch time of 5 to 15 s for 9 h for the first block, followed by a switch time of 15 to 60 s for 11 h and 42 s for the second block, at a gradient of 5,6 V/cm and an included angle of 120° (for a total running time of 20 h and 42 s). *Sma*I-digested *S. aureus* NCTC8325 and the MidRange PFG marker I (New England Biolabs) were used as markers.

Outcome measures and statistical analyses

A dog was considered an MRSP-carrier if at least one of the six carriage swabs yielded MRSP, irrespective of the site. A household was defined as contaminated if MRSP was isolated from at least one of the five contact plates.

Results from carriage swabs and environmental samples were analysed as percentages of total numbers of index dogs, contact dogs and environments sampled at the different occasions. Carriage results over time were recorded as carrier indices (as adapted from 42), calculated using the total number of samples for group MM but excluding inclusion samples for groups H&T and H.

The efficacy of the two interventions was assessed by comparing the number of MRSP isolations before

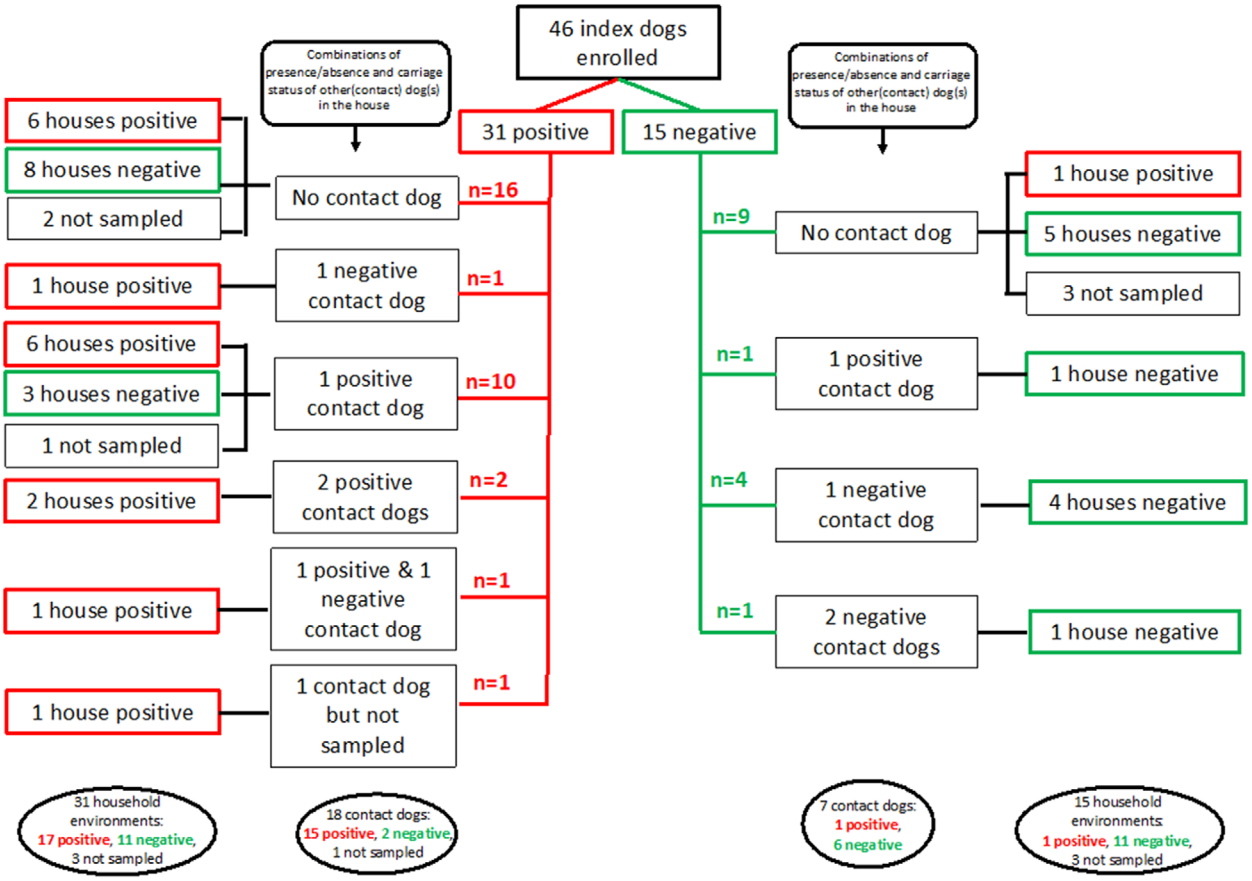

Figure x: Isolation of MRSP (positive or negative) from inclusion samples (carriage site swabs and contact plates) of 46 index dogs that had recovered from MRSP infection, from 25 contact dogs sharing the house with an index dog and from their household environments.

