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VETERINARY AND ANIMAL PRODUCTION - RESEARCH PAPER

Prevalence and antimicrobial susceptibility of *Streptococcus equi* isolated from horses in Santa Catarina state, Southern Brazil

Roberta F. Veiga[1] · Luiza N. Clarindo[1] · Arthur L. Fensterseifer[1] · Luis H. Pompelli[1] · Ricardo A. P. Sfaciotte[1] · David Germano G. Schwarz[1] · Lidiane R. Eloy[2] · Sandra M. Ferraz[1]

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Abstract
The objective of this study was to determine the prevalence of *Streptococcus equi* (*S. equi* subsp *equi* and *S. equi* subsp *zooepidemicus*) in the state of Santa Catarina and evaluate the antimicrobial susceptibility of the isolates. For this, 420 nasal swab samples were collected from randomly selected horses. Isolation and phenotypic characterization of the bacteria were performed by sowing on 5% sheep blood agar, followed by analysis of morphotinctorial characteristics and biochemical analysis. To differentiate the main beta-hemolytic *Streptococcus* in horses, the fermentation profiles of the sugar's lactose, maltose, sorbitol, and trehalose were used, which were confirmed at the subspecies level by the PCR technique. The antimicrobial susceptibility panel was defined by the disk diffusion method, testing 13 antimicrobials from ten different classes, all regularly used in equine medical clinics, followed by the calculation of the multiple antimicrobial resistance index. Ten strains of *S. equi* were isolated, with a prevalence of 2.38% (10/420). Of the total positive samples, 3% (3/10) were confirmed as belonging to *S. equi* subsp *equi* and 70% (7/10) were confirmed as belonging to *S. zooepidemicus.* Multidrug resistance was observed in 60% (6/10) of isolates. The antimicrobial with the greatest resistance was clindamycin with 70% (7/10), followed by beta-lactams, with 40% (4/10) resistance to penicillin and 30% (3/10) to ceftiofur. The isolates were 100% (10/10) sensitive to gentamicin, chloramphenicol, levofloxacin, and vancomycin. This was the first study carried out in the state, and based on these data, it can be said that Santa Catarina has a low prevalence of *S. equi* and the presence of multi-resistant strains of *S. equi* was confirmed in the equine herd in Santa Catarina.

Keywords Strangles · Equine adenitis · Epidemiology · Multidrug resistance · Upper respiratory tract

Introduction

Brazil stands out on the global equestrian scenery, having the third largest herd of horses in the world, with approximately 6 million animals, with the highest concentration of these animals in the Southeast region [1]. Among Brazilian states, Santa Catarina has the fifteenth largest national herd and stands out in the agricultural scenario due to its strategic position for the movement of animals through the southern region of Brazil and to neighboring countries, such as Argentina, Paraguay and Uruguay. In addition to being an important land route, the state also stands out as an international reference in the production of food of animal origin. This highlights the importance of constant vigilance regarding infectious diseases, with the state of Santa Catarina being a national reference in the control of these diseases.

Among cross-border control diseases, those of the respiratory system are of great importance due to their rapid spread in space and producing significant economic losses when they are not previously diagnosed and treated [2]. Diseases of the respiratory system are of great importance in equine farming, producing significant economic losses if their detection and treatment are not carried out early [3]. Among the pathologies that affect the respiratory tract, strangles and strangles-like diseases stand out. Although it

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✉ Roberta F. Veiga
vetrobertaveiga@gmail.com

[1] Departamento de Medicina Veterinária, Centro Agroveterinário, Universidade do Estado de Santa Catarina, Lages, SC 88520000, Brazil

[2] Departamento de Agrometeorologia e Forragicultura, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91540000, Brazil

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is not mandatory to report in Brazil, this disease causes substantial economic losses to the equine industry worldwide [2]. The losses caused by the disease are related to work interruption, decreased training performance, and treatment costs, in addition to the negative aesthetic impact due to the lymph node abscess [4].

