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1 Characterization of *Erysipelothrix rhusiopathiae* isolates from poultry, pigs,
2 emus, the poultry red mite and other animals

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1 Abstract

2 *Erysipelothrix rhusiopathiae* is the causative agent of erysipelas in mammals and birds,
3 especially pigs and poultry. In order to investigate the suitability of different subtyping
4 methods for genetic and phenotypic similarities among Swedish isolates of the organism, 45
5 isolates from poultry ($n=23$), pigs ($n=17$), emus ($n=2$) and the poultry red mite *Dermanyssus*
6 *gallinae* ($n=3$) were investigated by serotyping, pulsed-field gel electrophoresis (PFGE) and
7 antimicrobial susceptibility testing. Sequence analysis of the 16S rRNA gene was performed
8 on eleven isolates from nine animal species. The results indicated a random scattering of
9 serotypes throughout the dendrogram based on PFGE banding patterns following *Sma*I
10 digestion. In three cases, isolates with an identical PFGE pattern were of differing serotypes.
11 No differentiation into subgroups by antimicrobial susceptibility testing by broth
12 microdilution was possible as results were similar for all isolates. The Minimum Inhibitory
13 Concentrations for most antimicrobials, including penicillin and oxytetracycline, were low.
14 The 16S rRNA gene sequences (1443 nts) from eight of eleven selected isolates of
15 *Erysipelothrix* spp. were identical to that of the type strain *E. rhusiopathiae* ATCC 19414^T.
16 The other three isolates differed from the type strain by two or three nucleotides. While this
17 method may be useful for identification of *Erysipelothrix* spp., it is unsuitable for
18 epidemiological investigations. Similarities in PFGE banding patterns between isolates from
19 chickens and mites supported the hypothesis that *D. gallinae* may act as a reservoir and vector
20 for *E. rhusiopathiae*. Further PFGE studies on *E. rhusiopathiae* isolates are appropriate to
21 investigate the epidemiology of poultry erysipelas.

22

23 **Keywords:** *Erysipelothrix* sp.; poultry; pigs; PFGE; serotyping; antimicrobial susceptibility

24

1 **Introduction**

2 *Erysipelothrix (E.) rhusiopathiae* is a gram-positive rod-shaped bacterium belonging to the
3 family *Erysipelotrichaceae* (Verborg et al., 2004). Different serotypes of the bacterium occur
4 worldwide and infection has been reported in many species (Brooke and Riley, 1999).

5 Healthy pigs can carry *E. rhusiopathiae* in lymphoid tissues, and may therefore constitute a
6 reservoir of the bacterium (Wood, 1999). In turkey and chicken flocks *E. rhusiopathiae* may
7 cause severe disease outbreaks (erysipelas) (Eriksson et al., 2003). A closely related species,
8 *E. tonsillarum* is considered to be non-pathogenic for pigs and chickens (Takahashi et al.,
9 1994) but may cause clinical disease in dogs (Takahashi et al., 2000).

10
11 Serotyping is the traditional tool for subtyping of *E. rhusiopathiae*. Pulsed-field gel
12 electrophoresis (PFGE) has been used for examining the genetic diversity among strains of *E.*
13 *rhusiopathiae* (Opriessnig et al., 2004) and for studies of clonality during outbreaks of
14 erysipelas in poultry (Købke et al., 2005). Antimicrobial susceptibility testing has been useful
15 for subtyping in other bacterial species as subtypes have been correlated to susceptibility
16 groups (Aspevall et al., 2006). In previous studies antimicrobial susceptibility testing of *E.*
17 *rhusiopathiae* has been performed almost exclusively on isolates from pigs (Yamamoto et al.,
18 2001). 16S rRNA gene sequencing is often used for phylogenetic studies and characterization
19 of bacterial isolates. For some groups of bacteria nucleotide differences between sequences
20 make the method suitable for epidemiological investigations (Heldtander et al., 2001).

21
22 The purpose of this study was to investigate the suitability of different subtyping methods for
23 genetic and phenotypic similarities between Swedish isolates of *E. rhusiopathiae* from
24 poultry, pigs, emus and the poultry red mite *Dermanyssus gallinae*. In addition, sequence
25 analysis of the 16S rRNA gene was performed on eleven isolates from nine animal species.