FIGURE 1 Isolation of Meticillin-resistant *Staphylococcus pseudintermedius* (MRSP; positive or negative) from inclusion samples (carriage sites and contact plates) of 46 index dogs that had recovered from MRSP infection, from 25 contact dogs sharing the house with an index dog and from their household environments

and after for dogs receiving the respective intervention for at least one cycle.

Data were collected, analysed and plotted using Microsoft Excel v.1808 and IBM SPSS Statistics v26. Frequencies were compared using two-tailed Fisher's exact tests with a p-value of < 0.05 to indicate significance.

RESULTS

Enrolled dogs and households

Between May 2016 and January 2020, inclusion boxes were posted to veterinary practices for 55 dogs. Material was returned for 46 of them (participation rate 83.6%); five dogs were lost to follow-up, one dog died before samples could be taken, one developed heart problems that prevented sampling and two dogs were excluded after review of medical notes showed that the original MRSP had been isolated from carriage sites. At the end of the study, results from a total of 120 sampling events involving 46 index dogs were available for analysis with a mean observation period of 3.9 months (range one to 42 months, median one month). Among those, longitudinal sampling was ended prematurely due to Covid-19 (Coronavirus disease 2019) related restrictions for 10 index dogs (two in H&T, four in H, four in MM).

At the time of enrolment, index dogs had a mean age of 5.6 years (range 0.5–13.3 years), 19 were female (41.3%; 9 entire, 10 neutered) and 27 male (58.7%; 7 entire, 20 neutered) and 43 dogs represented 26 pure breeds, while three dogs were crossbred. Types of MRSP infections that had resolved before study begin were surface and superficial pyoderma ($n = 16$), deep pyoderma ($n = 9$), otitis ($n = 7$), surgical complications and traumatic wounds ($n = 8$) and other infections ($n = 6$, four of them involving eyes). Twenty-five index dogs were single dogs in their households, 17 lived with one contact dog and four with two (25 contact dogs).

MRSP from inclusion samples and subsequent group allocation

Inclusion samples yielded MRSP from 31/46 (67.4%) index dogs, from 16/24 (66.7%) contact dogs and from 18/40 (45%) households (Figure 1). MRSP contamination of the environment was associated with the number of MRSP-positive dogs in the house

(p = 0.047), where 20% (8/40) of households containing one MRSP-positive dog yielded environmental contamination, but 46% (11/24) with more than one MRSP-positive dog had MRSP isolated from environmental sites.

In total, 32 index dogs (31 yielding MRSP from their inclusion sample themselves, plus one carriage-negative index dog where only the contact dog carried MRSP) were randomly allocated to one of the two intervention groups resulting in 16 dogs in group H&T and 16 in group H. The remaining 14 index dogs and their respective six contact dogs underwent monthly sampling (monthly monitoring, MM).

Eight index dogs were later crossed over into the respective other intervention group after they had remained MRSP carriers after one intervention or into the monitoring (MM) group following a negative carriage sample. Six of those dogs crossed over between groups once, two dogs twice (Table S1).

Clearance of MRSP through interventions

In group H&T, 18 index dogs (16 allocated, two crossed over from group H) started the intervention. Nine dogs completed all three treatment and hygiene cycles, and five of them (55.6%) sampled negative for MRSP carriage at their last sampling events (Table 1). A further two dogs that left the study early (one lost to follow-up after one cycle, the other after two cycles due to Covid-19 restrictions) sampled negative at their respective last sample and without detected household contamination. For seven MRSP-positive index dogs, no samples were received beyond inclusion (five lost to follow-up, one died, one due to Covid-19 restrictions). No adverse reactions from treatment or compliance problems were reported by any of the owners.

