Case-control risk factor study of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infection in dogs and cats in Germany

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Abstract

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has emerged as a highly drug-resistant small animal veterinary pathogen. Although often isolated from outpatients in veterinary clinics, there is concern that MRSP follows a veterinary-hospital-associated epidemiology. This study's objective was to identify risk factors for MRSP infections in dogs and cats in Germany. Clinical isolates of MRSP cases (n=150) and methicillin-susceptible *S. pseudintermedius* (MSSP) controls (n=133) and their corresponding host signalment and medical data covering the six months prior to staphylococcal isolation were analyzed by multivariable logistic regression. The identity of all MRSP isolates was confirmed through demonstration of *S. intermedius*-group specific *nuc* and *mecA*. In the final model, cats (compared to dogs, OR 18.5, 95%CI 1.8-188.0, P=0.01), animals that had been hospitalised (OR 104.4, 95%CI 21.3-511.6, P<0.001), or visited veterinary clinics more frequently (>10 visits OR 7.3, 95%CI 1.0-52.6, P=0.049) and those that had received topical ear medication (OR 5.1, 95% CI 1.8-14.9, P=0.003) or glucocorticoids (OR 22.5, 95%CI 7.0-72.6, P<0.001) were at higher risk of MRSP infection, whereas *S. pseudintermedius* isolates from ears were more likely to belong to the MSSP-group (OR 0.09, 95% CI 0.03-0.34, P<0.001). These results indicate an association of MRSP infection with veterinary clinic/hospital settings and possibly with chronic skin disease. There was an unexpected lack of association between MRSP and antimicrobial therapy; this requires further investigation but may indicate that MRSP is well adapted to canine skin with little need for selective pressure.

Keywords: pyoderma, otitis, veterinary, MRSP, antimicrobial resistance, infection control

Introduction

Staphylococcus pseudintermedius, belonging to the *Staphylococcus intermedius* group is a frequent opportunistic commensal and the most important staphylococcal pathogen in dogs and cats and frequently affects the skin, ears and wounds (Devriese et al., 2005; Holm et al., 2002; White et al., 2005). Until recently, treatment of the great majority of *S. pseudintermedius* infections caused few problems in small animal veterinary practice as a wide range of authorized antimicrobial drugs showed good efficacy both *in vitro* and *in vivo* (Beco et al., 2012; Lloyd et al., 1996; Pellerin et al., 1998; Rantala et al., 2004). However, the emergence of methicillin-resistant *S. pseudintermedius* (MRSP) over the past ten years and its continuing spread worldwide (Gortel et al., 1999; Jones et al., 2007; Morris et al., 2006; Loeffler et al., 2007; Ruscher et al., 2009), present significant clinical challenges to veterinary surgeons. In addition, MRSP has implications for public health as it can spread between people and pets via direct and indirect contact and rarely MRSP infections in humans have been described (Campagnile et al., 2007; Gerstadt et al., 1999; Stegmann et al., 2010; van Duijkeren et al., 2011 a & b).

Resistance to methicillin in staphylococci is encoded by the gene *mecA* which confers resistance to all β-lactam antibiotics (Chambers, 1997). Epidemiologically, the significance of *mecA*-positive staphylococci is greatest in the context of nosocomial infections. Such isolates are likely to emerge as a consequence of antimicrobial selection pressure in hospitals and are typically multidrug-resistant. In MRSP, several other resistance genes have been identified which often render all clinically relevant pet-authorized systemic antimicrobial drugs ineffective (Kadlec and Schwarz, 2012). For canine pyoderma, it has been shown that most MRSP infections can still be resolved

with topical antibacterial therapy and/or with the help of more ‘exotic’ or less frequently used antimicrobials but that treatment may be prolonged and may be more frequently associated with adverse effects (Bryan et al., 2012; Loeffler et al., 2007). Knowledge of risk factors that contribute to MRSP infection becomes highly relevant since early identification of predisposed patients should facilitate implementation of infection control and prevention strategies.