Streptococcus equi subsp *equi* (S. *equi)*, a Gram-positive, beta-hemolytic bacteria belonging to Lancefield group C [5], are the etiologic agent of strangles and strangles-like disease. Although there are systemic forms, those respiratory diseases mainly affect the upper respiratory tract, being considered the most diagnosed equine infectious disease worldwide [6]. Consequently, efforts to improve diagnosis and prevention are of paramount importance to the equine industry [7]. The infection can occur in horses of all ages, but the youngest exhibit more severe clinical signs. On the other hand, older animals are less affected and recover more quickly due to their better immunological status [3], as they have acquired immunity after exposure [8].

Although *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) shares similar morphotintorial characteristics with S. equi [5], it acts as a mucosal commensal, causing opportunistic diseases, especially in conditions of low immunity [7]. This subspecies, not host-adapted, is a frequently isolated pyogenic bacterium from equine joints, lymph nodes, lungs, and nasal cavities, leading to a strangles-like disease [7]. Interestingly, both *S. equi* and *S. zooepidemicus* are challenging to differentiate in field practice.

In this sense, recognizing the carrier status is extremely important for the correct control and eradication of the strangles in potential endemic properties, and the identification of silent carriers in the herd has been associated with the appearance of new cases [9]. These persistent carriers can transmit the microorganism to susceptible horses, compromising the eradication of disease outbreaks [10]. Preventing transmission capacity is even more important since the therapeutic approach to this disease depends on the clinical signs and severity of the disease [4]. Most cases of strangles do not require treatment beyond adequate rest and good management conditions [11], but in clinically severe cases, treatment may include the use of antimicrobials [10].

Penicillin is the antimicrobial of choice for the treatment of acute strangles and other Streptococci diseases; although *S. equi* is considered sensitive to most antimicrobials [10, 12], the emergence of resistant strains is inevitable [13]. The emergence of penicillin resistance has been previously reported [14], justifying the monitoring of susceptibility profiles. In Japan, the presence of multidrug resistance (resistance to beta-lactams, macrolides, and quinolones) in a new species of alpha-hemolytic *Streptococcus* demonstrated the possibility of horizontal transfer of resistance between *Streptococcus* [15]. Associated with this, few data are available on the antimicrobial resistance of *S. equi* and *S. zooepidemicus*, particularly regarding beta-lactams and its transmission capacity between horses in Brazil.

Interestingly, horses are potential reservoirs of multidrug-resistant microorganisms that can be shared with humans through direct or indirect contact [16]. Furthermore, *S. equi* is currently considered a potential zoonotic agent [17], although with a low incidence in humans [18], and may be associated with bacteremia, sepsis, and meningitis in immunocompromised hosts, positioning strangles as a disease of importance in One Health.

Therefore, the present study aimed to verify the prevalence of *S. equi* and *S. zooepidemicus* in the equine herds in the state of Santa Catarina, southern Brazil, and evaluate its sensitivity and multiresistance profile to antimicrobials.

Materials and methods

Research ethics committee

The present study was approved by the Ethics Committee on the Use of Animals of the Universidade do Estado de Santa Catarina (UDESC) under protocol 1476210622 at the Centro de Ciências Agroveterinárias (CAV).

Study area and target population

The state of Santa Catarina has an area of 95,730,684 km² and is in the southern region of Brazil, bordering Paraná state to the north, Rio Grande do Sul state to the south, and Argentina to the west. Santa Catarina is located between the parallels 25° 57′ 41″ S and 29° 23′ 55″ S and between the meridians 48° 19′ 37″ W and 53° 50′ 00″ W, in the southern temperate zone of the planet [19]. The state was geographically divided into six mesoregions by the Brazilian Institute of Geography and Statistics (IBGE) [20]: Grande Florianópolis, Vale do Itajaí, Norte Catarinense, Sul Catarinense, Serrana, and Oeste Catarinense. According to the Santa Catarina Integrated Agricultural Development Company (CIDASC), through the Santa Catarina Agricultural Defense Management System database [21], the Santa Catarina equine herd consists of 106,354 animals, distributed as follows: Grande Florianópolis with 11,186 animals, Vale do Itajaí with 17,282, Norte Catarinense with 15,453, Sul Catarinense with 15,242, Serrana with 17,271, and Oeste Catarinense with 29,920 animals.

