1   **Case-control risk factor study of methicillin-resistant *Staphylococcus***

2   ***pseudintermedius* (MRSP) infection in dogs and cats in Germany**

3   GEORG LEHNER [a], MONIKA LINEK [a], ROSS BOND [b], DAVID H. LLOYD [b],

4   ELLEN PRENGER-BERNINGHOFF [c], NINA THOM [d], IRIS STRAUBE [e], KRISTIEN

5   VERHEYEN [f], ANETTE LOEFFLER [b]

6   [a]*Tierärztliche Spezialisten Hamburg, Rodigallee 85, 22043 Hamburg, Germany*

7   [b]*Department of Clinical Sciences and Services, The Royal Veterinary College,*

8   *University of London, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9*

9   *7TA, United Kingdom*

10   [c]*Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-University,*

11   *Giessen, Frankfurter Strasse 85 – 89, 35392 Giessen, Germany*

12   [d]*Small Animal Teaching Hospital, Dermatology Unit, Justus-Liebig-University,*

13   *Giessen, Frankfurter Strasse 85 – 89, 35392 Giessen, Germany*

14   [e]*SynlabVet, Labor Hamburg, Schillerstrasse 29, 21502 Geesthacht, Germany*

15   [f]*Department of Production and Population Medicine, The Royal Veterinary College,*

16   *University of London, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9*

17   *7TA, United Kingdom*

18   Corresponding author: Anette Loeffler, Department of Veterinary Clinical Sciences,

19   The Royal Veterinary College, University of London, Hawkshead Lane, North Mymms,

20   Hatfield, Hertfordshire, AL9 7TA, United Kingdom, Phone: + 44 1707 666333, Fax:

21   +44 1707 666298, Email: aloeffler@rvc.ac.uk

22   **Abstract**

23   Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has emerged as a highly

24   drug-resistant small animal veterinary pathogen. Although often isolated from

25   outpatients in veterinary clinics, there is concern that MRSP follows a veterinary-

26   hospital-associated epidemiology. This study's objective was to identify risk factors for

27   MRSP infections in dogs and cats in Germany. Clinical isolates of MRSP cases (n=150)

28   and methicillin-susceptible *S. pseudintermedius* (MSSP) controls (n=133) and their

29   corresponding host signalment and medical data covering the six months prior to

30   staphylococcal isolation were analyzed by multivariable logistic regression. The identity

31   of all MRSP isolates was confirmed through demonstration of *S. intermedius*-group

32   specific *nuc* and *mecA*. In the final model, cats (compared to dogs, OR 18.5, 95%CI 1.8-

33   188.0, P=0.01), animals that had been hospitalised (OR 104.4, 95%CI 21.3-511.6,

34   P<0.001), or visited veterinary clinics more frequently (>10 visits OR 7.3, 95%CI 1.0-

35   52.6, P=0.049) and those that had received topical ear medication (OR 5.1, 95% CI 1.8-

36   14.9, P=0.003) or glucocorticoids (OR 22.5, 95%CI 7.0-72.6, P<0.001) were at higher

37   risk of MRSP infection, whereas *S. pseudintermedius* isolates from ears were more

38   likely to belong to the MSSP-group (OR 0.09, 95% CI 0.03-0.34, P<0.001). These

39   results indicate an association of MRSP infection with veterinary clinic/hospital settings

40   and possibly with chronic skin disease. There was an unexpected lack of association

41   between MRSP and antimicrobial therapy; this requires further investigation but may

42   indicate that MRSP is well adapted to canine skin with little need for selective pressure.

43

44   **Keywords:** pyoderma, otitis, veterinary, MRSP, antimicrobial resistance, infection

45   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.

92

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

116   phenotypic methods described below and when the corresponding questionnaire had

117   been returned by the submitting veterinary surgeon for analysis; they were excluded if

118   no or insufficiently completed questionnaires had been returned after two weekly

119   reminder follow-up phone calls.

120   *Questionnaires*

121   When reporting *S. pseudintermedius* isolation, the laboratories invited the submitting

122   veterinary surgeons to participate in the study and to complete a questionnaire.

