Streptococcus canis, the underdog of the genus

Davide Pagnossin a,b,*, Andrew Smith c,d, Katarina Oravcová a, William Weir b

a Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK
b School of Veterinary Medicine, University of Glasgow, Glasgow, UK
c Bacterial Respiratory Infection Service, Scottish Microbiology Reference Laboratory, Glasgow Royal Infirmary, Glasgow, UK
d College of Medical, Veterinary & Life Sciences, Glasgow Dental Hospital & School, University of Glasgow, Glasgow, UK

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ABSTRACT

Streptococcus canis is a multi-host pathogen that causes disease of varying severity in a wide range of mammals, including humans. Dogs and cats appear to be the primary hosts and may play a role in transmitting infection to humans. The broader epidemiology of S. canis, however, is still poorly understood, as are its virulence mechanisms, antimicrobial resistance (AMR) and population structure. In this review we gather existing knowledge on S. canis, describing its epidemiology in animals and humans and present information on virulence factors, classification schemes and AMR prevalence. We describe the main ecological niches of S. canis in companion animals, discuss potential risk factors for infection in humans and propose a multi-host transmission cycle. We show that current knowledge on S. canis virulence determinants is limited and sometimes contradictory. We illustrate the different typing systems proposed to classify S. canis. We also report the range of known AMR phenotypes and the emergence of new mechanisms of resistance. Finally, we discuss the zoonotic potential of S. canis, highlighting the need for further evidence in this area. Streptococcus canis may be regarded as a neglected pathogen of one health concern. Further research is needed for its better understanding and effective control.

1. Introduction

The name Streptococcus canis was first used in 1937 to identify streptococci implicated in infection in dogs (Stafseth et al., 1937). Only in the late 1980s was the name formally ascribed to a bacterial species with defined phenotypic characteristics (Devriese et al., 1986), when it was described as Gram positive, β-haemolytic, Lancefield group G pyogenic coccus that could infect dogs and cattle (Devriese et al., 1986). Numerous biochemical and physiological traits of the newly identified species were reported (Devriese et al., 1986).

Streptococcus canis was initially thought to be solely a canine and bovine pathogen (Devriese et al., 1986) but has since been isolated from a range of mammals including cats, rats, rabbits, minks, foxes, Japanese raccoon dogs, kinkajous, seals, sea lions, otters, badgers and humans (Richards et al., 2012; Numberger et al., 2021). It might be possible that S. canis is able to cause disease in all the above mentioned species, making it one of the streptococcal pathogens with the widest host range (Fulde and Valentín-Weigand, 2012). Despite its broad host tropism, S. canis has not been given the same attention as other streptococci (Fulde and Valentín-Weigand, 2012) and this is probably due to the limited number of confirmed cases of infection in humans (Lam et al., 2007). Since most streptococcal isolates from human samples are not identified to the species level, however, the true disease burden of S. canis disease in humans is difficult to estimate (Lam et al., 2007).

Knowledge around S. canis epidemiology in human and veterinary medicine is based on a restricted number of studies, summarised in Table 1. It remains unclear how and to what extent inter-species transmission occurs, as well as which risk factors predispose humans to disease. While the zoonotic potential of S. canis is now widely accepted, the scientific evidence remains limited. Virulence mechanisms, population structure and antimicrobial resistance (AMR) determinants are also poorly characterised in S. canis. This review aims to gather all available knowledge on S. canis to contextualise some of the unanswered questions surrounding this multi-host pathogen. As the tiles of a mosaic form a bigger picture only when put together in an ordered pattern, we trust this review will offer a partial yet clearer picture of S. canis, presenting the topic in a critical and accessible way.

* Corresponding author at: Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK.
E-mail address: d.pagnossin.1@research.gla.ac.uk (D. Pagnossin).

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2. Epidemiology and clinical presentations of *S. canis* infection

2.1. Companion animals

In dogs and cats, *S. canis* is regarded as an opportunistic pathogen that can colonise the skin and mucosae of asymptomatic individuals (Lysková et al., 2007a; Timoney et al., 2017). When implicated in disease, *S. canis* is mainly associated with skin infections (Devriese et al., 1986), with the most common isolation sites being the oral and nasal cavities, the external ear canal, rectum and the genital mucosae (Fig. 1) (Devriese et al., 1992; Lysková et al., 2007a). However, infection in dogs and cats may sometimes result in severe clinical syndromes such as arthritis (Iglauer et al., 1991), myocarditis (Matsuue et al., 2007), necrotising fasciitis, pneumonia, meningitis, sepsis and streptococcal toxic shock syndrome (STSS) (Prescott et al., 1995; Pesavento et al., 2007).