Risk factors such as antimicrobial therapy, surgical interventions and chronic disease have been suspected for MRSP infection in pets based on the initially observed clinical presentations in chronic skin and wound infections in hospitalised animals.. Antimicrobial therapy during the 30 days prior to sampling was recently identified as a risk factor for MRSP infection in 56 hospitalised dogs in a North American case-control study while other medication (topical antibacterial therapy, glucocorticoids), animal signalment, clinical characteristics and veterinary interventions (such as concurrent disease, type of infection, surgery, hospitalisation) were not associated with outcome (Weese et al., 2012). For MRSP carriage in dogs and cats admitted to a veterinary hospital, previous hospitalisation and antimicrobial therapy in the six months before sampling have been proposed as risk factors (Nienhoff et al., 2011 a & b). Studies on risk factors for MRSP infection in cats have not been published to the authors’ knowledge.

This study aimed to identify risk factors for MRSP infection in dogs and cats in two regions in Germany with a particular focus on exploring a possible veterinary care-associated epidemiology.

Materials & Methods

Study groups

Privately owned dogs and cats with *S. pseudintermedius* infection were eligible for inclusion in a prospective unmatched 1:1 case-control study. Cases with MRSP infection and controls with methicillin-susceptible *S. pseudintermedius* (MSSP) infection were identified based on bacterial isolation from clinical samples. Samples had been submitted for bacterial culture and antimicrobial susceptibility testing to one of two laboratories in Germany (SynlabVet, Geestacht, Germany and Institute for Hygiene and Infectious Diseases, Justus-Liebig University, Giessen, Germany). SynlabVet Laboratory received submissions directly from general veterinary practices in the surrounding area and from a dermatology referral centre (Tierärztliche Spezialisten, Hamburg, Germany). The Giessen university laboratory received submissions from general veterinary practices in the surrounding area as well as samples from dermatology, surgery and internal medicine referral services within the university teaching hospital. Samples had been taken by veterinary surgeons as part of their diagnostic investigations into suspected canine and feline bacterial infection. All MRSP isolates identified between October 2010 and October 2011 inclusive were considered. MSSP isolates were selected throughout the study period using simple randomization on www.randomizer.org.

Enrolment criteria

Animals were enrolled with their *S. pseudintermedius* isolate if their original bacterial isolate had been preserved (lyophilised or frozen in tryptone soya broth with 20% glycerol) by the diagnostic laboratory, when its identity had been confirmed by the

phenotypic methods described below and when the corresponding questionnaire had been returned by the submitting veterinary surgeon for analysis; they were excluded if no or insufficiently completed questionnaires had been returned after two weekly reminder follow-up phone calls.

Questionnaires

When reporting *S. pseudintermedius* isolation, the laboratories invited the submitting veterinary surgeons to participate in the study and to complete a questionnaire. Questionnaires were returned by the participants via the laboratories to the lead investigator (GL) by fax, email or post. Cases and controls were coded and pet and owner details were deleted on receipt by the lead investigator (GL) to ensure confidentiality. Where the Hamburg dermatology referral centre or a referral service at Giessen University had submitted samples to the laboratories, copies of the animal's referral medical history were used to complete the questionnaire and referring general practitioners were contacted if this information was incomplete. Data were collected on each animal's i) signalment, ii) medical history including clinical presentation, sample site, previous veterinary consultations (not including the visit at the time of sampling) and hospitalisation and iii) medication prescribed in the six months prior to *S. pseudintermedius* isolation. One course of antibacterial therapy was defined as the same antibacterial therapy given on consecutive days, while a change of drug or an interruption of treatment of at least one day constituted different courses.

Microbiological identification of MRSP and MSSP

Initial identification of *S. pseudintermedius* by the diagnostic laboratories was based on routine bacteriological methods used for phenotypic identification of *S. intermedius*-group (SIG) isolates (Barrow and Feltham, 2004). Methods specified for use by both

laboratories included assessment of colony morphology and haemolysis on sheep blood agar, the detection of clumping factor by slide coagulation test with rabbit plasma or by a commercial agglutination test (Pasteurex Staph Plus®, Bio-Rad, Munich, Germany) and a Voges-Proskauer-reaction. As all SIG isolates had originated from dogs or cats, they were assumed to represent *S. pseudintermedius* (Bannoehr and Guardabassi, 2012). Resistance to methicillin and antimicrobial agents commonly used for therapy in small animal patients was determined through disc diffusion tests using oxacillin (OX 1 C Mast Diagnostica GmbH, Reinfeld, Germany) on Mueller-Hinton agar (Merck, Darmstadt, Germany) or with MRSA-Ident-Agar (Heipha, Eppelheim, Germany) and with VITEK2 (Biomérieux, Nürtingen, Germany). Breakpoints for disc diffusion tests were according to Din 58940-3, supplement 1 (DIN, 2011).