123   Questionnaires were returned by the participants via the laboratories to the lead

124   investigator (GL) by fax, email or post. Cases and controls were coded and pet and

125   owner details were deleted on receipt by the lead investigator (GL) to ensure

126   confidentiality. Where the Hamburg dermatology referral centre or a referral service at

127   Giessen University had submitted samples to the laboratories, copies of the animal's

128   referral medical history were used to complete the questionnaire and referring general

129   practitioners were contacted if this information was incomplete. Data were collected on

130   each animal's i) signalment, ii) medical history including clinical presentation, sample

131   site, previous veterinary consultations (not including the visit at the time of sampling)

132   and hospitalisation and iii) medication prescribed in the six months prior to *S.*

133   *pseudintermedius* isolation. One course of antibacterial therapy was defined as the same

134   antibacterial therapy given on consecutive days, while a change of drug or an

135   interruption of treatment of at least one day constituted different courses.

136   *Microbiological identification of MRSP and MSSP*

137   Initial identification of *S. pseudintermedius* by the diagnostic laboratories was based on

138   routine bacteriological methods used for phenotypic identification of *S. intermedius-*

139   group (SIG) isolates (Barrow and Feltham, 2004). Methods specified for use by both

140 laboratories included assessment of colony morphology and haemolysis on sheep blood

141 agar, the detection of clumping factor by slide coagulation test with rabbit plasma or by

142 a commercial agglutination test (Pasteurex Staph Plus®, Bio-Rad, Munich, Germany)

143 and a Voges-Proskauer-reaction. As all SIG isolates had originated from dogs or cats,

144 they were assumed to represent *S. pseudintermedius* (Bannoehr and Guardabassi,

145 2012). Resistance to methicillin and antimicrobial agents commonly used for therapy in

146 small animal patients was determined through disc diffusion tests using oxacillin (OX 1

147 C Mast Diagnostica GmbH, Reinfeld, Germany) on Mueller-Hinton agar (Merck,

148 Darmstadt, Germany) or with MRSA-Ident-Agar (Heipha, Eppelheim, Germany) and

149 with VITEK2 (Biomérieux, Nürtingen, Germany). Breakpoints for disc diffusion tests

150 were according to Din 58940-3, supplement 1 (DIN, 2011).

151 All *S. pseudintermedius* isolates were re-grown at the end of the enrolment period and

152 posted to the Royal Veterinary College on nutrient agar slopes or plates. Phenotypic

153 tests for initial genus and species identification were repeated from subcultures on 5%

154 ovine blood agar (Oxoid, Basingstoke, UK) as previously described (Loeffler et al.,

155 2007). The identity of MRSP isolates was confirmed phenotypically after growth on

156 mannitol salt agar containing 4% oxacillin (MSAox) (Oxoid, Basingstoke, UK) and

157 genotypically through demonstration of *mecA* after polymerase chain reaction (Brakstad

158 et al., 1993). In addition, methicillin-resistant isolates were differentiated genetically

159 from non-pigmented strains of MRSA by demonstration of the *S. intermedius*-

160 groupthermonuclease gene, *nuc* (Becker et al., 2005), and those negative for *S.*

161 *intermedius*-group *nuc* were tested for *S. aureus*-specific *nuc* (Baron et al., 2004).

162 *Data analyses*

163　Data were collected for 20 variables into Microsoft, Excel for Mac 2011, Version 14.3.0

164　spread sheets. All descriptive statistical analyses were performed using SPSS 17.0

165　software for Windows, whereas regression analyses were done using Stata/IC 11.2.

166　Variables listed in Table 1 were analysed by univariable logistic regression for their

167　association with MRSP infection. Referral practice origin of all submissions　was also

168　compared by chi-squared test. Continuous variables were initially categorised (based on

169　quartiles) to assess the shape of their association with the outcome, using likelihood

170　ratio tests to assess departure from linear trend where appropriate. Variables with a

171　likelihood ratio test p-value of <0.20 in univariable analysis were considered for

172　inclusion in a multivariable model, built using a forward stepwise approach. The model

173　building process started with variables most strongly associated with the outcome in

174　univariable analysis, adding exposure variables one by one to assess the presence and

175　direction of potential confounding. To adjust for potential clustering of cases by origin

176　of sample submission, origin was included in the multivariable model as a random

177　effect. This variable was categorised according to samples coming from general

178　practitioners (Synlab); dermatology referral centre (Synlab); dermatology referral

179　service (Giessen); internal medicine referral service (Giessen); surgery referral service

180　(Giessen) and external general practitioners (Giessen). All variables not included in the

181　final model were then forced back into the model, one by one, to check for their

182　statistical significance when adjusted for the other variables in the model. Pair-wise

183　interactions were tested for between variables included in the final multivariable model.