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Materials and Methods

Isolates investigated

Altogether 45 isolates of *E. rhusiopathiae* were characterized by serotyping, PFGE and antimicrobial susceptibility testing. Twenty-five isolates from chickens ($n=20$), turkeys ($n=3$) and emus ($n=2$) were collected from outbreaks of erysipelas during 1998 to 2004. The isolates from outbreaks in chickens were from flocks housed in indoor litter based systems (14 isolates from 11 farms) or on organic farms where the birds had access to outside pens and/or pasture (6 isolates from 3 farms). Two of the isolates from turkeys were from backyard flocks whereas the third isolate represented an outbreak among commercial breeder turkeys housed indoors. All avian isolates were from birds diagnosed with septicaemia at routine necropsy and represented one to four randomly selected isolates from all farms from which *E. rhusiopathiae* were isolated at the National Veterinary Institute (SVA, Uppsala, Sweden) during the study period. An additional three isolates from the hematophagous poultry red mite (*D. gallinae*) were collected during outbreaks in flocks of laying hens as described by Nordenfors and Chirico (2001). Seventeen porcine isolates of *E. rhusiopathiae*, collected between 1996 and 2003 from pigs in seventeen different herds geographically scattered over Sweden, were also investigated. The isolates from pigs were selected from the strain collection at the National Veterinary Institute (SVA, Uppsala, Sweden) based on a history of characteristic macroscopic lesions of erysipelas noted at necropsy or slaughter. The isolates were compared with the type strain of *E. rhusiopathiae* (ATCC 19414^T) which was obtained from the Culture Collection at the University of Göteborg (CCUG) as CCUG 221^T.

Sequence analysis of the 16S rRNA gene and serotyping were performed on eleven isolates from the strain collection at the SVA from nine different animal species previously diagnosed

1 as infected with *E. rhusiopathiae* (Table 1). One of these (Bd 6531/96, from a dog) was
2 previously typed and deposited at CCUG (CCUG 36858). These isolates were derived from
3 Swedish cases diagnosed between 1981 and 2003, and chosen for analysis with the aim to
4 give as wide variation as possible as regards year of isolation and host. Three isolates from
5 laying hens were included as erysipelas is considered an emerging disease in this population
6 in Sweden. All isolates were stored in serum broth with 15% glycerol at -70°C , until further
7 testing.

8

9 *Culture and biochemical identification method*

10 Samples were cultured on horse blood agar plates (blood agar base no. 2 [LabM], 39.5 g/L;
11 citrated horse blood [SVA], 50 ml/L) and on bromcresol purple lactose agar plates (balanced
12 peptone, 10 g/L [LabM]; NaCl, 5 g/L [Merck]; sodium ammoniumphosphate, 1 g/L [Merck];
13 Lab Lemco Powder, 4 g/L [Oxoid]; Agar no. 2, 10 g/L [LabM]; lactose solution 20%, 50 ml/L
14 [SVA]; bromcresol purple solution 1.6%, 1 ml/L [SVA]). Bacterial growth was examined
15 after 24 and 48 h incubation at 37°C in 5% CO_2 . Colonies were identified by colony
16 morphology, Gram staining and biochemical tests (Quinn et al., 1994). Isolates identified as
17 *E. rhusiopathiae* were catalase-negative and oxidase-negative. The isolates usually fermented
18 lactose, glucose and maltose, were H_2S positive and arginine was hydrolysed. The isolates did
19 not ferment rhamnose or sucrose and did not produce indole or urease. The isolates were
20 stored as described above. Each strain was streaked on 5% horse blood agar plates and
21 incubated at 37°C overnight, before further analyses were made.

22

23 *Serotyping*

24 All isolates were serotyped by the tube agglutination method at the National Veterinary
25 Institute, Copenhagen, Denmark, as previously described by Wellmann et al. (1983).