Sample collection and survey design

For the epidemiological survey, all six mesoregions of the state were covered, considering the total number of animals in the Santa Catarina herd as the study population [21], and the number of samples was defined through the sufficiency test, which was determined according to Thursfield [22], by the following formula:

$$n = \frac{Z^2.P.Q.N}{e^2.(N-1) + Z^2.P.Q}$$

where n = size sample; Z = confidence interval; P = population belonging to the studied category; Q = population that does not belong to the studied category; N = population size; e = maximum error. As there was no previous study of the prevalence of strangles in Santa Catarina, a prevalence of 50% was considered, with a confidence limit of 5%. In this way, a minimum total of samples from 383 animals was obtained. However, for the present study, 420 animals distributed across properties in the state of Santa Catarina were used. All sample calculations were performed using the *samplingbook* package [23] of the RStudio program (version 1.4.1717).

To define the municipalities included in the present study, it was stipulated that the municipalities with the largest equine population in each region would be part of the study, with a minimum number of 700 animals in the municipality to be considered in the present study, reaching a minimum of three municipalities of each region. As there was no statistical difference between the number of animals per region, a standard of 70 samples was stipulated in each region, totaling 420 samples in the state and obtaining a safety margin in the number of samples. The properties were selected randomly and represented different breeding and management profiles (Fig. 1). The horses representing different breeds, both sexes and aged between 8 months and 15 years.

At each property visited, consent to carry out the sampling was obtained by signing the free and informed consent form. The selection of animals on the properties was non-probabilistic and for convenience, as not all animals were permitted to be collected by the owners due to participation in sporting events or training, for example. Data on the animals and properties were obtained during visits to the

Fig. 1 Location of properties in the six mesoregions in the state of Santa Catarina, for the study of the prevalence of *S. equi* in horses

properties. The sample collections were conducted during the local winter, between July and August 2022.

Samples were collected using sterile swabs from the nasal cavity in the upper respiratory tract of animals with and without clinical signs. Before collection, the external regions of the nostrils were cleaned with a paper towel, and the rostral-median region of both nostrils were lightly rubbed with the swab for approximately 15 s. After collection, the samples were placed in transport medium (15% agar-agar) inside a refrigerated isothermal box (temperature between 2º and 8º C) and sent to the Animal Microbiological Diagnosis Center (CEDIMA) of CAV/UDESC, for bacterial isolation and identification.

Bacterial isolation and characterization

Samples were plated in 5% sheep blood agar and incubated at 37 °C for 48 h. After isolation, macro and microscopic analysis of the colonies were carried out. Bacterial colonies that presented morphotinctorial characteristics compatible with the genus *Streptococcus* were subjected to biochemical tests to identify the species, with esculin, bile-esculin, CAMP, Voges-Proskauer (VP), and 6.5% NaCl tests being carried out, in addition to resistance to bacitracin and sulfamethoxazole + trimethoprin (STX) [24, 25]. Still, the fermentation of the sorbitol, trehalose, maltose, and lactose was analyzed [26]. The fermentation tests made it possible to differentiate the subspecies of *S. equi* subsp. *equi*, *S. equi* subsp. *zooepidemicus*, and *S. equisimilis.*

DNA extraction and PCR detection of *Streptococcus equi* isolates

The isolates that showed phenotypic characteristics for *S. equi* were cultivated in BHI broth at 37ºC for 24 h. For bacterial DNA extraction, 200µL of the inoculum was transferred to a sterile microtube. Then 500 µl of chloroform: isoamyl alcohol (24:1) was added and incubated in a water bath at 56º for 30 min. Subsequently, it was centrifuged at 12,000 rpm for 10 min and the supernatant was transferred to a new sterile microtube. Then, 600 µl of 70º alcohol was added and centrifuged again at 13,500 rpm for 20 min. After this procedure, the supernatant was carefully discarded by inversion and 200 µL of Milli -Q water was added to the sample. DNA concentration measurements were performed in Nanodrop (ThermoFischer ®, Waltham, USA). To perform the PCR assay, the DNA concentration was adjusted to 20-100ng/µl.