184　Reliability of estimates in the random effects model was assessed by checking the

185　sensitivity of quadrature approximation (*quadchk* command in Stata). The level of

186　statistical significance was set at $p<0.05$.

187

188 **Results**

189 *Enrolment*

190 During the study period, 2130 *S. pseudintermedius* isolates were identified. MRSP

191 accounted for 11.6% (248/2130) of *S. pseudintermedius* submissions overall with 10%

192 (109/1090) isolated at Synlab Vet Hamburg and 13.3% (139/1040) at Giessen university

193 laboratory. At the latter laboratory, MRSP was more frequent (χ^2 40.8, P<0.001) in

194 submissions from the referral services at the university teaching hospital (87/360,

195 24.2%) compared with those submitted by non-university veterinary clinics (52/680,

196 7.7%).

197 The return rate for questionnaires was 66.1% (164/248) for MRSP infected animals and

198 80.6% (133/165) for MSSP control animals. The identities of eleven methicillin-

199 resistant isolates classified as MRSP using phenotypic methods could not be confirmed

200 genetically (three were identified as MRSA) and three MRSP strains were lost. In total,

201 283 animals were enrolled including 150 cases and 133 controls.

202 *Animals*

203 Most *S. pseudintermedius* infections had been diagnosed in dogs (266/283, 94%) for

204 both cases and controls (Table 1). However, of the 17 affected cats, only one was in the

205 control group. Sex and numbers of different breeds were evenly distributed with 92

206 (61.3%) males and 58 (38.6%) females amongst the cases and 71 (53.3%) males and 62

207 (46.6%) females amongst the controls. Of the dogs with MRSP infection, 133 (88.6%)

208 were pure-bred (50 different breeds) and 17 (11.3%) were cross breeds. Of the MSSP

209 infected dogs, 108 (81.2%) were pure-bred dogs (50 different breeds) and 25 (18.7%)

210 were cross breeds. The majority of cats (14/17, 82.3%) were domestic short-haired cats.

211 Cases had a mean age of 6.5 years (range 1-18 years, SD 3.8) and a mean bodyweight of

212   24.9 kg (range 3-85, SD 16.2); controls had a mean age of 6.4 years (range 1-16, SD

213   3.8) with a mean bodyweight of 25.9 kg (range 3-85, SD 15.3).

214   *Clinical characteristics of* S. pseudintermedius *infection*

215   Of all *S. pseudintermedius* submissions, 212/283 (74.9%) were from surface sites (skin

216   and wounds) while other affected organs included the respiratory tract in 25 (8.8%), the

217   urogenital tract in 25 (8.8%) and miscellaneous organs in 21 patients (7.4%). Surface

218   sites from which *S. pseudintermedius* was isolated included pyoderma lesions (35%,

219   99/283), ear canals (17%, 48/283), claws and claw folds (3.5%, 10/283) and post-

220   surgical wounds (19.4%, 55/283; 38 and 17 from sites of orthopaedic and soft tissue

221   surgery, respectively). Interestingly, four of the pyoderma lesions had been recorded at

222   previous intravenous catheter sites with all yielding MSSP. Of the 16 MRSP positive

223   cats, 71% were hospitalised and 50% had a history of surgery, 70% had non-cutaneous

224   diseases and in 25% MRSP was isolated from the urogenital tract. MRSP was less often

225   found in ears (8.7%, 13/150) than MSSP (26.3%, 35/133)(Table 1).

226   Antibacterial agents had been used systemically in 78.1% (221/283) of animals prior to

227   sampling and topically (excluding ear drops) in 15.9% (45/283). Overall, 46.9%

228   (133/283) had received more than one course of systemic antimicrobials in the six

229   months prior to sampling. Four courses of antimicrobials had been prescribed to 18.6%

230   (28/150) of MRSP cases and to 6.7% (9/133) of MSSP controls over the same period .

231   Cephalosporin and fluoroquinolone therapies were both associated with MRSP infection

232   in the univariable analysis (Table 1). In total, 21.2% (60/283) of animals had received

233   medicated ear drop preparations in the six months prior to *S. pseudintermedius*

234   isolation.