1

2 *DNA preparation and pulsed-field gel electrophoresis*

3 One horse blood agar plate of bacteria with an overnight culture was harvested for each
4 isolate. The bacteria were suspended in 250 µl of lysis buffer (10 mM Tris-HCl, pH 8.0, 1 M
5 NaCl, 200 mM EDTA, 0.5% Sarcosyl and 0.2% sodium deoxycholate) and immediately
6 mixed with an equal volume of 1.5% low melt point agarose, and the mixture was left to
7 solidify in agarose plug moulds at 4°C for 10 minutes. Gel plugs were incubated overnight in
8 2.5 ml of lysis buffer, supplemented with lysozyme (Roche; Basle, Switzerland) to a final
9 concentration of 1.0 mg/ml, in a shaking incubator at 37°C. New lysis buffer was added, this
10 time supplemented with proteinase K (Roche) to a final concentration of 1.0 mg/ml, and
11 incubation was performed for 20 h in a shaking incubator at 54°C. After three washes with
12 pre-warmed double-distilled water and four washes with pre-warmed TE-buffer (10 mM Tris,
13 1 mM EDTA, pH 7.4) at 54°C for 15 min each, genomic DNA was digested with the
14 macrorestriction enzyme *Sma*I (GE Healthcare; Amersham, England), according to the
15 manufacturer's instructions. Plug slices were loaded onto a 1.2% agarose gel, and genomic
16 DNA was separated by electrophoresis for 20 h in a CHEF DRII apparatus (Biorad; Hercules,
17 Cal., USA), while the pulse switch time was increased from 0.5 s to 40 s. PFGE patterns were
18 analysed with the GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). A
19 dendrogram was prepared by the Unweighted Pair Group Method with Arithmetic mean
20 (UPGMA), dice coefficient and 0.5% optimization with 1.4% band position tolerance.

21

22 *Antimicrobial susceptibility testing*

23 A 96-well broth microdilution panel, VetMIC™ Large animal panel (National Veterinary
24 Institute, SVA, Uppsala) was used for antimicrobial susceptibility testing with concentration

1 ranges as per Table 2. The antimicrobial agents were dried in two-fold serial dilutions in the
2 panel according to Table 2.

3
4 All *E. rhusiopathiae* isolates were recovered from frozen (-70°C) stocks and subcultured at
5 least twice on horse blood agar plates before susceptibility testing. One well-filled 1- μl loop
6 of colony material from an overnight culture was suspended in 4 ml of NaCl. From the
7 suspension, two 20- μl loopfuls were transferred to 10 ml of brain heart infusion broth [Difco]
8 supplemented with 10% fetal calf serum, giving a final inoculum density of 1×10^6 CFU/ml.
9 Each well in the VetMICTM panel was inoculated with 50 μl . The wells were sealed with
10 transparent adhesive tape and then incubated at 37°C for 18 h. The Minimum Inhibitory
11 Concentration (MIC) was read as the lowest concentration where no visible growth, or the
12 most significant reduction of growth, was observed. The assays were performed in duplicate
13 for each isolate.

14
15 For purity control, each bacterial suspension was streaked on a horse blood agar plate and
16 incubated at 37°C for 18 h. Two wells were used as growth controls in each panel without
17 antimicrobial agents, but containing dried dilution buffers. The inoculation density was
18 determined by viable counts on horse blood agar plates after incubation at 37°C for 24 h. The
19 type strain *E. rhusiopathiae* ATCC 19414^T, was included in each test run. The reference
20 strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were
21 tested in duplicate. The three reference strains were tested as described for the clinical isolates
22 of *E. rhusiopathiae*.

23
24 As a further quality check of the panels, tests were performed with the reference strains *E.*
25 *faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC

1 27853 and *S. aureus* ATCC 29213. These four strains were tested in cation-adjusted Mueller
2 Hinton broth, precultured for 4 h at 37°C and then diluted to a final inoculum density of $1 \times$
3 10^5 CFU/ml and incubated at 37°C for 16–18 h.

4

5 *Sequence analysis of the 16S rRNA gene*

6 The 16S rRNA gene of the eleven selected isolates was amplified with primers suitable for
7 members of the phylum *Firmicutes* (Johansson et al., 2006). The amplicons were used for
8 cycle sequencing with labelled terminators (Big Dye; Applied Biosystems, Foster City, Cal.,
9 USA) and with the sequencing primers described by Johansson et al. (2006). The sequencing
10 products were analysed with an ABI Prism 3100 genetic analyser (Applied Biosystems) and
11 contigs (clones with contiguous nucleotide sequences) were generated by using the Contig
12 Express program included in the Vector NTI Suite (InforMax, Bethesda, Md, USA). The
13 contigs were edited manually by using the Genetic Data Environment software (Smith, 1992)
14 before further analysis and deposition in GenBank. The sequences of the 16S rRNA gene of
15 the isolates were used for similarity searches in GenBank by applying the BLASTn program.