In a 2007 study, 6.5% of healthy dogs (n = 35/539) and 5.9% of healthy cats (n = 10/169) tested positive for carriage of *S. canis*, which was isolated principally from the rectum of both species, the praeputium of dogs and the oral cavity of cats (Lysková et al., 2007a). In the same study, it was isolated from various body sites in 22.2% of dogs. Among clinically ill dogs, it was frequently isolated from those with signs of gastrointestinal disease, urogenital infection, otitis externa and rhinitis. In clinically ill cats, *S. canis* was isolated from just two of 42 specimens. However, since co-infection with other pathogens was not considered, it is impossible to determine whether *S. canis* was responsible for the clinical signs reported. As sampling was skewed towards canine samples and external ear canal specimens (Lysková et al., 2007a), this may have contributed to biases in the results reported.

Two other studies report a high prevalence of *S. canis*-associated otitis externa in pets. In one, *S. canis* was shown to be the third most common microorganism isolated from dogs with otitis externa (29.9% of the cases) (Lysková et al., 2007b), being found significantly more frequently in the ear canals of dogs with otitis externa than from healthy dogs (P < 0.001) (Lysková et al., 2007b). Another study revealed a prevalence of 20.83% from the ears of cats with otitis externa, although the sample size was very small (n = 24) (Dégi and Cristina, 2011).

In a work by Lamm et al., the prevalence of streptococcal isolation from all canine specimens submitted to a diagnostic laboratory was 20.5% (n = 499/2432), of which 22.4% (n = 106/499) were confirmed as *S. canis* (Lamm et al., 2010). A high proportion of the sampled dogs that tested positive for *Streptococcus* spp. (n = 267) showed co-infection with other pathogens, meaning that causative role of streptococci in those disease cases could not be established. The authors found that *S. canis* was the most common streptococcal species isolated from infection sites in dogs and that *S. canis* infection can be associated with dermatitis, septicaemia, placentitis and pneumonia (Lamm et al., 2010).

A more recent work by Guerrero et al. suggested an association between vaginal carriage of β-haemolytic streptococci and neonatal death in dogs (Guerrero et al., 2018). No significant difference in the frequency of vaginal isolation of *S. canis*, however, was found between dogs with healthy litters and dogs experiencing neonatal losses (Guerrero et al., 2018). The role of *S. canis* vaginal colonisation in canine fertility is still unclear and further studies are required.

*Streptococcus canis* outbreaks have also been reported in feline colonies and shelters. An outbreak of contagious arthritis due to *S. canis* in a cat breeding colony over a six-month period has been described (Iglauer et al., 1991). A high level of inbreeding among colony cats was suggested to have contributed to susceptibility to infection (Iglauer et al., 1991), although outbreaks have also been detected among shelter cats. Three outbreaks of *S. canis* infection in cat shelters were reported (Pesavento et al., 2007), two of which were characterised by skin ulceration, sinusitis and meningitis while a third outbreak was associated with necrotising fasciitis and sudden death (Pesavento et al., 2007).

Table 1 provides a summary of all case reports referenced in this subsection.

### Table 1

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population sampled</th>
<th>Site of isolation</th>
<th>Prevalence of isolation</th>
<th>Clinical manifestations</th>
<th>Biases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysková et al. (2007a)</td>
<td>Dogs and cats with ongoing infection</td>
<td>Various body sites</td>
<td>22.2% in dogs (39/176) and 4.8% in cats (2/42)</td>
<td>Otitis externa, skin infections, GIT infections, urogenital infections, rhinitis</td>
<td>Sampling bias in favour of canine and external ear canal specimens.</td>
</tr>
<tr>
<td>Lysková et al. (2007b)</td>
<td>Dogs with otitis externa</td>
<td>External ear canal</td>
<td>29.9% (29/97)</td>
<td>Otitis externa</td>
<td>Potentially undetected coinfections.</td>
</tr>
<tr>
<td>Galpéron et al. (2007)</td>
<td>People, patients of the University Hospital of Bordeaux, France</td>
<td>Various body sites</td>
<td>1.3% (80/6404) of all streptococcal isolations</td>
<td>Skin and soft tissue infections, bacteremia, urinary infections, osteoarticular infections, pneumonia</td>
<td>Frequent co-infections with other pathogens.</td>
</tr>
<tr>
<td>Lamm et al. (2010)</td>
<td>Dogs with ongoing infection</td>
<td>Various body sites</td>
<td>4.4% (106/2432)</td>
<td>Dermatitis, septicaemia, pneumonia, placentitis</td>
<td>Frequent co-infections with other pathogens.</td>
</tr>
<tr>
<td>Dégé et al., 2011</td>
<td>Cats with otitis externa</td>
<td>External ear canal</td>
<td>20.8% (5/24)</td>
<td>Otitis externa</td>
<td>Small sample size.</td>
</tr>
<tr>
<td>Timoney et al., 2017</td>
<td>Young healthy cats</td>
<td>Upper respiratory tract and reproductive tract</td>
<td>14.1% (25/177)</td>
<td>Asymptomatic carriage</td>
<td>Sampled cats all attended a small animal clinic for elective procedures.</td>
</tr>
<tr>
<td>Guerrero et al., 2018</td>
<td>Dogs who had delivered litters in which neonatal death occurred</td>
<td>Vagina</td>
<td>91% (21/23)</td>
<td>Neonatal death</td>
<td>Small sample size.</td>
</tr>
</tbody>
</table>