235   *Regression analyses*

236   Of the 20 variables investigated, 11 (species, number of visits to a veterinary clinic,

237   admission to hospital, surgery, isolation from wounds, concurrent disease, systemic

238   antimicrobial therapy, number of antibiotic courses, cephalosporins, fluoroquinolones

239   and systemic glucocorticoid therapy) significantly increased the odds of MRSP (at

240    $p < 0.05$) while 4 variables significantly decreased the odds of MRSP (seen at a referral

241   centre, isolation from a cutaneous site, isolation from ears and pruritus) (Table 1).

242   The final multivariable model is shown in Table 2. The risk for MRSP infection

243   compared with MSSP infection was higher in cats than in dogs and in animals that had

244   been hospitalised, had visited veterinarians more frequently and in those that had

245   received glucocorticoids. Animals from which *S. pseudintermedius* had been isolated

246   from ears were more likely to be in the control group. However, ear drops, when forced

247   back into the model manually were associated with MRSP isolation. No interactions

248   between variables included in the final model were identified and model estimates were

249   found to be reliable based on the quadrature sensitivity check.

250

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

274 contacted by hospital staff (Bergström et al., 2012) while animal-exposed areas such as

275 weighing scales have yielded large numbers of SIG isolates previously (Hamilton et al.,

276 2012). Based on the long-term survival ability of staphylococci on environmental

277 surfaces (Wagenvoort et al., 2000) and the good adherence of *S. pseudintermedius* to

278 canine corneocytes (Wooley et al., 2008), the veterinary intervention-associated risk

279 factors identified in this study can be considered biologically meaningful.

280 With cats less frequently colonised by *S. pseudintermedius*, including MRSP (Couto et

281 al., 2011, Nienhoff et al., 2011b) and less often suffering from staphylococcal

282 infections, numbers of cats in this study were low (6%, 17/283) as expected. Although

283 with a large confidence interval, cats showed a substantially increased risk for MRSP

284 infection though, which may imply that infection in cats is indeed more likely to be of

285 hospital-or clinic related origin.

286 Systemic glucocorticoid therapy was shown to predispose to MRSP carriage in dogs in

287 both the present and in a previous study (Nienhoff et al., 2011a). Since allergic skin

288 disease and the associated skin barrier dysfunction is known to favour staphylococcal

289 infection in dogs, and since allergy is commonly managed with gluocorticoids, it is

290 perhaps possible that glucocorticoid therapy favoured MRSP indirectly through the

291 need for repeated antimicrobial therapy and visits to veterinary clinics.

292 The lack of positive association between MRSP and antimicrobial therapy in the final

293 model was surprising in view of the more frequent antimicrobial prescription to MRSP

294 animals found in the univariable analysis. However, the link between antimicrobial

295 therapy and MRSP infection has not always been reported consistently. Antimicrobial

296 drug therapy was the most important risk factor for methicillin-resistance in

297 staphylococci in dogs with superficial pyoderma (Eckholm et al., 2013) and in a study

298   of dogs in referral hospitals in Canada (Weese et al., 2012), antimicrobial therapy was

299   the only variable associated with MRSP infection, albeit related to the 30 days prior to

300   sampling. In contrast, a longitudinal study found no association between the

301   development of MRSP infection and prior antimicrobial therapy (Beck et al., 2012).

302   Strain-specific variation or the longer time window for antimicrobial therapy in our

303   study may explain these differences. It is possible that analysis of more recent

304   antimicrobial therapy might have shown a stronger effect on MRSP selection as

305   adaption to different niches may occur more rapidly than anticipated. Alternatively,

306   MRSP may be equally well adapted to canine skin as MSSP with little need for

307   selective pressure (Beck et al., 2012).

308

309   Another unexpected finding was the association between topical ear medication and

310   MRSP isolation. It is likely though that some dogs may have had MRSP isolated from

311   other body sites and received ear drops for otitis associated with a chronic skin disease

312   such as allergy.

313   **Conclusion**

314   The identification of MRSP in 12% of *S. pseudintermedius* submissions to veterinary

315   laboratories and the strong association between MRSP and veterinary institutions

316   confirm MRSP as an important veterinary care-associated bacterium. In particular,

317   veterinary surgeons dealing with patients with chronic skin disease and with

318   hospitalised animals need to be aware as early recognition and implementation of

319   rigorous hygiene measures are paramount to limit spread and reduce the risk to other

320   pets and people.