16

17 **Results**

18 *Serotyping*

19 The most prevalent *E. rhusiopathiae* serotype from pigs, poultry, emus and *D. gallinae* (17 of
20 45 isolates) was serotype 1a while the second most prevalent (serotype 2b; $n=12$) was only
21 found among pig isolates. In addition, six isolates were reported as serotype 1ab (isolates that
22 reacted with sera for both serotype 1a and serotype 1b). Serotypes 1b, 4, 5 and 6 were
23 represented by two isolates each. Two of the pig isolates as well as five of the isolates
24 selected for 16S rRNA sequencing were not typeable. Detailed results are shown in Fig. 1 and
25 Table 1.

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Pulsed-field gel electrophoresis

A dendrogram based on PFGE-banding patterns is presented in Fig. 1. The isolates were distributed among several different PFGE patterns. Five PFGE groups with multiple identical isolates (100% PFGE homology) were found. Three of these groups contained more than one isolate from the same poultry or emu farm, together, in all three cases, with isolates from other farms. Two out of three isolates from the poultry red mite *D. gallinae* were found to be identical to the isolate from the corresponding outbreak in laying hens. The third mite isolate clustered closely (90% homology) to the corresponding poultry isolate. The other two of these five PFGE groups contained isolates from pigs that had no known epidemiological relationship to each other.

Antimicrobial susceptibility testing

The MIC distributions of all antimicrobial agents are presented in Table 2. The results were similar for all strains and differentiation into subgroups was not possible. For most of the antimicrobial agents tested, including penicillin and oxytetracycline, the MICs were low. The aminoglycosides gentamicin, neomycin, and streptomycin had uniformly high MICs, greater than or equal to the highest concentration tested. The MICs obtained for all the antimicrobial agents tested in duplicate did not differ for any isolate.

For the reference strain *E. rhusiopathiae* ATCC 19414^T, the MICs of all antimicrobial agents from four tests did not differ (Table 2). The MICs of the reference strains *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 were within the recommended ranges (National Committee for Clinical Laboratory Standards, 2002; Clinical and Laboratory Standards Institute, 2005).

1

2 *Sequence analysis of the 16S rRNA gene*

3 The results of the sequencing of the 16S rRNA gene of the eleven isolates are presented in
4 Table 1. The length of the sequences was 1443 nucleotides and they have been deposited in
5 GenBank under the accession numbers listed in Table 1. All eleven isolates have been
6 deposited in CCUG (Table 1). Eight of the sequences were identical to the 16S rRNA
7 sequence of *E. rhusiopathiae*, strain ATCC 19414^T. One of the sequences (from strain B
8 470/87) differed by two nucleotides from the sequence of the type strain. Two of the
9 sequences (from strains B 2344/87 and Bd 6531/96) were identical to the 16S rRNA sequence
10 of *E. tonsillarum*, strain ATCC 43339^T.

11

12 **Discussion**

13 In this study, isolates of *E. rhusiopathiae* were investigated by serotyping, PFGE,
14 antimicrobial susceptibility testing and 16S rRNA sequencing. PFGE was found to be a
15 suitable method for genotypic studies of *E. rhusiopathiae* and provided further support for the
16 previously reported hypothesis that *D. gallinae* may act as a vector of or reservoir for the
17 bacteria, especially among laying hens.

18 In our study, serotyping clearly distinguished strains from certain hosts as pigs were the only
19 host to carry serotype 2b and emus the only host with serotype 5. Therefore most of the pig
20 isolates were of a serotype not found in poultry while most of the poultry isolates were of a
21 serotype (1a or 1ab), less common in pigs. Interestingly, no correlation between serotype and
22 PFGE pattern could be observed as serotypes were randomly scattered throughout the
23 dendrogram. Furthermore, isolates with the same PFGE pattern were of different serotypes in
24 three cases. The significance of this finding is uncertain. However, based on the limited
25 number of serotypes found in our material we conclude that serotyping is less suitable for

1 studies on epidemiological relationships between flocks. PFGE however appears to be a
2 useful method for this purpose. In five cases, PFGE linked isolates from different farms with
3 no known epidemiological connection. This could be due to lack of historical epidemiological
4 information on possible contacts between these farms or to a limitation of the PFGE method
5 in separating the strains. This latter problem could be overcome by using one or several
6 additional restriction enzymes.