All *S. pseudintermedius* isolates were re-grown at the end of the enrolment period and posted to the Royal Veterinary College on nutrient agar slopes or plates. Phenotypic tests for initial genus and species identification were repeated from subcultures on 5% ovine blood agar (Oxoid, Basingstoke, UK) as previously described (Loeffler et al., 2007). The identity of MRSP isolates was confirmed phenotypically after growth on mannitol salt agar containing 4% oxacillin (MSAox) (Oxoid, Basingstoke, UK) and genotypically through demonstration of *mecA* after polymerase chain reaction (Brakstad et al., 1993). In addition, methicillin-resistant isolates were differentiated genetically from non-pigmented strains of MRSA by demonstration of the *S. intermedius*-groupthermonuclease gene, *nuc* (Becker et al., 2005), and those negative for *S. intermedius*-group *nuc* were tested for *S. aureus*-specific *nuc* (Baron et al., 2004).

Data analyses

Data were collected for 20 variables into Microsoft, Excel for Mac 2011, Version 14.3.0 spread sheets. All descriptive statistical analyses were performed using SPSS 17.0 software for Windows, whereas regression analyses were done using Stata/IC 11.2. Variables listed in Table 1 were analysed by univariable logistic regression for their association with MRSP infection. Referral practice origin of all submissions was also compared by chi-squared test. Continuous variables were initially categorised (based on quartiles) to assess the shape of their association with the outcome, using likelihood ratio tests to assess departure from linear trend where appropriate. Variables with a likelihood ratio test p-value of <0.20 in univariable analysis were considered for inclusion in a multivariable model, built using a forward stepwise approach. The model building process started with variables most strongly associated with the outcome in univariable analysis, adding exposure variables one by one to assess the presence and direction of potential confounding. To adjust for potential clustering of cases by origin of sample submission, origin was included in the multivariable model as a random effect. This variable was categorised according to samples coming from general practitioners (Synlab); dermatology referral centre (Synlab); dermatology referral service (Giessen); internal medicine referral service (Giessen); surgery referral service (Giessen) and external general practitioners (Giessen). All variables not included in the final model were then forced back into the model, one by one, to check for their statistical significance when adjusted for the other variables in the model. Pair-wise interactions were tested for between variables included in the final multivariable model. Reliability of estimates in the random effects model was assessed by checking the sensitivity of quadrature approximation (*quadchk* command in Stata). The level of statistical significance was set at $p<0.05$.

Results

Enrolment

During the study period, 2130 *S. pseudintermedius* isolates were identified. MRSP accounted for 11.6% (248/2130) of *S. pseudintermedius* submissions overall with 10% (109/1090) isolated at Synlab Vet Hamburg and 13.3% (139/1040) at Giessen university laboratory. At the latter laboratory, MRSP was more frequent (χ^2 40.8, P<0.001) in submissions from the referral services at the university teaching hospital (87/360, 24.2%) compared with those submitted by non-university veterinary clinics (52/680, 7.7%).

The return rate for questionnaires was 66.1% (164/248) for MRSP infected animals and 80.6% (133/165) for MSSP control animals. The identities of eleven methicillin-resistant isolates classified as MRSP using phenotypic methods could not be confirmed genetically (three were identified as MRSA) and three MRSP strains were lost. In total, 283 animals were enrolled including 150 cases and 133 controls.

Animals

Most *S. pseudintermedius* infections had been diagnosed in dogs (266/283, 94%) for both cases and controls (Table 1). However, of the 17 affected cats, only one was in the control group. Sex and numbers of different breeds were evenly distributed with 92 (61.3%) males and 58 (38.6%) females amongst the cases and 71 (53.3%) males and 62 (46.6%) females amongst the controls. Of the dogs with MRSP infection, 133 (88.6%) were pure-bred (50 different breeds) and 17 (11.3%) were cross breeds. Of the MSSP infected dogs, 108 (81.2%) were pure-bred dogs (50 different breeds) and 25 (18.7%) were cross breeds. The majority of cats (14/17, 82.3%) were domestic short-haired cats. Cases had a mean age of 6.5 years (range 1-18 years, SD 3.8) and a mean bodyweight of

24.9 kg (range 3-85, SD 16.2); controls had a mean age of 6.4 years (range 1-16, SD 3.8) with a mean bodyweight of 25.9 kg (range 3-85, SD 15.3).