321

326

327 **Conflict of interest statement**

328 No conflict of interest has been declared.

329

330

331   **References**

332   Bannoehr, J., Guardabassi, L., 2012. *Staphylococcus pseudintermedius* in the dog:

333   taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Vet. Dermatol. 23,

334   253–269.

335   Baron, F., Cochet, M-F., Pellerin, J-L., Ben Zakour, N., Lebon, A., Navarro, A.,

336   Proudy, I., Le Loir, Y., Gautier, M., 2004. Development of a PCR test to differentiate

337   between *Staphylococcus aureus* and *Staphylococcus intermedius*. J. Food Prot. 67,

338   2302–2305.

339   Barrow GI, Feltham R., 2004. Cowan and Steel's manual for the identification of

340   medical bacteria. Cambridge University Press, Cambridge, pp. 21–42, 52–59.

341   Beck, K.M., Waisglass, S.E., Dick, H.L.N., Weese, J.S., 2012. Prevalence of meticillin-

342   resistant *Staphylococcus pseudintermedius* (MRSP) from skin and carriage sites of dogs

343   after treatment of their meticillin-resistant or meticillin-sensitive staphylococcal

344   pyoderma. Vet. Dermatol. 23, 369–378.

345   Becker, K., von Eiff, C., Keller, B., Brück, M., Etienne, J., Peters, G., 2005.

346   Thermonuclease gene as a target for specific identification of *Staphylococcus*

347   *intermedius* isolates: use of a PCR-DNA enzyme immunoassay. Diagn. Microbiol.

348   Infect. Dis. 51, 237–244.

349   Beco, L., Guaguère, E., Lorente Méndez, C., Noli, C., Nuttall, T., Vroom, M., 2013.

350   Suggested guidelines for using systemic antimicrobials in bacterial skin infections: part

351   2-- antimicrobial choice, treatment regimens and compliance. Vet. Rec. 172, 156-160.

352   Bergström, A., Gustafsson, C., Leander, M., Fredriksson, M., Grönlund, U., Trowald-

353   Wigh, G., 2012. Occurrence of methicillin-resistant staphylococci in surgically treated

354   dogs and the environment in a Swedish animal hospital. J. Small Anim. Pract. 53, 404–

355   410.

356   Brakstad, O.G., Maeland, J.A., Tveten, Y., 1993. Multiplex polymerase chain reaction

357   for detection of genes for *Staphylococcus aureus* thermonuclease and methicillin

358   resistance and correlation with oxacillin resistance. APMIS. 101, 681–688.

359   Bryan, J., Frank, L.A., Rohrbach, B.W., Burgette, L.J., Cain, C.L., Bemis, D.A., 2012.

360   Treatment outcome of dogs with meticillin-resistant and meticillin-susceptible

361   *Staphylococcus pseudintermedius* pyoderma. Vet. Dermatol. 23, 361–369.

362   Campanile, F., Bongiorno, D., Borbone, S., Venditti, M., Giannella, M., Franchi, C.,

363   Stefani, S., 2007. Characterization of a variant of the SCC*mec* element in a bloodstream

364   isolate of *Staphylococcus intermedius*. Microb. Drug Resist. 13, 7-10.

365   Chambers, H.F., 1997. Methicillin resistance in staphylococci: molecular and

366   biochemical basis and clinical implications. Clin. Microbiol. Rev. 10, 781-791.

367   Couto, N., Pomba, C., Moodley, A., Guardabassi, L., 2011. Prevalence of meticillin-

368   resistant staphylococci among dogs and cats at a veterinary teaching hospital in

369   Portugal. Vet. Rec. 169, 72–73.

370   Devriese, L.A., Vancanneyt, M., Baele, M., Vaneechoutte, M., De Graef, E., Snauwaert,

371   C., Cleenwerck, I., Dawyndt, P., Swings, J., Decostere, A., Haesebrouck, F., 2005.

372   *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals.

373   Int. J. Syst. Evol. Microbiol. 55, 1569-1573.

374   Din 58940-3, supplement 1, 2011. Medical microbiology - Susceptibility testing of

375   microbial pathogens to antimicrobial agents - Part 3: Agar diffusion test.