7
8 A novel finding in this study was that isolates with different PFGE patterns came from the
9 same laying hen farms (farms B and F in Fig. 1). The five isolates from farm B represent
10 outbreaks in four different organic laying hen flocks in the years 1998, 1999, 2001 and 2004.
11 For farm F, the five isolates represent almost simultaneous outbreaks on the farm in separate
12 flocks, housed in a litter-based aviary system, in 2001 to 2002. The differing PFGE patterns
13 might be attributable to a genetic instability of *E. rhusiopathiae* in a flock over time. This has
14 been discussed previously by Cross and Claxton (1979) regarding changes of serotype.
15 Another explanation could be that the outbreaks were caused by different strains with
16 different PFGE types. Extended field studies or experimental challenge studies need to be
17 undertaken to ascertain the genetic stability of *E. rhusiopathiae*.

18
19 In two outbreaks on different farms (farms B and F), isolates with identical PFGE-banding
20 patterns were found in laying hens and *D. gallinae*. The relationship between isolates from
21 mites and affected laying hens found by PFGE (Fig. 1) supports our previously reported
22 hypothesis that mites may act as a vector of, or reservoir host for *E. rhusiopathiae* (Chirico et
23 al., 2003). *D. gallinae* is very difficult to control and has so far proved to be extremely
24 difficult to eradicate from commercial laying hen operations (J. Chirico, personal
25 communication).

1
2 Pig and poultry isolates were randomly scattered throughout the PFGE dendrogram, and were
3 not separated into two distinct populations, which could suggest that pigs may act as a source
4 of the infection for poultry. However, no identical pairs of isolates were found between the
5 two species, thus we cannot draw the conclusion that the same isolate of *E. rhusiopathiae* can
6 infect both pigs and poultry.

7
8 Antimicrobial susceptibility testing was not a useful method for subtyping of *E.*
9 *rhusiopathiae*, as most isolates from both poultry and pigs in this study gave similar results.
10 To our knowledge this is the first report of antimicrobial susceptibility of chicken isolates of
11 *E. rhusiopathiae*. In previous studies, the agar dilution method has been used on isolates from
12 pigs (Takahashi et al., 1984, 1987; Yamamoto et al., 1999, 2001) and a variety of sources
13 including humans (Fidalgo et al., 2002). However, for most laboratories this method is a time-
14 consuming and tedious procedure for the often small number of isolates involved. In this
15 study, broth microdilution was a reliable and reproducible method for susceptibility testing of
16 *E. rhusiopathiae*. The MIC endpoints were easy to read and the method was readily
17 performed. In the present investigation, all isolates had low MICs for penicillin, the drug of
18 choice for treating *E. rhusiopathiae* infections (Prescott, 2006). All isolates also had low
19 MICs of oxytetracycline, the antimicrobial most widely used in poultry (Hofacre, 2006). Field
20 experience from Sweden supports this finding, as some flocks with acute outbreaks of
21 erysipelas have been successfully treated with oxytetracycline, which is the only approved
22 antimicrobial for layers in Sweden today (Eriksson et al, 2003). In previous studies on isolates
23 from pigs the MICs of oxytetracycline varied (Takahashi et al., 1984, 1987; Yamamoto et al.,
24 1999, 2001).

25

1 The 16S rRNA genes of eleven isolates from nine different Swedish animal species were
2 sequenced in this study. The fact that these sequences contained only minor nucleotide
3 differences indicates that the method is unsuitable for epidemiological investigations of
4 erysipelas outbreaks. Moreover, there are only three nucleotide differences between the 16S
5 rRNA sequences of the type strains of *E. rhusiopathiae* and *E. tonsillarum*. The results should
6 therefore be interpreted with caution, as rather few 16S rRNA sequences of *E. rhusiopathiae*
7 and *E. tonsillarum* have, so far, been deposited in GenBank. Furthermore, three of the
8 sequences deposited in GenBank as *E. rhusiopathiae* (AB055907, AB055909 and AB055910)
9 contained more nucleotide differences to the type strain than to *E. tonsillarum*. Notably, two
10 isolates in this study, from a rat (Feinstein and Eld, 1989) and a dog, were identified
11 biochemically as *E. rhusiopathiae*, based on their inability to ferment sucrose, but results
12 obtained by 16S rRNA sequencing indicated that these isolates were actually *E. tonsillarum*.
13 Our finding that sucrose-negative strains of *E. tonsillarum* might be misdiagnosed as *E.*
14 *rhusiopathiae* are consistent with those of Okatani et al. (2000) who questioned both the
15 fermentation of sucrose and serotyping as appropriate methods for distinguishing the two
16 species. A simple method suitable for differentiating *E. tonsillarum* from *E. rhusiopathiae* in
17 routine diagnostic laboratories is needed.