Clinical characteristics of S. pseudintermedius *infection*

Of all *S. pseudintermedius* submissions, 212/283 (74.9%) were from surface sites (skin and wounds) while other affected organs included the respiratory tract in 25 (8.8%), the urogenital tract in 25 (8.8%) and miscellaneous organs in 21 patients (7.4%). Surface sites from which *S. pseudintermedius* was isolated included pyoderma lesions (35%, 99/283), ear canals (17%, 48/283), claws and claw folds (3.5%, 10/283) and post-surgical wounds (19.4%, 55/283; 38 and 17 from sites of orthopaedic and soft tissue surgery, respectively). Interestingly, four of the pyoderma lesions had been recorded at previous intravenous catheter sites with all yielding MSSP. Of the 16 MRSP positive cats, 71% were hospitalised and 50% had a history of surgery, 70% had non-cutaneous diseases and in 25% MRSP was isolated from the urogenital tract. MRSP was less often found in ears (8.7%, 13/150) than MSSP (26.3%, 35/133)(Table 1).

Antibacterial agents had been used systemically in 78.1% (221/283) of animals prior to sampling and topically (excluding ear drops) in 15.9% (45/283). Overall, 46.9% (133/283) had received more than one course of systemic antimicrobials in the six months prior to sampling. Four courses of antimicrobials had been prescribed to 18.6% (28/150) of MRSP cases and to 6.7% (9/133) of MSSP controls over the same period . Cephalosporin and fluoroquinolone therapies were both associated with MRSP infection in the univariable analysis (Table 1). In total, 21.2% (60/283) of animals had received medicated ear drop preparations in the six months prior to *S. pseudintermedius* isolation.

Regression analyses

Of the 20 variables investigated, 11 (species, number of visits to a veterinary clinic, admission to hospital, surgery, isolation from wounds, concurrent disease, systemic antimicrobial therapy, number of antibiotic courses, cephalosporins, fluoroquinolones and systemic glucocorticoid therapy) significantly increased the odds of MRSP (at $p<0.05$) while 4 variables significantly decreased the odds of MRSP (seen at a referral centre, isolation from a cutaneous site, isolation from ears and pruritus) (Table 1).

The final multivariable model is shown in Table 2. The risk for MRSP infection compared with MSSP infection was higher in cats than in dogs and in animals that had been hospitalised, had visited veterinarians more frequently and in those that had received glucocorticoids. Animals from which *S. pseudintermedius* had been isolated from ears were more likely to be in the control group. However, ear drops, when forced back into the model manually were associated with MRSP isolation. No interactions between variables included in the final model were identified and model estimates were found to be reliable based on the quadrature sensitivity check.

Discussion

This study confirms that MRSP should be regarded as a hospital-associated pathogen in small animal veterinary practice and it provides data that emphasise the need for rigorous hygiene measures and awareness of MRSP as an important contagion.

The estimates reported here need to be interpreted with caution for categories where numbers were low, e.g. cats. An additional bias may have been introduced by the higher questionnaire return rate from control animals. This could be due to concern over certain clinics becoming associated with multidrug-resistance.

Definitions for nosocomial (healthcare-associated) infection are difficult to extrapolate to a veterinary environment where pets are often seen as outpatients. However, a veterinary-care-associated epidemiology will be likely for diseases where an increased risk of infection is found associated with veterinary interventions or institutions (Johnson, 2002). The strong association between MRSP infection and hospitalisation, frequent visits to veterinary practices and likely involvement in patients with chronic skin disease indicates that MRSP is an opportunistic pathogen thriving in patients that require repeated veterinary medication or intervention. This supports previous studies into carriage of MRSP in pets and of infection with other multidrug-resistant staphylococci (Nienhoff et al., 2011 a & b; Eckholm et al., 2013; Soares-Magalhães et al., 2010).

An important reason for a veterinary-care-related acquisition of MRSP may be the opportunity for transmission in veterinary clinics and hospitals via contaminated environment, vector activity of staff or through other colonised or infected pets. Contamination of veterinary hospitals with MRSP has been documented for surfaces

contacted by hospital staff (Bergström et al., 2012) while animal-exposed areas such as weighing scales have yielded large numbers of SIG isolates previously (Hamilton et al., 2012). Based on the long-term survival ability of staphylococci on environmental surfaces (Wagenvoort et al., 2000) and the good adherence of *S. pseudintermedius* to canine corneocytes (Wooley et al., 2008), the veterinary intervention-associated risk factors identified in this study can be considered biologically meaningful.