In group H, 21 index dogs started (16 allocated and five after crossing over from either group H+T (n = 4) or from group MM (n = 1)), six completed all three cycles, and two of them (33.3%) sampled negative for MRSP carriage at the end of the intervention (Table 2). Another four dogs (one with a contact dog) completed two cycles and all five dogs (index and contact) yielded MRSP from at least one of the two samples, two dogs from both; their environments were all negative after the second cycle (three dogs were lost to follow-up, the fourth could not be sampled due to Covid-19 restrictions). For the remaining seven dogs, no further samples were received.

Environmental contamination was infrequent in both intervention groups during the cycles (9/36, 25% in H & T; 7/24, 29.2% in H), and overall, the environment was found not contaminated in 32/60 (53.3%) sampling events, despite at least one carrier dog identified in the house; in the reverse, a contaminated house without concurrent isolation of MRSP from dogs was found once. Although fewer households (H&T and H combined) showed contamination after the three cycles (2/15, 13.3%) than at inclusion (5/15, 33.3%), this change was not significant (p = 0.2).

The lasting absence of MRSP from carriage samples was infrequent in dogs that had carried MRSP at inclusion. Three dogs yielded negative samples on three consecutive events (two in H&T, one in H), a further three dogs sampled negative on two consecutive events (one in H&T, two in H), but MRSP was subsequently recovered again from all.

Monthly monitoring

Eighteen carriage-negative index dogs (14 at inclusion, four after crossing over) were enrolled into group MM (Table 3). Four dogs were lost to follow-up after inclusion, for another four dogs, only one further sample was received.

Results from the 10 index dogs were available for a total of 47 sampling events covering between three and 12 months (mean 5.8 months; Table 3). Two dogs, both free of clinical signs of MRSP infection for less than one month at inclusion, remained MRSP-free throughout their observation periods of 12 months, compatible with a non-carrier status. Another two index dogs, also enrolled within one month of resolution of infection, yielded MRSP once during their observation periods and were classified as occasional carriers, while the remaining six index dogs, free of infection for between one and 10 months (mean 4.3 months) showed intermittent carriage.

Genetic relatedness of MRSP isolated over time

Thirty-four MRSP isolates, originating from eight index dogs, isolated at least three months apart from the inclusion isolate, were analysed. Within each household, PFGE patterns of isolates were either indistinguishable or closely related to each other over time (Figure 2).[28]

DISCUSSION

With a total of 120 sampling events including at least 11 samples each and spanning observation periods of between one and 12 months, this is the largest longitudinal study on MRSP carriage to date and the first to describe the effects of topical antimicrobial therapy on MRSP carriage in dogs recovered from MRSP infection. While clinical studies remain challenging due to the extra demand on clinician's efforts beyond disease management, the high participation rate of 84% at inclusion was thought to reflect a growing awareness and concern about antimicrobial resistance. However, despite the initial enthusiasm during enrolment, subsequent drop-outs and a lack of control over adherence to treatment and hygiene instructions remain

TABLE 1 MRSP isolation from nine index dogs, eight contact dogs and their environments at inclusion and after three cycles of hygiene and topical antibacterial treatment (group H&T)

	Inclusion sample			After Cycle 1			After Cycle 2			After Cycle 3			Index dog	
Study number	Index dog	Contact dog(s)	House	Index dog	Contact dog(s)	House	Index dog	Contact dog(s)	House	Index dog	Contact dog(s)	House	Carrier index	Carriage pattern***
1	1	–	0	0	–	0	0	–	0	0	–	0	0	Cleared
2*	1	1; 1	0	0	0; 0	0	0	0; 0	0	0	0; 0	0	0	
3*	1	1; 1	0	0	0; 0	1	1	1; 1	1	1	0; 0	0	0.67	Intermittent
4	1	1	0	1	0	0	1	1	0	0	1	1	0.67	
5	1	1	0	0	1	0	1	1	1	0	1	1	0.33	
6	1	–	0	1	–	0	0	–	0	0	–	0	0.33	
7	1	1	1	1	1	1	1	1	0	1	1	0	1	Persistent
8	1	1	1	1	1	0	1	1	1	1	1	0	1	
9**	1	–	0	1	–	0	1	–	0	1	–	0	1	
Positive samples/ total number of samples	9/9	8/8	2/9	5/9	3/8	2/9	6/9	6/8	3/9	4/9	4/8	2/9		