The PCR reaction was carried out as previously described by Alber [27]. Briefly, targets for the *sodA* gene were used to confirm the samples as belonging to the *S. equi* species and both *seeH* g and *seeI* genes for the *S. equi equi* or *S. zooepidemicus* subspecies, respectively. The sequence of the primers used is described in Table 1. Individual reactions were performed to analyze each target gene. The calculation for the reaction composition was performed for a final volume of 20 µl containing 10pmol of each primer, dNTP 20mmol each, PCR buffer (Tris- HCl −20mM, KCl −50mM), MgCl2 (1.5mM) Taq DNA polymerase (1U), MiliQ water to complete the final volume and 2 µl of bacterial DNA.

Amplification was carried out in a thermocycler (Verity 96 well Thermal Cycler - Applied Biosystems by Thermofisher ®) for the different reactions, following the temperature profile: 3 min cycle at 94ºC for initial denaturation, followed by 30 cycles of denaturation at 94 ºC for 30 s, annealing at 59 ºC for 30 s and extension at 72 ºC for 40s. The last cycle was at 72ºC for 5 min of final extension, followed by holding at 4ºC.

The amplified products were subjected to electrophoresis at 100 V and 400 mA for 30 min, in a 2% agarose gel. 7µL of sample labeled with 2µL of GelRed ® nucleic were used acid gel stain 2X (Uniscience) and 1µL 6X Blue/Orange Loading Dye (Thermofischer®). The molecular marker used was 100 bp DNA Ladder stained with 6X Blue/Orange Loading Dye (Thermofischer®). The gel was visualized using an L-Pix EX transilluminator (Loccus ®).

For positive control in biochemical tests, sugar fermentation and PCR, isolates of *Streptococcus equi* subsp *equi* ATCC33398 and *Streptococcus equi* subsp *zooepidemicus* ATCC 700400 were used.

Table 1 Sequence of primers used for molecular confirmation of species and subspecies of *Streptococcus equi* isolates, with gene *sodA* being confirmatory for *S. Equi* and genes *seeH* and *seeI* confirmatory for *S. equi* subsp *equi*

Gene	Oligonucleotide sequence	Size (bp)
sodA	F: CAG CAT TCC TGC TGA CAT TCG TCA GG A: CTG ACC AGC CTT ATT CAC AAC CAG CC	235
seeH	F: AGC ATG ATT CTA ACT TAA TTG AAG CCG A: TAG CAT GCT ATT AAA GTC TCC ATT GCC	503
seeI	F: GAA GGT CCG CCA TTT TCA GGT AGT TTG A: GCA TAC TCT CTC TGT CAC CAT GTC CTG	520

Antimicrobial sensitivity profile analysis and multidrug resistance

To verify the antimicrobial resistance profile of the isolates, the disk diffusion method was used on Muller-Hinton agar plus 5% defibrinated sheep blood [12]. Ten classes of antimicrobials were selected for the sensitivity test (TSA), using as criteria the 13 antimicrobials most commonly used for the treatment of respiratory tract infections and other bacterial infections in horses. The following antimicrobials were used: Beta-lactams: penicillin G (10U) and ceftiofur (30 µg); Aminoglycosides: gentamicin (120 µg) and

streptomycin (300 µg); Macrolides: erythromycin (15 µg); Lincosamides: clindamycin (2 µg); Ansamycins: rifampicin (5 µg); Phenicols: chloramphenicol (30 µg); Fluorquinolones: enrofloxacin (5 µg) and levofloxacin (5 µg); Tetracyclines: tetracycline (30 µg); Sulfonamides: trimethoprim-sulfamethoxazole (25 µg); Glycopeptides: Vancomycin (30 µg). Zones of growth inhibition around each antimicrobial disc were measured and interpreted using criteria established by the human and animal CSLI [12]. For the evaluation of multi-resistant microorganisms, the criteria provided by the CLSI were used, when the tested isolate is resistant to at least one antimicrobial from three different classes [12, 28].