2.2. Production animals

In cattle, *S. canis* is a recognised cause of mastitis (Chaffer et al., 2005; Hassan et al., 2005; Tikofsky and Zadoks, 2005). Although the prevalence of Group G *Streptococcus* mastitis is thought to be low (Wilson et al., 1997), *S. canis* mastitis outbreaks have been reported, with herd prevalence as high as 38% (Chaffer et al., 2005). In a case of *S. canis* sub-clinical mastitis outbreak that affected 22% (n = 11/49) of a dairy herd, pulse field gel electrophoresis (PFGE) genotyping revealed the isolates were either identical or very closely related, suggesting a clonal spread of *S. canis* that may be explained by cow-to-cow transmission (Hassan et al., 2005). A study by Tikofsky and Zadoks described another mastitis outbreak that affected 13% (n = 12/90) of lactating cows in a dairy herd (Tikofsky and Zadoks, 2005). The origin of the outbreak was thought to be a cow with chronic sinusitis due to *S. canis* infection. The cat, whose infection predated the outbreak, lived in close contact with the herd. All bovine and feline *S. canis* isolates showed the same ribotype pattern, supporting the hypothesis that the cat was the outbreak source and that infection subsequently spread from cow to cow (Tikofsky and Zadoks, 2005). A similar case of an outbreak of bovine sub-clinical *S. canis* mastitis associated with a cat was reported by Ebil et al. (Ebil et al., 2021). In this instance, strains of the same multilocus sequence type (MLST) were isolated from nine cows and one cat living in contact with the herd, but no directionality of transmission could be determined.
These reports highlight the potential spread of infectious agents from pets to cattle, which should be considered when assessing biosecurity measures in dairy farms. Both reports, however, rely on low discrimination methods to assess the genetic relatedness of bovine and feline isolates (Salipante et al., 2015; Tsang et al., 2017), so should not be considered as conclusive evidence of cats being the source of infection in outbreak scenarios. Importantly, S. canis mastitis outbreaks have been documented in dairy herds that were not in contact with dogs and cats, suggesting alternative routes of herd infection may occur (Chaffer et al., 2005).

Kröl et al. demonstrated the contagious potential of S. canis among cows (Kröl et al., 2015). Relatedness of the outbreak isolates was confirmed by random amplified polymorphic DNA (RAPD) analysis and PFGE. The authors also showed that S. canis was capable of causing long-term sub-clinical mastitis that persisted for up to 14 months (Kröl et al., 2015). A summary of S. canis mastitis case reports in dairy cattle is shown in Table 3.

### Table 2

Case reports of S. canis infection in companion animals reviewed for this study.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Host species</th>
<th>Number of cases</th>
<th>Clinical manifestations</th>
<th>Suggested predisposing factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iglauer et al.</td>
<td>Cat</td>
<td>6</td>
<td>Arthritis</td>
<td>Possible genetic predisposition due to high inbreeding</td>
</tr>
<tr>
<td>Prescott et al.</td>
<td>Dog</td>
<td>3</td>
<td>Necrotising fasciitis</td>
<td>Trauma</td>
</tr>
<tr>
<td>Matuu et al.</td>
<td>Cat</td>
<td>1</td>
<td>Myocarditis</td>
<td>Acquired mitral stenosis associated with congenital malformation of the mitral valve complex</td>
</tr>
<tr>
<td>Pesavento et al.</td>
<td>Cat</td>
<td>&gt; 150 (3 outbreaks)</td>
<td>Skin ulceration, sinusitis, meningitis, necrotising fasciitis</td>
<td>Indirect contact with dogs and concomitant upper respiratory tract infections</td>
</tr>
</tbody>
</table>