Note: 1: positive; 0: negative; –: not sampled or no contact dog present.
Abbreviation: H&T, household hygiene protocol.
*Two contact dogs in the household.
**Dog(s) crossed over from another group and then completed the second intervention.
***Definition of carriage pattern for this study (for dogs where more than two samples were available) adapted from Eriksen et al. 1995.[42]

TABLE 2 MRSP isolation from six index dogs, five contact dogs and their environments at inclusion and after three cycles of hygiene measures (group H)

	Inclusion sample			After Cycle 1			After Cycle 2			After Cycle 3			Index dog	
	Index dogs	Contact dog(s)	House	Index dog	Contact dog(s)	House	Index dog	Contact dog(s)	House	Index dog	Contact dog(s)	House	Carrier index	Carriage pattern***
5**	0	1	1	0	Not sampled	0	0	Not sampled	0	0	1	0	0	Clear
3*, **	1	1; 1	1	1	1; 1	0	0	1; 1	0	0	0; 1	0	0.33	Intermittent
10**	1	0	0	0	0	0	0	Died	0	1	Died	0	0.33	
8**	1	1	1	1	1	1	1	1	1	1	1	0	1	Persistent
9	1	–	0	1	–	1	1	–	0	1	–	0	1	
11	1	–	0	1	–	0	1	–	1	1	–	0	1	
Positive samples/ total number of samples	5/6	4/5	3/6	4/6	3/4	2/6	3/6	3/3	2/6	4/6	3/4	0/6		

Note: 1: positive; 0: negative; –: not sampled or no contact dog present.
Abbreviation: H, hygiene alone.
*Two contact dogs in the household.
**Dog(s) crossed over from another group and then completed the second intervention.
***Definition of carriage pattern for this study (for dogs where more than two samples were available) adapted from Eriksen et al. 1995.

TABLE 3 MRSP isolation from longitudinal carriage sampling of 18 index dogs in the monthly monitoring (MM) group and findings from contact dogs and environments

		Index dogs									Contact dogs	Environment
Dog study number	**Monitoring period (months)**	**Time of sampling and number of MM events**							**Carrier index**	**Carriage pattern*****	**MRSP positive samples/all samples**	**MRSP positive samples/all samples**
		Inclusion	1	2	3	4	5	6				
12	12	0	0	0	0	0	0	0	0	Non-carrier	0/7*	0/7
13	12	0	0	0	0	0	0	–	0	Non-carrier	0/6	0/6
5**	5	0	0	0	1	0	0	–	0.17	Occasional	0/6	0/6
6**	4	0	0	0	1	0	–	–	0.2	Occasional	–	0/5
9**	4	0	1	1	1	1	–	–	0.8	Intermittent	–	0/5
14	4	0	0	1	1	–	–	–	0.5	Intermittent	–	1/3
15	7	0	0	1	1	–	–	–	0.5	Intermittent	–	4/4
2**	3	0	1	1	0	–	–	–	0.5	Intermittent	2/4*	1/4
16	4	0	1	1	–	–	–	–	0.67	Intermittent	–	0/3
10	3	0	1	1	–	–	–	–	0.67	Intermittent	0/2	0/2
17	1	0	1	–	–	–	–	–	Observation period was too short		–	0/2
18	4	0	0	–	–	–	–	–		0/2	0/2	
19	3	0	0	–	–	–	–	–		0/2	0/1	
20	1	0	0	–	–	–	–	–		–	0/2	
21	0	0	–	–	–	–	–	–		–	0/1	
22	0	0	–	–	–	–	–	–		–	–	
23	0	0	–	–	–	–	–	–		–	–	
24	0	0	–	–	–	–	–	–		–	0/1	

Note: 1: positive; 0: negative; –: not sampled or no contact dog present.
*Two contact dogs in the household, results for the two contact dogs identical on each occasion.
**Dog(s) crossed over from another group after their last intervention sample was negative for MRSP (= Inclusion sample for MM).
***Definition of carriage pattern for this study (for dogs where more than two samples were available) adapted from Eriksen et al. 1995.