Statistical analysis

For point prevalence (instant prevalence) analysis, the standard formula Prevalence = Number of isolates/population sampled x 100 was used [23]. The sample sufficiency test was carried out using the *samplingbook package* [22]. Descriptive statistics were performed using absolute frequency (*table* function) and relative frequency (*prop.table* function). All statistical analyzes were performed using the RStudio statistical program (version 1.4.1717).

Results

Streptococcus equi isolates and prevalence study

Of the 420 nasal swab samples collected from horses, ten were positive for *S. equi*, determining a prevalence in the Santa Catarina equine herd of 2.38% (10/420) and 23.07% (9/39) per property for *S. equi.* It was observed that 93.6% (393/420) of the horses sampled did not show clinical respiratory signs, while 6.4% (27/420) did (Table 2). Of the samples, 53% (223/420) were female and 47% (197/420) were male. Additionally, 3.8% (16/420) were foals under 1-year-old, while 96.2% (404/420) were adults aged 1 to 9 years. As for the positive animals, 80% (8/10) did not show clinical respiratory signs at the time of collection and 80% (8/10) were female.

Table 2 Total number of horses sampled, according to clinical signs and sex in Santa Catarina, Brazil

		Total of horses
Clinical Signs	Yes	27
	No	393
Sex	Female	223
	Male	197

Of the total positive samples, 3% (3/10) were confirmed as belonging to *S. equi* subsp *equi* and 70% (7/10) were confirmed as belonging to *S. equi* subsp *zooepidemicus*. Positive isolates were confirmed phenotypically and through PCR. For the isolates of *S. equi* subsp *equi*, 1 isolate was from the Vale do Itajaí region, 1 from the Oeste region and one from the Serrana region. For the *S. equi* subsp *zooepidemicus* isolates, 2 were isolated from the Vale do Itajaí region, 3 from the Oeste region and 2 from the Serrana region. In 20% (2/10) of the animals sampled, the presence of clinical respiratory signs were observed at the time of collection (Table 3).

Only on one property, located in the Serrana region, were both subspecies of *S. equi* isolated. In the other properties, only one subspecies of the microorganism was found.

Antimicrobial susceptibility testing and multidrug resistance

In the present study, the routine use of beta-lactams was verified in the treatment of infectious diseases, especially in respiratory tract conditions, with the preference for the use of penicillins and streptomycin observed in 48.7% (19/39) and ceftiofur in 17.95% (7/39) of the properties visited. Trimethoprim-sulfamethoxazole is the treatment of choice in 46.15% (18/39) of the properties visited. The results of

Table 3 *Streptococcus equi* isolates, regarding region, municipality, age, clinical signs and history of respiratory disease outbreak in horses from Santa Catarina, Southern Brazil

Isolated	Region	Municipality	CS	Age	Outbreak history on the property	Outbreak resolved	Bacteria isolated
1	Vale do Itajaí	Camboriú	Yes	1 y	Yes	No	*S. equi equi*
2	Oeste	Xanxerê	No	8 m	Yes	Yes	*S. equi equi*
3	Serrana	Lages	Yes	5 y	No	NA	*S. equi equi*
4	Vale do Itajaí	Gaspar	No	4 y	No	NA	*S. equi zooepidemicus*
5	Vale do Itajaí	Itajai	No	4 y	No	NA	*S. equi zooepidemicus*
6	Oeste	Chapecó	No	2 y	No	NA	*S. equi zooepidemicus*
7	Oeste	Chapecó	No	7 y	No	NA	*S. equi zooepidemicus*
8	Oeste	Concordia	No	7 y	No	NA	*S. equi zooepidemicus*
9	Serrana	Lages	No	9 y	No	NA	*S. equi zooepidemicus*
10	Serrana	Lages	No	6 y	Yes	Yes	*S. equi zooepidemicus*

y = years; m = month. CS = clinical signs. NA = Not applied

the antimicrobial susceptibility test (TSA) of *S. equi* isolates may be seen in Table 4.