### Table 3

Case reports of S. canis infection in dairy cattle with subclinical mastitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of cases</th>
<th>Herd size</th>
<th>Proportion of herd affected</th>
<th>Suggested risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaffer et al.</td>
<td>26</td>
<td>69</td>
<td>38%</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Hassan et al.</td>
<td>11</td>
<td>49</td>
<td>22%</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Tikofsky and Zadoks</td>
<td>46 Group G Streptococcus cases.</td>
<td>90</td>
<td>51% Group G Streptococcus. 1.3% confirmed S. canis</td>
<td>Direct contact with an infected cat</td>
</tr>
<tr>
<td>Tikofsky and Zadoks</td>
<td>12 confirmed S. canis cases</td>
<td>76</td>
<td>22%</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Kröl et al.</td>
<td>17</td>
<td>76</td>
<td>22%</td>
<td>Direct contact with an infected cat</td>
</tr>
<tr>
<td>Eibl et al.</td>
<td>9</td>
<td>59</td>
<td>15%</td>
<td>Direct contact with an infected cat</td>
</tr>
</tbody>
</table>

(Eibl et al., 2021). These reports highlight the potential spread of infectious agents from pets to cattle, which should be considered when assessing biosecurity measures in dairy farms. Both reports, however, rely on low discrimination methods to assess the genetic relatedness of bovine and feline isolates (Salipante et al., 2015; Tsang et al., 2017), so should not be considered as conclusive evidence of cats being the source of infection in outbreak scenarios. Importantly, S. canis mastitis outbreaks have been documented in dairy herds that were not in contact
2.3. Role in human health

*Streptococcus canis* appears to be rarely isolated from humans, although the actual infection burden is hard to estimate (Lam et al., 2007). It shares the same Lancefield classification (group G) with other β-haemolytic streptococci, such as *S. dysgalactiae* and *S. anginosus*, recognised to infect humans. The determination of Lancefield antigenic group is often sufficient for diagnostic and public health purposes and for this reason the prevalence of *S. canis* infection is likely to be underestimated (Lam et al., 2007).

In a retrospective study carried out at the University Hospital of Bordeaux from 1997 to 2002, *S. canis* was confirmed in 1% (n = 80/6404) of all *Streptococcus*-positive samples submitted for culture (Galpérine et al., 2007). Clinical and microbiological data available for a subset of cases (n = 54) revealed that *S. canis* was mainly involved in skin and soft tissue infection (n = 35), and occasionally implicated in bacteremia (n = 5), urinary tract infection (n = 3), osteoarticular infection (n = 2), pneumonia (n = 1) and asymptomatic carriage (n = 8). Toxic shock was noted in two patients. The majority of the cases for which clinical data was available were confirmed as community acquired (n = 39) and mortality attributable to *S. canis* infection was 3.7% (n = 2/54). Most patients had comorbidities that predated infection and the majority of *S. canis*-positive samples for which data were available (n = 42/54) contained additional bacterial pathogens (Galpérine et al., 2007). It is, therefore, impossible to determine to what extent the presence of *S. canis* contributed to pathology. Fig. 2 illustrates the common sites of *S. canis* isolation in humans (Galpérine et al., 2007).

Sporadic cases of human infection were described in the literature as case reports, with clinical manifestations such as purulent skin infection (Bert and Lambert-Zechovsky, 1997; Whatmore et al., 2001; Lam et al., 2007), cellulitis (Takeda et al., 2001; Lam et al., 2007), septicaemia (Bert and Lambert-Zechovsky, 1997; Takeda et al., 2001; Whatmore et al., 2001; Ohtaki et al., 2013; Taniyama et al., 2017), endocarditis (Amsallem et al., 2014; Lacave et al., 2016; Malisová et al., 2019), arthritis and bone infection (Tarabichi et al., 2018; McGuire et al., 2021). The majority of case reports of *S. canis* infection involve patients above 60 years of age, with various comorbidities or previous trauma. Notably, a proportion of cases describe prior interactions with dogs (Takeda et al., 2001; Lam et al., 2007; Ohtaki et al., 2013; Amsallem et al., 2014; Lacave et al., 2016; Taniyama et al., 2017; Tarabichi et al., 2018; Malisová et al., 2019; McGuire et al., 2021), in particular dog bites or scratches (Takeda et al., 2001; Taniyama et al., 2017; Tarabichi et al., 2018). However, more direct evidence to support the hypothesis that dogs may be a source of *S. canis* zoonotic infection was presented in only one report, which described a woman developing *S. canis* septicemia two weeks after a dog bite (Takeda et al., 2001). *Streptococcus canis* was also isolated from the dog’s oral cavity and both human and canine strains shared the same PFGE pattern, suggesting a canine-to-human transmission event (Takeda et al., 2001). Although generally reliable, PFGE results are occasionally discordant with higher resolution methods such as whole genome sequencing (Salipante et al., 2015). Further evidence is required to clarify the role dogs play in the transmission of *S. canis* to humans.