376   Eckholm, N.G., Outerbridge, C.A., White, S.D., Sykes, J.E., 2013. Prevalence of and

377   risk factors for isolation of meticillin-resistant *Staphylococcus* spp. from dogs with

378   pyoderma in northern California, USA. Vet. Dermatol. 24, 154–162.

379   Fazakerley, J., Nuttall, T., Sales, D., Schmidt, V., Carter, S.D., Hart, C.A., McEwan,

380   N.A., 2009. Staphylococcal colonization of mucosal and lesional skin sites in atopic and

381   healthy dogs. Vet. Dermatol. 20, 179–184.

382   Gerstadt, K., Daly, J.S., Mitchell, M., Wessolossky, M., Cheeseman, S.H., 1999.

383   Methicillin-resistant *Staphylococcus intermedius* pneumonia following coronary artery

384   bypass grafting. Clin. Infect. Dis. 29, 218-219.

385   Goodacre, R., Harvey, R., Howell, S.A., Greenham, L.W., Noble, W.C., 1997. An

386   epidemiological study of *Staphylococcus intermedius* strains from dogs, their owners

387   and veterinary surgeons. J. Anal. Appl. Pyrolysis 44, 49–64.

388   Gortel, K., Campbell, K.L., Kakoma, I., Whittem, T., Schaeffer, D.J., Weisinger, R.M.,

389   1999. Methicillin resistance among staphylococci isolated from dogs. Am. J. Vet. Res.

390   60, 1526–1530.

391   Hamilton, E., Kaneene, J.B., May, K.J., Kruger, J.M., Schall, W., Beal, M.W.,

392   Hauptman, J.G., DeCamp, C.E., 2012. Prevalence and antimicrobial resistance of

393   *Enterococcus* spp. and *Staphylococcus* spp. isolated from surfaces in a veterinary

394   teaching hospital. J. Am. Vet. Med. Assoc. 240, 1463–1473.

395 Holm, B.R., Petersson, U., Mörner, A., Bergström, K., Franklin, A., Greko, C., 2002.

396 Antimicrobial resistance in staphylococci from canine pyoderma: a prospective study of

397 first-time and recurrent cases in Sweden. Vet. Rec. 151, 600–605.

398 Johnson, J.A., 2002. Nosocomial infections. Vet. Clin. Small Anim. 32, 1101-1126.

399 Jones, R.D., Kania, S.A., Rohrbach, B.W., Frank, L.A., Bemis, D.A., 2007. Prevalence

400 of oxacillin and multidrug-resistant staphylococci in clinical samples from dogs 1,772

401 samples (2001-2005). J. Am. Vet. Med. Assoc. 230, 221–227.

402 Kadlec, K., Schwarz, S., 2012. Antimicrobial resistance of *Staphylococcus*

403 *pseudintermedius*. Vet. Dermatol. 23, 276–282.

404 Lloyd, D.H., Lamport, A.I., Feeney, C., 1996. Sensitivity to antibiotics amongst

405 cutaneous and mucosal isolates of canine pathogenic staphylococci in the UK, 1980–96.

406 Vet. Dermatol. 7, 171–175.

407 Loeffler, A., Linek, M., Moodley, A., Guardabassi, L., Sung, J.M.L., Winkler, M.,

408 Weiss, R., Lloyd, D.H., 2007. First report of multiresistant, *mecA*-positive

409 *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral

410 clinic in Germany. Vet. Dermatol. 18, 412–421.

411 Morris, D.O., Rook, K.A., Shofer, F.S., Rankin, S.C., 2006. Screening of *Staphylococ-

412 cus aureus*, *Staphylococcus intermedius*, and *Staphylococcus schleiferi* isolates obtained

413 from small companion animals for antimicrobial resistance: a retrospective review of

414 749 isolates (2003–04). Vet. Dermatol. 17, 332–7.

415 Nienhoff, U., Kadlec, K., Chaberny, I., Verspohl, J., Gerlach, G-F., Kreienbrock, L.,

416 Schwarz, S., Simon, D., Nolte, I., 2011a. Methicillin-resistant *Staphylococcus*

417   *pseudintermedius* among dogs admitted to a small animal hospital. Vet. Microbiol. 150,

418   191–197.