18

19 **Conclusions**

20 PFGE was in our study found to be a suitable method for epidemiological studies of outbreaks
21 of erysipelas. Our results further support the previously proposed hypothesis that *D. gallinae*
22 may act as a reservoir for and possibly as a vector of *E. rhusiopathiae*. Collecting isolates
23 from affected poultry flocks for further epidemiological studies by PFGE is recommended. To
24 establish the stability in PFGE-patterns extended field studies or experimental challenge

1 studies, need to be undertaken. Sequence analysis of the 16S rRNA gene might be useful for
2 differentiation between *E. rhusiopathiae* and *E. tonsillarum* but this requires further study.

3

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8

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13

1 **Figure captions**

2 Figure 1. Dendrogram for 45 field isolates from poultry, pigs, emus, the poultry red mite
3 (*Dermanyssus gallinae*) and the type strain of *Erysipelothrix rhusiopathiae* ATCC 19414^T,
4 based on pulsed-field gel electrophoresis banding patterns produced by *Sma*I restriction
5 digestion.

6

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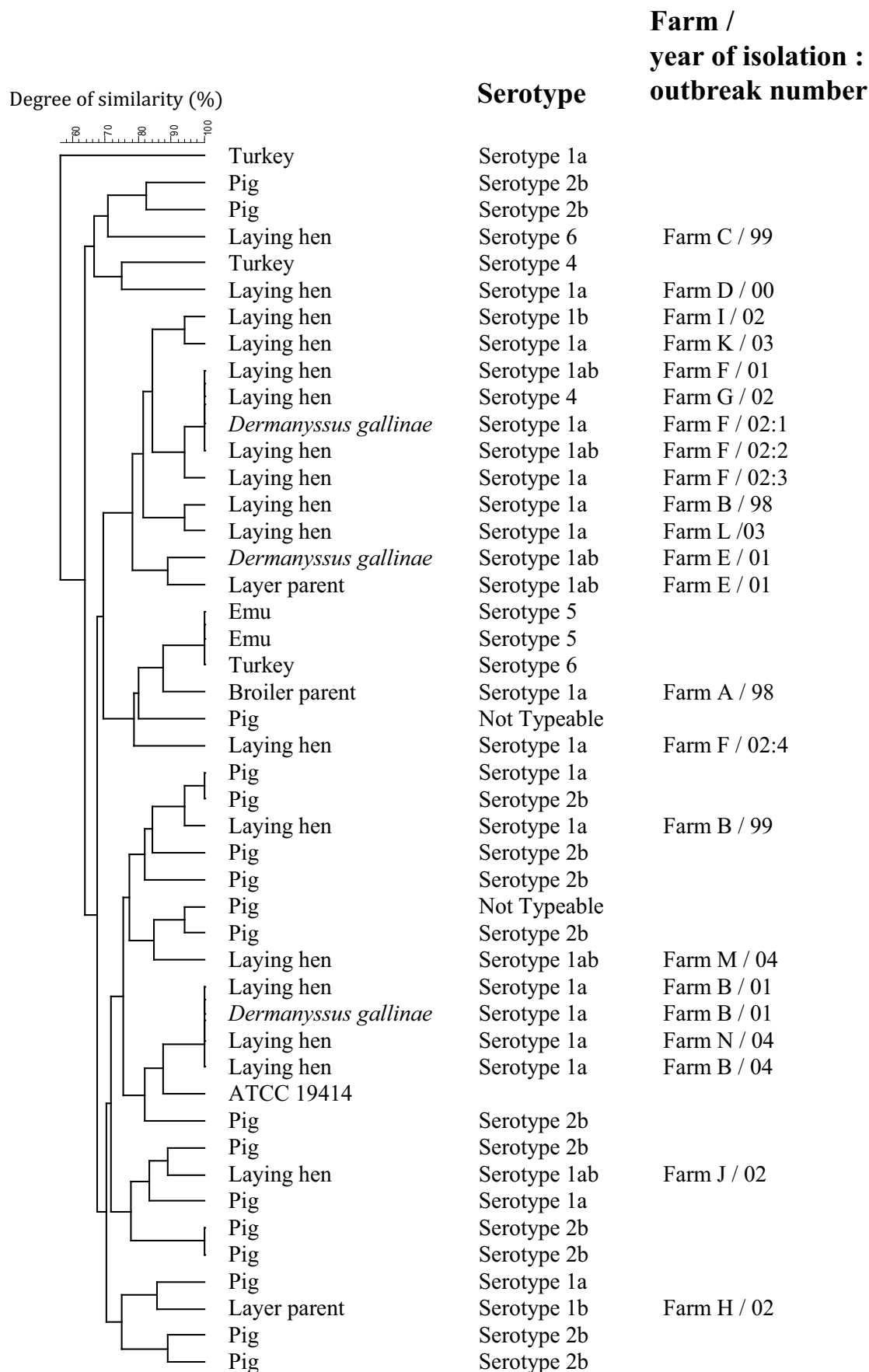