The reviewed case reports of human *S. canis*-associated disease are summarised in Table 4.

Based on the epidemiological studies and clinical reports available, a transmission cycle for *S. canis* including the environment, human, canine, feline and bovine hosts is hypothesised and is visually represented in Fig. 3.

2.4. Genotyping of *S. canis*

The MLST scheme developed for *S. canis* is based on allelic variation of seven housekeeping genes, namely gki, gtr, murl, mutS, recP, xpt and yqiZ (Pinho et al., 2013). As alluded to in the previous section, there is no evidence that links STs to specific clinical disease manifestation. Only a single study suggested an association between ST-4, 8, 11, 12, 13, 14, 17, 27 and 38, all belonging to clonal complex 13 (CC-13), and canine ulcerative keratitis (Enache et al., 2020) but this is based on a small number of cases and is not statistically supported. With regards to STs and species-specificity, it was shown that isolates sharing the same ST may be isolated from multiple species, including humans, companion animals and wildlife (Pinho et al., 2013).

A genotyping system based on allelic diversity of the *scm* gene has been proposed (Pinho et al., 2019). According to this scheme, 41 allelic variants are grouped into 12 SCM types, forming two major groups. Group I SCM variants (SCM types 1–7) have an IgG binding domain and are most commonly isolated from diseased patients. Group II SCM proteins (SCM type 8–12) lack this domain, which is thought to have anti-phagocytic activity, and the role of group II SCM in pathogenesis is not yet fully understood. MLST has been shown to be a good predictor of SCM type, although the converse is not true (Pinho et al., 2019).

Fukushima et al. suggested an alternative SCM-based typing scheme, which currently encompasses 15 types (Fukushima et al., 2018, 2020a). Based on this scheme, SCM types 1–9 are classified as group I (corresponding to group I in the scheme by Pinho et al.) and types 10–15 are classified as group II (group II also for Pinho et al.). The author of this scheme suggests that SCM group I strains are more commonly isolated in Japan (Fukushima et al., 2020a). As with the scheme of Pinho et al., MLST was shown to be a good predictor of SCM type, although, again, the opposite was not the case. Notably, a significantly higher prevalence of macrolide/lincosamide genetic resistance determinants and fluoroquinolone-resistant phenotype was detected among group I compared to group II strains (Fukushima et al., 2020a).

Recently, an association was found between high-frequency CIA and Fukushima SCM types 10 and 11, as well as high-frequency CIA and STs 21 and 41 (Yoshida et al., 2021). It should be noted, however, that a limited number of isolates were tested (n = 40) and therefore the resulting low frequency or absence of some SCM types and STs might have been a source of bias. Moreover, the threshold value used to separate low-frequency from high-frequency CIA isolates was arbitrarily chosen with the CIA value for almost one fifth of the isolates tested was just above or just below the threshold value (Yoshida et al., 2021).
that it is broadly sensitive to commonly used antibiotics, which may never demonstrated to our knowledge, may occur. Environmental contamination and cats, implying that faecal contamination of the environment, although 2001). Streptococcus canis can be frequently isolated from the rectum of dogs respectively (A: Tikofsky et al., 2017). The main host species of S. canis appear to be dogs and cats. Dogs and cats have been reported as a potential source of infection for humans and cattle, implying that fecal contamination of the environment, although never demonstrated to our knowledge, may occur. Environmental contamination may be a source of infection not only for dogs and cats but also for other susceptible species, namely wildlife and humans. In the diagram, S. canis transmission is represented through solid arrows (direct route) and dashed arrows (indirect route). Question marks are added next to transmission routes that have not been proven yet.

remains uncertain, therefore, whether an association exists between CIA and specific strains of S. canis.

A third SCM-based classification scheme has been described by Timoney et al. 2017. Four SCM types were detected among S. canis isolates (n = 25) from healthy and diseased cats. SCM type 1 strains were most commonly derived from diseased cats, while SCM type 4 strains were almost exclusively isolated from healthy individuals. The authors concluded that type 1 strains were strongly associated with disease and that type 4 strains were avirulent in cats. However, type 1 strains were also isolated from healthy cats and one type 4 strain was implicated in a case of bacteremia, suggesting both types can be associated with either clinical disease or asymptomatic carriage (Timoney et al., 2017).

Fig. 4 summarises the three main genotyping schemes proposed for S. canis.