419   Nienhoff, U., Kadlec, K., Chaberny, I.F., Verspohl, J., Gerlach, G.F., Schwarz, S.,

420   Kreienbrock, L., Nolte, I., Simon, D., 2011b. Methicillin-resistant *Staphylococcus*

421   *pseudintermedius* among cats admitted to a veterinary teaching hospital. Vet. Microbiol.

422   153, 414-416.

423   Pellerin, J.L., Bourdeau, P., Sebbag, H., Person, J.M., 1998. Epidemiosurveillance of

424   antimicrobial compound resistance of *Staphylococcus intermedius* clinical isolates from

425   canine pyodermas. Comp. Immunol. Microbiol. Infect. Dis. 21, 115–133.

426   Rantala, M., Lahti, E., Kuhalampil, J., Pesonen, S., Järvinen, A.K., Saijonmaa-

427   Koulumies, Honkanen-Buzalski, T., 2004. Antimicrobial resistance in *Staphylococcus*

428   spp., *Escherichia coli* and *Enterococcus* spp. in dogs given antibiotics for chronic

429   dermatological disorders, compared with non-treated control dogs. Acta. Vet. Scand.

430   45, 37–45.

431   Ruscher, C., Lübke-Becker, A., Wleklinski, C-G., Šoba, A., Wieler, L.H., Walther, B.,

432   2009. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated

433   from clinical samples of companion animals and equidaes. Vet. Microbiol. 136, 197–

434   201.

435   Soares-Magalhães, R.J., Loeffler, A., Lindsay, J., Rich, M., Roberts, L., Smith, H.,

436   Lloyd, D.H., Pfeiffer, D.U., 2010. Risk factors for methicillin-resistant *Staphylococcus*

437   *aureus*(MRSA) infection in dogs and cats: a case-control study. Vet. Res. 41, 55–67.

438 Stegmann, R., Burnens, A., Maranta, C.A., Perreten, V., 2010. Human infection

439 associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. J.

440 Antimicrob. Chemother. 65, 2047–2048.

441 van Duijkeren, E., Catry, B., Greko, C., Moreno, M.A., Pomba, M.C., Pyörälä, S.,

442 Ruzauskas, M., Sanders, P., Threlfall, E.J., Torren-Edo, J., Törneke, K., 2011a.

443 Scientific Advisory Group on Antimicrobials (SAGAM). Review on methicillin-

444 resistant *Staphylococcus pseudintermedius*. J. Antimicrob. Chemother. 66, 2705-2714.

445 Van Duijkeren, E., Kamphuis, M., van der Mije, I.C., Laarhoven, L.M., Duim, B.,

446 Wagenaar, J.A., Houwers, D.J., 2011b. Transmission of methicillin-resistant

447 *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets,

448 humans and the environment in households and veterinary clinics. Vet. Microbiol. 16,

449 1–6.

450 Wagenvoort, J.H., Sluijsmans, W., Penders, R.J., 2000. Better environmental survival of

451 outbreak vs. sporadic MRSA isolates. J. Hosp. Infect. 45, 231–234.

452 Weese, J.S., Faires, M.C., Frank, L.A., Reynolds, L.M., Battisti, A., 2012. Factors

453 associated with methicillin-resistant versus methicillin-susceptible *Staphylococcus*

454 *pseudintermedius* infection in dogs. J. Am. Vet. Med. Assoc. 240, 1450–1455.

455 White, S.D., Brown, A.E., Chapman, P.L., Jang, S.S., Ihrke, P.J., 2005. Evaluation of

456 aerobic bacteriologic culture of epidermal collarette specimens in dogs with superficial

457 pyoderma. J. Am. Vet. Med. Assoc. 226, 904–908.

458 Woolley, K.L., Kelly, R.F., Fazakerley, J., Williams, N.J., Nuttall, T.J., McEwan, N.A.,

459 2008. Reduced *in vitro* adherence of *Staphylococcus* species to feline corneocytes

460 compared to canine and human corneocytes. Vet. Dermatol. 19: 1-6.

461 **Table 1:** Univariable analysis of putative risk factor variables from animals with MRSP

462 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

463   Information was based on questionnaire data referring to the six months prior to *S.*

464   *pseudintermedius* isolation. Ref.: reference category; OR: odds ratio; CI: confidence

465   interval. *Modelled as ordered categories (none, 1-5, 6-10, >10).

466

467

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.