Table 1. Sequence analysis of the 16S rRNA gene from Swedish *Erysipelothrix* sp. isolates from various animal species

Strain/year	Serotype ^a	Host	Result of 16S rRNA sequencing	Accession number in GenBank	CCUG accession number ^b
B 3950/81	2	Pig (<i>Sus scrofa domesticus</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050038	CCUG 56459
B 1877/84	2	Hare (<i>Lepus</i> sp.)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050039	CCUG 56460
B 470/87	NT	Mink (<i>Mustela vison</i>)	Two nucleotide differences to the type strain of <i>E. rhusiopathiae</i> ^c	EF050040	CCUG 56461
B 2344/87	NT	Rat (<i>Rattus norvegicus</i>)	Identical to the type strain of <i>E. tonsillarum</i> ^d	EF050041	CCUG 56462
B 1688/92	NT	Sheep (<i>Ovis aries</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050042	CCUG 56463
B 1667/97	2	Harbour seal (<i>Phoca vitulina</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050043	CCUG 56464
B 2962/01	NT	White-tailed eagle (<i>Haliaeetus albicilla</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050044	CCUG 56465
B 2669/02	1ab	Laying hen (<i>Gallus gallus domesticus</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050045	CCUG 56466
B 781/03	1a	Laying hen (<i>Gallus gallus domesticus</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050046	CCUG 56467
Bd 6531/96	NT	Dog (<i>Canis lupus familiaris</i>)	Identical to the type strain of <i>E. tonsillarum</i> ^d	EF050047	CCUG 36858
Bd 4708/99	6	Laying hen (<i>Gallus gallus domesticus</i>)	Identical to the type strain of <i>E. rhusiopathiae</i> ^c	EF050048	CCUG 56468

^a NT, Not typeable

^b Culture Collection, University of Göteborg, Sweden; www.ccug.se

^c *E. rhusiopathiae* ATCC 19414^T

^d *E. tonsillarum* ATCC 43339^T

Table 2. Results of MIC determination of eleven antimicrobial agents for 45 *Erysipelothrix rhusiopathiae* isolates from poultry, pigs, emus and mites by broth microdilution using the VetMIC™ Large animal panel (SVA, Uppsala) MICs for the reference strain *E. rhusiopathiae* ATCC 19414^T are also included

Antimicrobial agent	MIC (µg/ml)			
	Range tested	Range results	MIC ₅₀ ^a	MIC ₉₀ ^a
Ampicillin	1-8	≤ 1	≤ 1	≤ 1
Ceftiofur	0.25-2	≤0.25	≤0.25	≤0.25
Enrofloxacin	0.12-1	≤0.12 – 0.25	≤0.12	0.25
Florfenicol	2-16	4-8	4	8
Gentamicin	2-16	> 16	> 16	> 16
Neomycin	4-32	> 32	> 32	> 32
Oxacillin	0.5-1	≤0.5	≤0.5	≤0.5
Oxytetracycline	1-8	≤1	≤1	≤1
Penicillin	0.12-1	≤ 0.12	≤ 0.12	≤ 0.12
Spiramycin	4-32	≤4	≤4	≤4
Streptomycin	4-32	32 - >32	>32	>32

^aMIC₅₀ and MIC₉₀ are the concentrations at which 50% and 90% of the isolates respectively were inhibited.