2.5. Virulence mechanisms

The knowledge on pathogenesis and virulence mechanisms of S. canis is currently limited. This may be explained by the low prevalence of infection in humans and production animals together with the fact that it is broadly sensitive to commonly used antibiotics, which may contribute to it being given a low priority (Galpérint et al., 2007; Pinho et al., 2013). However, the health threat represented by S. canis should not be underestimated, particularly in light of the severe disease cases reported in humans and the documented acquisition of AMR (Takeda et al., 2001; Galpérint et al., 2007; Lam et al., 2007; Lacave et al., 2016; Tan et al., 2016; Fukushima et al., 2020b; McGuire et al., 2021). Potential virulence determinants of S. canis are summarized in Table 5. The presence of sequences homologous to well-characterised S. pyogenes virulence genes was assessed in the genome of S. canis,
including 15 isolates from dogs diagnosed with STSS and/or necrotising fasciitis, by Southern hybridisation (DeWinter et al., 1999). Genes homologous to the S. pyogenes slo and emm, encoding streptolysin O and the M protein, respectively, were detected in the genome of the majority of isolates analysed. However, no matches were found to eight other S. pyogenes virulence genes (speA, speB, speC, speF, scpA, hasA, ska and ssa). Resistance to phagocytosis and presence of surface fibrillae were also observed as S. canis virulence characteristics (DeWinter et al., 1999).

More recently, genomics has been used to characterise virulence of S. canis with 34 candidate virulence genes detected (Richards et al., 2012). Most of these virulence genes constitute part of the S. pyogenes pangeneome and have been implicated in tissue invasion. The carriage of slo and emm homologous genes, already described by De Winter et al. (DeWinter et al., 1999), was confirmed in S. canis. While an orthologue for S. pyogenes exotoxin streptolysin S (SLS) was identified, no genes encoding pyrogenic exotoxins (i.e. those responsible for toxic shock syndrome) were found, suggesting alternative mechanisms in the pathogenesis of S. canis. Some similarity with S. agalactiae and S. pneumoniae virulence genes, such as those encoding CAMP factor and neuroaminidase B, was also found in the S. canis genome analysed (Richards et al., 2012).

Components of the arginine deiminase system (ADS) have been characterised in S. canis genome, giving insights into a metabolic pathway that could have a role in colonisation and disease (Hitzmann et al., 2013). ADS, which is responsible for the catabolism of arginine and production of ATP, citrulline, ornithine, ammonia and carbon dioxide, has been shown to be involved in virulence of Streptococcus suis by increasing its tolerance to adverse environments (Fulde et al., 2011a). Three enzymes of the S. canis ADS are localised on the cell surface, with possible implications for its virulence, so further investigation is warranted (Hitzmann et al., 2013).

The ability of S. canis to invade host cells was recently demonstrated (Yoshida et al., 2021). In S. pyogenes, cell invasion ability (CIA) is mediated by surface proteins such as fibronectin-binding proteins (FBPs) (Walker et al., 2014). The presence of genes with homology to S. pyogenes FBPs in the genome of S. canis has been shown together with experimental evidence of CIA in human and animal S. canis isolates (Yoshida et al., 2021). All 43 isolates tested showed intracellular invasion, but CIA was highly variable. Due to the lack of required clinical data no link could be made between levels of CIA and disease severity (Yoshida et al., 2021) and the role of CIA in S. canis pathogenesis, thus, remains unknown.

The most extensively studied virulence factor of S. canis is the M-like protein SCM (Fulde et al., 2011a). Experimental evidence showed that the S. canis SCM protein binds to plasminogen of humans, pigs, goats, cats and dogs. Interaction with plasminogen facilitates bacterial adherence and tissue invasion, the latter occurring through fibrinogen and fibrin degradation (Fulde et al., 2011a). SCM was also shown to cooperate in plasminogen recruitment with another surface-expressed virulence factor, enolase, and to have anti-phagocytic activity (Fulde et al., 2013). The scm gene has been confirmed as universally present in the S. canis population, although with substantial allelic variation (Pinho et al., 2019). In particular, some scm variants lack the putative IgG binding domain which is thought to contribute to the anti-phagocytic activity of SCM (Bergmann et al., 2017; Pinho et al., 2019). Moreover, some scm alleles are associated with lower binding affinity to plasminogen than others (Fulde et al., 2013; Pinho et al., 2019).

Although according to some studies SCM appears to be linked to S. canis virulence (Fulde et al., 2011a, 2013), recent findings, based on comparisons between a wildtype strain and an SCM-deficient mutant, questioned the role of SCM in clinical infection (Cornax et al., 2021). The SCM-deficient mutant showed reduced ability to form biofilms compared to the wildtype, but haemolytic activity and survivability in the presence of aminising and oxidising agents were not impacted by the lack of scm. There was no effect on survival after exposure to canine macrophages, human neutrophils and human whole blood or the ability to induce an immune response through cytokine production from human monocytes. When tested in vivo, the wildtype strain and the mutant were equally virulent in mouse models of dermal and systemic infection. The SCM-deficient strain, however, showed reduced adhesion and persistence in a murine model of vaginal colonisation when compared to the wildtype, suggesting that SCM might confer fitness advantages in particular anatomical sites (Cornax et al., 2021). Overall, the role of SCM as a virulence factor in S. canis is unclear with recent evidence suggesting a marginal involvement in disease progression.