The isolates showed 100% sensitivity to gentamicin (aminoglycoside class), levofloxacin (fluorquinolones), chloramphenicol (phenicols) and vancomycin (glycopeptides). The greatest resistance was observed to clindamycin, with 70% (7/10) of isolates demonstrating resistance, followed by the beta-lactam class, where 40% (4/10) demonstrated resistance to penicillin and 30% (3/10) for ceftiofur. Resistance to streptomycin and erythromycin was 10% (1/10). For rifampicin and sulfamethoxazole + trimethoprim, resistance was 50% (5/10) and 20% (2/10) respectively. As for tetracycline and enrofloxacin, we obtained resistance in 20% (2/10) and 10% (1/10) respectively, with both showing an intermediate sensitivity pattern in 10% (1/10) of the isolates.

It was observed that 10% (1/10) of the samples demonstrated sensitivity to 100% of the antimicrobials tested, while 60% (6/10) of the positive samples showed a multiresistance pattern according to the referenced criteria.

Discussion

The state of Santa Catarina is strategically located as a transit route for animals from the state of Rio Grande do Sul to Paraná and other Brazilian states. In this way, the control of equine diseases, such as strangles, within the state has an impact on the maintenance of animal and human health throughout Brazil and neighboring countries. In the present study, it was found that the prevalence of *S. equi* in Santa Catarina was similar (2.38%) to that found in the state of Rio Grande do Sul (2.37%), also located in southern Brazil [30], and in Jammu and Kashmi region (2.87%), India [30]. In both studies, samples were collected through nasal swabs from clinical and subclinical animals. The difference between the present study and previous studies is the large sampling of subclinical animals and the collection of material only from the nasal cavity. Obviously, when we compare our results with studies that evaluated the prevalence in animals with clinical signs and the collection of material from characteristic lesions, and guttural pouches, which made the isolation of the pathogen easier, these results are even greater, with prevalence of 18.4%, 45.2%, and 13.8% [31–33], respectively. Therefore, apparently, in the state of Santa Catarina, low prevalence of S. *equi* in horses without clinical signs were verified, demonstrating that clinically affected animals are the main transmitters of the agent to susceptible hosts.

It is important to note that this study used nasal swabs to collect samples from animals both with and without clinical signs, which may explain the low prevalence found in the

Table 4 Antimicrobial susceptibility profile of *Streptococcus equi* isolated from horses in Santa Catarina, Southern Brazil, using the disk diffusion method to different classes of antimicrobials

Sample	Beta-lac		Aminog		Mac	Linco	Ans	Fen	Fluorq		tetra	Sulfa	Glyco
	PEN	CFT	GEN	EST	ERI	CLI	RIF	CLO	ENO	LEV	TET	SUT	VAN
1	S	S	S	S	S	S	S	S	S	S	S	S	S
2	R	S	S	S	S	R	R	S	S	S	S	S	S
3	S	S	S	S	S	S	R	S	S	S	S	S	S
4	S	S	S	R	S	S	S	S	R	S	I	S	S
5	R	S	S	S	R	R	R	S	S	S	S	S	S
6	S	R	S	S	S	R	R	S	I	S	R	R	S
7	R	R	S	S	S	R	S	S	S	S	S	R	S
8	S	R	S	S	S	R	S	S	S	S	R	S	S
9	R	S	S	S	S	R	R	S	S	S	S	S	S
10	S	S	S	S	S	R	S	S	S	S	S	S	S

Beta-lac = beta-lactams; Aminog = aminoglycosides; Mac = macrolides; Lin = lincosamides; Ans = ansamycins; Phen = phenicols; Fluorq = fluoroquinolones; Tetra = tetracyclines; Sulf = sulfonamides; Glyco = glycopeptides; PEN = penicillin G; CFT = ceftiofur; GEN = gentamicin; EST = streptomycin; ERI = erythromycin; CLI = clindamycin; RIF = rifampicin; CLO = chloramphenicol; ENO = enrofloxacin; LEV = levofloxacin; TET = tetracycline; SUT = trimethoprim-sulfatoxazole; VAN = vancomycin; R = resistant; I = intermediate; S = sensitive. Isolates 1 to 3 refer to *S. equi* subsp *equi*. Isolates 4 to 10 refer to *S. equi* subps. *zooepidemicus*

present study. The literature supports this method [8], and other studies using the same technique also reported a low prevalence of *S. equi* [13, 30].