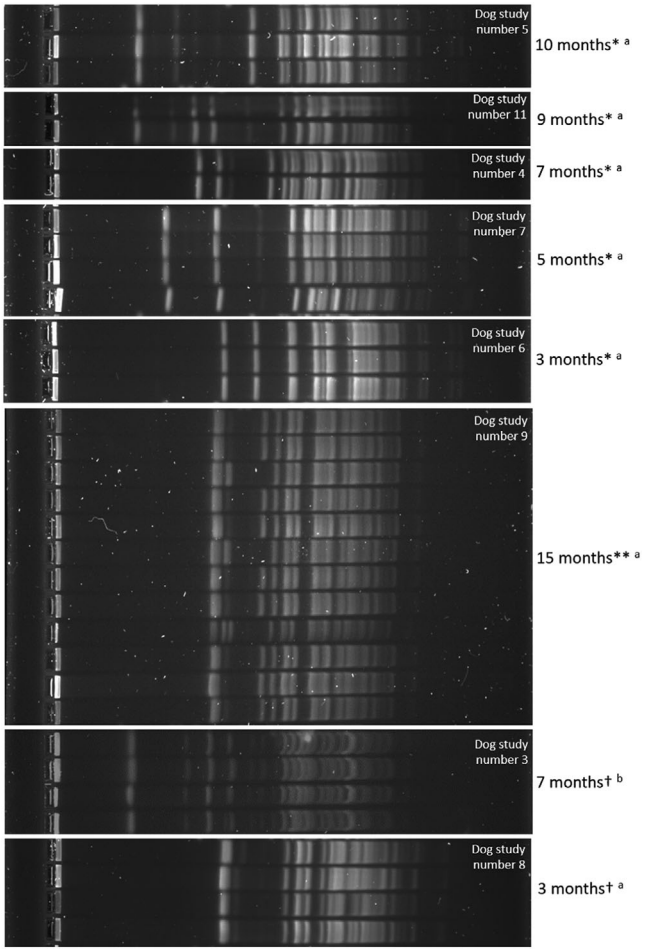

FIGURE 2 Pulsed-field gel electrophoresis (PFGE) patterns of 34 MRSP isolate from eight index dogs sampled over time, digested with [a] *Sma*I or [b] *Cfr*9I. A *Sma*I-digested *S. aureus* NCTC8325 and the MidRange PFG marker I (New England Biolabs) were used for reference but are not shown. Intervals between sampling events spanned at least three months and are detailed on the right. PFGE patterns are designated as identical (*), identical at the start and end of the sampling period with other closely related patterns in between (**) or closely related (†)

notable limitations of this study and reflect the difficulties associated with long-term compliance required for longitudinal studies.

Important findings from this study include the high MRSP prevalence rates of 67% in both index and contact dogs at inclusion. This was higher than the 48% reported in 102 dogs that had recovered from superficial MRSP pyoderma[7]; the rates were also higher than the 26% in 16 index dogs and 19% of their contact animals in another study especially since index dogs during that study continued to show clinical signs of MRSP infection concurrently.[8] These differences appear substantial and may be explained by more sensitive sampling methods of swabbing six sites in our study, compared to three and two sites in the other two studies.

Household contamination with MRSP was also high with 45% of households yielding MRSP, compared to 18% in Laarhoven's study where samples were collected over a six-month period with MRSP carrier dogs in the house and without hygiene interventions.[8] In both these studies, MRSP was even isolated when dogs sampled negative at the same event. These seemingly inconsistent findings are thought to reflect survivability of staphylococci on surfaces, shown for MRSA to exceed 12 months.[29] Hygiene interventions alone had little impact on MRSP contamination, indicating that the presence of carrier dogs was the determining factor for isolation of MRSP from environmental surfaces and that sampling dogs should be prioritised over sampling environmental surfaces for clinical purposes, especially when funds for diagnostic tests are limited. Despite the missing evidence for the efficacy of hygiene measures alone in this study though, recommending cleaning and disinfection for households with MRSP infected or carrier dogs cannot be wrong. Transmission routes and directions are complex, and for *S. aureus*, data have shown that contaminated surfaces are important sources for MRSA transmission [30] and that decontamination of household fomites can help to prevent recurrence of *S. aureus* skin infections.[31]