A correlation between molecular characteristics of bacterial strains and clinical outcome of infection has not yet been shown for S. canis. Evidence based on limited numbers of isolates from dogs with toxic shock syndrome and/or necrotising fasciitis suggested that there was no specific genotype associated with severe disease in dogs (DeWinter and Prescott, 1999). Another study, which included more isolates from dogs and cats also failed to demonstrate a connection (Kruger et al., 2010). Further studies, using larger sample sizes and high-resolution genotyping are required to clarify the association between molecular characteristics of S. canis strains and clinical disease.

A visual summary of S. canis virulence factors is provided in Fig. 5.

### Table 5

<table>
<thead>
<tr>
<th>Virulence traits</th>
<th>Evidence provided</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine deiminidase system (ADS)</td>
<td>Experimental evidence and bioinformatic analysis</td>
<td>Hitzmann et al. (2013)</td>
</tr>
<tr>
<td>Christine and Munch-Peterson (CAMP) factor</td>
<td>Detection of homologous gene based on WGS bioinformatic analysis</td>
<td>Richards et al. (2012)</td>
</tr>
<tr>
<td>Intracellular invasion</td>
<td>Experimental evidence</td>
<td>Yoshida et al. (2021)</td>
</tr>
<tr>
<td>Neuroaminidase B</td>
<td>Detection of homologous gene based on WGS bioinformatic analysis</td>
<td>Richards et al. (2012)</td>
</tr>
<tr>
<td>Resistance to phagocytosis</td>
<td>Experimental evidence – hypotised role of M protein</td>
<td>DeWinter et al. (1999)</td>
</tr>
<tr>
<td>Streptococcus canis M-like (SCM) protein</td>
<td>Detection of homologous gene based on Southern hybridisation</td>
<td>DeWinter et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>Detection of homologous gene based on WGS bioinformatic analysis</td>
<td>Richards et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Experimental evidence – adherence and tissue invasion, plasminogen-mediated</td>
<td>Fulde et al., (2011a)</td>
</tr>
<tr>
<td>Streptolysin O (SLO)</td>
<td>Detection of homologous gene based on WGS bioinformatic analysis</td>
<td>Richards et al. (2012)</td>
</tr>
<tr>
<td>Streptolysin S (SLS)</td>
<td>Detection of homologous gene based on WGS bioinformatic analysis</td>
<td>Richards et al. (2012)</td>
</tr>
<tr>
<td>Surface fibrillae</td>
<td>Direct observation through electron microscopy</td>
<td>DeWinter et al. (1999)</td>
</tr>
</tbody>
</table>

2.6. Antimicrobial susceptibility

Streptococcus canis infections are successfully treated with ampicillin, amoxicillin and clavulanic acid or vancomycin in human medicine and amoxicillin and clavulanic acid or penicillin in veterinary medicine (Takeda et al., 2001; Tikofsky and Zadoks, 2005; Lam et al., 2007;
The most commonly encountered AMR phenotype among S. canis strains is tetracycline resistance, which is expressed by 30–40% of all the isolates and associated with the carriage of tet(M), tet(O), tet(S), tet(K) and tet(L) genes (Galpérine et al., 2007; Lysková et al., 2007b; Pinho et al., 2013; Fukushima et al., 2020b; Yoshida et al., 2021).

Although less frequent, macrolide, lincosamide and streptogramin (MLS) resistance phenotypes have been detected in S. canis strains, particularly in association with the presence of the erm(A), erm(B), mef(A) and aadA genes (Galpérine et al., 2007; Lysková et al., 2007b; Pinho et al., 2013; Fukushima et al., 2020b; Yoshida et al., 2021). Occasional resistance to gentamicin and rifampicin has also been reported in S. canis (Galpérine et al., 2007).

The occurrence of fluoroquinolone resistance associated with specific amino acid substitutions in the quinolone resistance-determining region (QRDR) of the gyrA, gyrB, parC and parE genes has recently been documented in a small number of resistant strains (Fig. 6) (Fukushima et al., 2020b). Table 6 shows the proportion of Fukushima SCM group I and SCM group II strains sharing a macrolide/lincosamide resistance genotype and expressing a fluoroquinolone-resistant phenotype (Fukushima et al., 2018).