The 23.07% (9/39) of prevalence of properties found in the present study are considerably higher than those obtained in a previous study [29], which found a prevalence of 5.86% (20/341) and also differs from that found in the study by Jaramillo-Morales [33], with 60% (9/15). The high prevalence of positive properties found in this study may be related to the lack of biosecurity techniques observed, since 89.75% (35/39) of the properties visited did not employ any quarantine method for new animals and/or isolation of animals with clinical signs.

Curiously, only 2 of the 10 isolates were from horses with clinical signs, which reinforces the importance of knowing the subclinical carrier status of the pathogen and its potential for the emergence of new cases [9]. Curiously, the ages of the positive animals ranged between 8 months and 9 years (Table 3). These findings are consistent with previously published studies in which equines of all ages were susceptible to infection, but commonly observed in young horses or those for which the etiologic agent was never detected [10].

Interestingly, the positive horses for *S. equi* subsp *equi* belonged to 3 different regions of the state. The properties have severe characteristics in common, such as predominantly extensive management and as they were breeding farms. None of the properties employ quarantine for new animals introduced to the farm and sharing equipment, such as brushes, halters, water troughs were part of the routine. According to Durham [34], bacteria can remain alive on different surfaces for different periods, but due the constant colonization of these surfaces, infection control becomes even more difficult. The fact that one of the positive animals did not present clinical signs but had previous contact with the microorganism in an outbreak already resolved on the property reinforces that the survival of the microorganism may be greater than that found in previous studies, pointing to a survival of *S. equi* for approximately 30 days [35]. According to Frost [36], the pathogen survives for about 7 days outside the host, and a later study [37] demonstrated survival for 5 days on different surfaces.

Another interesting point was the isolation of an animal with clinical signs, but with no history of the disease on the property and no record of the animal's movement (entry and exit) in the last six months. As bacteria are transmitted directly through contact with infected horses or indirectly through contaminated equipment such as training equipment, stalls/grazing objects and water buckets [36], environmental contamination with bacteria excreted from an infected horse may represent a significant source of contagion [34]. This animal was probably a silent carrier, as it was subclinical, but could shed *S. equi* intermittently in its environment for months or even years, thus serving as a persistent source of infection for other horses [37].

In this epidemiological context, the rapid and accurate identification of horses infected by *S. equi* is essential for implementing appropriate biosecurity procedures to control the strangles [6]. Following treatment or an adaptive immune response, most horses recover from the illness within a few weeks [5]. However, in approximately 10% of convalescent horses, dried and hardened residual abscess material in the guttural pouches or sinus tracts forms chondroids, which may harbor live *S. equi* [38].

Biosecurity practices on equestrian properties in Santa Catarina are scarce and insufficient, allowing various individuals easy access to the animals. Similarly, the absence of quarantine facilities and implementation of biosecurity measures in all visited equestrian properties may increase the risk of introducing S. equi and other pathogens. Therefore, limiting animal exposure remains the best method to prevent S. equi infections [10]. Additionally, biosecurity measures should include, but not be limited to, quarantine and screening of new arrivals, appropriate disinfection and cleaning of equipment, isolation, and monitoring of clinically affected animals and their contacts [9].

In the present study, the routine use of certain antimicrobials as the first choice for infectious diseases was observed, such as penicillins and streptomycin in 48.8% and ceftiofur in 16.43% of the properties visited. This may justify the detection of resistance in 40% of isolates to penicillin, 10% to streptomycin, and 30% to ceftiofur. Many veterinarians and international literature still consider S. equi generally sensitive to beta-lactam antimicrobials [10, 39], justifying the empirical use of these medications in routine equine medicine. However, a study conducted in Canada [31] demonstrated the emergence of resistance in S. equi subsp. zooepidemicus isolates to penicillin (5% of samples were considered resistant), while S. equi subsp. equi maintained 100% sensitivity to penicillin and ceftiofur. Indeed, the present study demonstrated that streptococcus resistance to beta-lactams is emerging, and caution must be exercised when using these antimicrobials.