With cats less frequently colonised by *S. pseudintermedius*, including MRSP (Couto et al., 2011, Nienhoff et al., 2011b) and less often suffering from staphylococcal infections, numbers of cats in this study were low (6%, 17/283) as expected. Although with a large confidence interval, cats showed a substantially increased risk for MRSP infection though, which may imply that infection in cats is indeed more likely to be of hospital-or clinic related origin.

Systemic glucocorticoid therapy was shown to predispose to MRSP carriage in dogs in both the present and in a previous study (Nienhoff et al., 2011a). Since allergic skin disease and the associated skin barrier dysfunction is known to favour staphylococcal infection in dogs, and since allergy is commonly managed with gluocorticoids, it is perhaps possible that glucocorticoid therapy favoured MRSP indirectly through the need for repeated antimicrobial therapy and visits to veterinary clinics.

The lack of positive association between MRSP and antimicrobial therapy in the final model was surprising in view of the more frequent antimicrobial prescription to MRSP animals found in the univariable analysis. However, the link between antimicrobial therapy and MRSP infection has not always been reported consistently. Antimicrobial drug therapy was the most important risk factor for methicillin-resistance in staphylococci in dogs with superficial pyoderma (Eckholm et al., 2013) and in a study

of dogs in referral hospitals in Canada (Weese et al., 2012), antimicrobial therapy was the only variable associated with MRSP infection, albeit related to the 30 days prior to sampling. In contrast, a longitudinal study found no association between the development of MRSP infection and prior antimicrobial therapy (Beck et al., 2012). Strain-specific variation or the longer time window for antimicrobial therapy in our study may explain these differences. It is possible that analysis of more recent antimicrobial therapy might have shown a stronger effect on MRSP selection as adaption to different niches may occur more rapidly than anticipated. Alternatively, MRSP may be equally well adapted to canine skin as MSSP with little need for selective pressure (Beck et al., 2012).

Another unexpected finding was the association between topical ear medication and MRSP isolation. It is likely though that some dogs may have had MRSP isolated from other body sites and received ear drops for otitis associated with a chronic skin disease such as allergy.

Conclusion

The identification of MRSP in 12% of *S. pseudintermedius* submissions to veterinary laboratories and the strong association between MRSP and veterinary institutions confirm MRSP as an important veterinary care-associated bacterium. In particular, veterinary surgeons dealing with patients with chronic skin disease and with hospitalised animals need to be aware as early recognition and implementation of rigorous hygiene measures are paramount to limit spread and reduce the risk to other pets and people.

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Conflict of interest statement

No conflict of interest has been declared.

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Table 1: Univariable analysis of putative risk factor variables from animals with MRSP infection (cases, n=150) and controls with MSSP infection (n=133).

Variable level	MRSP n (%)	MSSP n (%)	OR	95% CI	*p*-value
Species					
Dog	134 (89.3)	132 (99.2)	Ref.		
Cat	16 (10.7)	1 (0.8)	15.8	2.1-120.6	0.008
Sex					
Female	58 (38.7)	62 (46.6)	Ref.		
Male	92 (61.3)	71 (53.4)	1.4	0.9-2.2	0.18
Age in years					
≤ 34 to 6	41 (27.3)	37 (27.8)	Ref.		
7 to 9	37 (24.7)	36 (27.1)	0.9	0.5-1.8	0.82
≥ 10	41 (27.3)	30 (22.6)	1.2	0.6-2.4	0.53
	31 (20.7)	30 (22.6)	0.9	0.5-1.8	0.84
Body weight					
≤ 15 kg	58 (38.8)	43 (32.3)	Ref.		
> 15 kg	92 (61.3)	90 (67.7)	0.8	0.5-1.2	0.27
Visits to a veterinary institution prior to sampling visit*					
0	4 (2.7)	8 (6.0)	Ref.		
1 to 5	57 (38.0)	91 (68.4)	1.3	0.4-4.4	0.72
6 to 10	41 (27.3)	26 (19.6)	3.2	0.9-11.5	0.08
> 10	48 (32.0)	8 (6.0)	12.0	2.9-49.4	0.001
Seen at referral centre					
No	73 (48.6)	88 (66.2)	Ref.		
Yes	77 (51.3)	45 (33.8)	0.5	0.3-0.8	0.003
Admission to hospital					
No	76 (50.7)	131 (98.5)	Ref.		
Yes	74 (49.3)	2 (1.5)	63.8	15.2-267.2	<0.001
Surgery					
No	90 (60.0)	126 (94.7)	Ref.		
Yes	60 (40.0)	7 (5.3)	12.0	5.2-27.5	<0.001