2.7. Zoonotic potential

The ability of S. canis to colonise and cause disease in a variety of mammals is well documented (Richards et al., 2012). Human infections are understood to be rare, although there has recently been an increase in reported cases (Takeda et al., 2001; Galpérine et al., 2007; Lam et al., 2007; Lacave et al., 2016; Tan et al., 2016; McGuire et al., 2021), and little is known about epidemiology in humans. Since dogs and cats are recognised as the main host species of S. canis, it is likely that human infection can result from direct ‘pet-to-people’ transmission, making S. canis a potentially zoonotic pathogen (Richards et al., 2012). This hypothesis has been supported by reports of human infections following dog bites and other forms of interaction with companion animals (Bert and Lambert-Zechovsky, 1997; Takeda et al., 2001; Lam et al., 2007). It remains unclear, however, whether all S. canis strains possess the same multi-species tropism profile or whether adaptation has occurred.

Fig. 5. Schematic representation of the three main classification systems proposed for S. canis (Pinho et al., 2013, 2019; Fukushima et al., 2020a).

Fig. 6. Amino acid substitutions observed in the QRDR regions of gyrA, gyrB, parC and parE in thirteen fluoroquinolone-resistant isolates of S. canis. Percentages and fractions represent the proportion of fluoroquinolone-resistant isolates carrying that mutation. Fluoroquinolone resistance was confirmed when the MIC for Levofloxacin by Etest was >1 µg/mL (Fukushima et al., 2020b).

Preliminary evidence based on MLST classification suggests that S. canis strains of the same ST can be found in both animals and humans (Pinho et al., 2013, 2019), inferring lack of host adaptation and zoonotic potential. However, it may be argued that MLST fails to represent
accurately the diversity of bacterial populations when compared to more discriminatory genomic methods (Tsang et al., 2017). Better evidence is required to aid our understanding of the epidemiology of S. canis and provide insight into public health risks.

3. Conclusion

Historically considered a canine pathogen, S. canis is now known to cause disease in a variety of mammals, including humans. Dogs, however, appear to be the primary host and it is considered that this bacterium is part of the skin and mucosal microbiota of healthy individuals. Clinical manifestations of S. canis infection range from mild superficial inflammation to severe invasive disease in dogs, cats, and humans. In cattle, S. canis is responsible for sub-clinical mastitis, which may have an important impact on productivity and animal welfare. Risk factors for S. canis infection are currently unknown, although in humans most cases involve elderly individuals with comorbidities. Direct interaction with dogs, particularly via bites and scratches, is thought to be an important driver of infection in humans, but evidence to support this hypothesis is currently limited. Mechanisms underlying S. canis pathogenesis remain unclear although putative virulence genes have been detected in its genome. The most well-characterised candidate is the SCM protein, which showed virulence potential in vitro but proved to have only a minor involvement in disease development in a murine model. Nevertheless, scm plays an important role in S. canis strain classification, being used by three typing schemes, supplementing the established MLST scheme. The most common AMR in S. canis are towards tetracyclines and MLS, although they are not first line antibiotics for the treatment of streptococcal infections. The recent acquisition of fluoroquinolone resistance conferring mutations should be the subject of future monitoring. Direct transmission of S. canis strains from one host species to another appears likely, although it has only been partially demonstrated and requires confirmation through high-discriminatory genotyping methods. This is important given the relatively high prevalence of asymptomatic S. canis colonisation in dogs. In conclusion, S. canis infections are rare in humans compared to those caused by other bacteria but their real incidence might be underestimated by limitations in diagnostic laboratories, where streptococcal infections are rarely identified to the species level. Regardless of its true disease burden, S. canis infections can be life-threatening in humans and companion animals alike and with important questions on transmission, zoonotic importance and AMR potential still unanswered, this pathogen is a worthy focus of continued research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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References


Table 6

Proportion of S. canis group I and group II SCM strains sharing a specific AMR genotype or phenotype in a study by Fukushima et al. Only AMR profiles with a statistically significant difference in prevalence between SCM groups are reported. Statistical significance is displayed as p value < 0.05 calculated through the Fisher’s exact probability test (two-sided) (Fukushima et al., 2018).

<table>
<thead>
<tr>
<th>AMR profile</th>
<th>Group I SCM</th>
<th>Group II SCM</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolide/lincosamide</td>
<td>29% (15/55)</td>
<td>7.5% (3/40)</td>
<td>p = 0.0013</td>
</tr>
<tr>
<td>resistance genotype</td>
<td>12.5% (7/55)</td>
<td>0% (0/40)</td>
<td>p = 0.0389</td>
</tr>
<tr>
<td>Fluoroquinolone resistance</td>
<td>55%</td>
<td></td>
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<tr>
<td>phenotype</td>
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