The intermittent carriage patterns identified in the majority of dogs and specifically the recurrent isolation of MRSP from carriage sites even in the two dogs that had yielded three negative consecutive carriage samples is concerning and complicates the design of infection control policies for small animal practices. Possible explanations for intermittent carriage include external sources for re-acquisition of MRSP, for example, from contact dogs or environmental surfaces, but these were not consistently present in the study dogs. Endogenous sources such as hidden gastrointestinal or throat carriage are identified as sources for intermittent *S. aureus* carriage in humans.[32] Reverse zoonotic transmission from contact humans is also possible as MRSP carriage has been described in owners and veterinarians of MRSP infected dogs.[2,33,34] Human MRSP carriage is considered rare and transient though[8] and was not investigated in this study. Last, increased or high minimum inhibitory concentrations (MICs) of fusidic acid and chlorhexidine might account for the recovery of MRSP populations after treatment. However, such resistance development is considered unlikely for topical application; results from a recent canine skin penetration study showed that when applied topically, fusidic acid skin surface concentrations greatly exceeded MICs for canine pathogenic staphylococci at common skin infection sites, and by extrapolation skin carriage sites.[35] Chlorhexidine has been widely used in the treatment of canine pyoderma, and evidence for clinical efficacy even over several weeks of treatment is good.[36]

Protective equipment and isolation measures are currently recommended when dealing with MRSP-infected or colonised pets,[1] but an end point of when these measures can be relaxed has not yet been defined. Unfortunately, the results from this study suggest that one, two or even three negative carriage samples one month apart may not be sufficient to ensure non-carriage. Screening post infection is already rarely done by clinicians for various reasons including cost, time and possibly a lack of urgency in an outpatient setting and in dogs where clinical signs have already been resolved. In human medicine, the need for highly predictive but economical and feasible MRSA carriage

testing protocols has led to the development of a 'culture rule' whereby the results from two sampling events are combined with a quantitative assessment of growth and anti-staphylococcal antibody profiles to predict a risk of reinfection and contagion.[37,38] This has not yet been explored for dogs and in the absence of such data, sampling at strategically chosen time points (e.g., before elective hospitalisation), swabbing as many sites as possible with pooling of swabs to reduce cost might be the most pragmatic compromise on screening in the meantime. The results also suggest that rigorous hygiene and practice infection control measures (and responsible antimicrobial prescribing) are indicated long-term when attending to dogs with a history of MRSP infection.

However, the main finding from this study is that elimination of MRSP carriage with topical antimicrobial therapy and household hygiene measures is possible, at least for some dogs and temporarily. Whether and when dogs should receive antimicrobial therapy to clear MRSP carriage though requires careful clinical and ethical evaluation and should only be considered in the context of good antimicrobial stewardship. The overall impact of antimicrobial therapy itself on colonisation with meticillin-resistant staphylococcal pathogens was highlighted in a longitudinal study of 31 dogs where prolonged therapy extended MRSP carriage in dogs[20] and by a recent systematic review showing that good antibiotic stewardship could reduce the incidence of colonisation with MRSA in humans.[39] In view of the lack of evidence for long-term clearance of carriage, analogous with decolonisation for MRSA carriage in human medicine, the routine use of antimicrobial agents for the decolonisation of by then healthy dogs would be controversial. In contrast, such interventions may be justifiable, for example, before elective surgeries as commonly done in human medicine[40,41] or in 'high-risk' household situations to protect vulnerable owners from exposure. In addition, more research is needed to further characterise and identify markers for persistent carriage in dogs so that decolonisation strategies can be tailored to those with a higher risk of significant MRSP dispersal.

CONCLUSION

MRSP carriage was a frequent sequel to MRSP infection and dogs recovered from infection remained a risk for contagion for many months afterwards, even after repeated negative carriage samples. Topical antimicrobial therapy could temporarily eliminate carriage but recurrence was frequent. These findings can be used to inform the design of infection control policies but also emphasise the need for responsible antimicrobial prescribing and for diagnostic and clinical efforts to prevent the recurrence of infections.

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ETHICS STATEMENT

The study was approved by the Royal Veterinary College (RVC) Ethics and Welfare Committee (URN 2012 1166). Written informed consent was obtained from owners at enrolment.

CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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