Sulfamethoxazole-trimethoprim is the option of choice in 45% of the properties visited when treating various infectious diseases. This factor may be contributing to the emergence of resistance to these drugs, even in commensal and opportunistic microorganisms. Regarding sensitivity to trimethoprim-sulfamethoxazole, Clark et al. [31] found a sensitivity of 79% for *S. equi*, compared of the 80% found in the present study. Both results differ from those found by Jaramillo-Morales et al. [33] and Kirinus et al. [40], where 100% of *S. equi* isolates were sensitive to trimethoprim-sulfamethoxazole. It has been reported by many veterinarians

that animals with equine adenitis recovered better when given trimethoprim-sulfadiazine treatment [16].

Interestingly, to *S. equi*, the resistance to most antimicrobials is low, except for aminoglycosides, including gentamicin, and this resistance is constantly observed [10]. However, the findings in the present study demonstrate sensitivity to aminoglycosides, including gentamicin, in 100% (10/10) of isolates, although resistance to streptomycin was observed in 10% (1/10) of the samples analyzed. The fact that most commercial presentations of penicillins are associated with streptomycin may be contributing to the development of this resistance. This result demonstrates the importance of veterinarians aligning their treatment with clinical suspicion and basing the choice of drug on antimicrobial susceptibility tests in their region of operation.

When analyzing the resistance rate to multiple antimicrobials of *S. equi* isolates, 60% (6/10) of the isolates showed multidrug resistance. On the other hand, 10% (1/10) of the isolates were sensitive to all antimicrobials tested. Studies carried out in the state of Rio Grande do Sul, Brazil [40] found 13% (5/38) of multidrug resistance and 39.4% (15/38) were sensitive to all antimicrobials tested (20 antimicrobials in 10 classes). In São Paulo, Brazil [12], 70% of samples identified were multidrug resistant to *S. equi*. The emergence of resistance to several antimicrobials is of considerable medical importance and may be related to their irrational and sometimes uncontrolled use in the treatment of infectious diseases [41].

The treatment of infections in the upper respiratory tract of horses depends on the stage and severity of the disease [10]. When it comes to cases of strangles, veterinary opinion remains divided regarding the usefulness of antimicrobial treatment [38]. Depending on the site of infection, penicillin is commonly viewed as the preferred choice for treating non-pneumococcal streptococcal disease [11], while other drugs are considered depending on ease of administration.

Regarding the animals colonized with *S. equi* subsp *zooepidemicus*, it is important to highlight that none of these animals showed clinical signs at the time of collection and that, unlike *S. equi* subsp *equi*, this microorganism is a mucosal commensal that produces opportunistic infections with clinical signs like equine adenitis [8]. However, the isolation of this microorganism with a high rate of multiresistance to antimicrobials is a warning sign, because of the transmission of resistance mechanisms between different species of *Streptococcus* has already been confirmed [15]. The fact that animals are colonized with these multidrug-resistant bacteria in their commensal microbiota may contribute to the development of resistance in pathogenic microorganisms.

The antimicrobial susceptibility profiles of beta-hemolytic *Streptococcus* are somewhat predictable and do not vary greatly between different geographic locations [42]. This statement is refuted by the present study, in which sensitivity profiles varied within regions of the same state. For this reason, it is extremely important that the veterinarian perform diagnostic tests and antibiograms to correctly identify the infectious agent and its sensitivity profile to antimicrobials before deciding on the course of treatment.

Equine veterinarians have many questions to consider when deciding whether antimicrobials are indicated for the treatment of a particular patient. Especially when it is analyzing the context of concern about the growing acquired resistance of bacteria isolated from humans and animals (including horses) to existing antimicrobials and the scarcity of new drugs [43].

Conclusion

The state of Santa Catarina has a low prevalence of *S. equi* (2.38%) and the presence of multi-resistant strains (60%) in its equine herd. An important finding was resistance in the beta-lactam group, with 40% resistance to penicillin and 30% to ceftiofur. These circulating isolates are even resistant to first-choice antimicrobials in the treatment of respiratory streptococcal infections in animals and humans, and the presence of resistance genes in the isolates should be investigated. This is the first scientific report on the prevalence and resistance of *Streptococcus equi* carried out in Santa Catarina state.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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