Wounds					
No	100 (66.6)	128 (96.2)	Ref.		
Yes	50 (33.3)	5 (3.8)	12.8	4.9-33.3	<0.001
Isolated from cutaneous site (skin, ears, claw and catheter sites)					
No	85 (56.7)	41 (30.8)	Ref.		
Yes	65 (43.3)	92 (69.2)	0.3	0.2-0.6	<0.001
Isolated from ears					
No	137 (91.3)	98 (73.7)	Ref.		
Yes	13 (8.7)	35 (26.3)	0.3	0.1-0.5	<0.001
Concurrent diseases					
No	47 (31.3)	75 (56.4)	Ref.		
Yes	103 (68.7)	58 (43.6)	2.8	1.7-4.6	<0.001
Pruritus					
No	92 (61.3)	59 (44.4)	Ref.		
Yes	58 (38.7)	74 (55.6)	0.5	0.3-0.8	0.004
Systemic antimicrobials					
No	18 (12)	44 (33.1)	Ref.		
Yes	132 (88.0)	89 (66.9)	3.6	2.0-6.7	<0.001
Number of antimicrobial courses					
0	18 (12.0)	44 (33.1)	Ref.		
1	45 (30.0)	43 (32.3)	2.6	1.3-5.1	0.008
≥ 2 courses	87 (58.0)	46 (34.6)	4.6	2.4-8.9	<0.001
Cephalosporins					
No	99 (66.0)	107 (80.5)	Ref.		
Yes	51 (34.0)	26 (19.5)	2.1	1.3-3.7	0.007
Fluoroquinolones					
No	105 (70.0)	113 (84.9)	Ref.		
Yes	45 (30.0)	20 (15.0)	2.4	1.3-4.3	0.004
Systemic glucocorticoids					
No	106 (70.7)	128 (96.2)	Ref.		
Yes	44 (29.3)	5 (3.8)	10.6	4.1-27.8	<0.001
Topical antimicrobials (shampoos, gels)					
No	127 (84.7)	111 (83.5)	Ref.	0.5-1.7	0.78

Yes	23 (15.3)	22 (16.5)	0.9		
Ear drops					
No	118 (78.7)	105 (78.9)	Ref.		
Yes	32 (21.3)	28 (21.1)	1.0	0.6-1.8	0.95

Information was based on questionnaire data referring to the six months prior to *S. pseudintermedius* isolation. Ref.: reference category; OR: odds ratio; CI: confidence interval. *Modelled as ordered categories (none, 1-5, 6-10, >10).

468 **Table 2:** Final mixed-effects multivariable regression model for putative risk factors for
469 MRSP infection in dogs and cats.

Variable	Value	OR	95% CI	Wald test *p*-value	LRT *p*-value
Species	Dog	Ref.			0.008
	Cat	18.5	1.8 – 188.0	0.01	
Visits to a veterinary institution prior to sampling visit	None	Ref.			0.004
	1 to 5	1.1	0.2 – 6.5	0.94	
	6 to 10	1.6	0.2 – 10.7	0.63	
	>10	7.3	1.0 – 52.6	0.049	
Admission to hospital	No	Ref.			<0.001
	Yes	104.4	21.3 – 511.6	<0.001	
Isolated from ears	No	Ref.			<0.001
	Yes	0.09	0.03 – 0.34	<0.001	
Systemic glucocorticoids	No	Ref.			<0.001
	Yes	22.5	7.0 – 72.6	<0.001	
Ear drops	No	Ref.			0.002
	Yes	5.1	1.8 – 14.9	0.003	
Sample origin					0.056[†]

470 Information was based on 283 questionnaires with data referring to the six months prior
471 to *S. pseudintermedius* isolation. Sample origin was included in the model as a random
472 effect. Ref.: reference category; OR: odds ratio; CI: confidence interval; LRT:
473 likelihood ratio test. [†]p-value derived from a test of the null hypothesis that there is no
474 correlation between samples from the same origin.