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Streptococcus equi subspecies equi from strangles suspected equines: molecular detection, antibiogram profiles and risk factors

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Abstract

Strangles, caused by *Streptococcus equi subspecies equi*, is a highly infectious disease of equines causing major health issues and financial losses. The aim of the study was to detect the presence of the *SeM* gene in *Streptococcus equi* isolated from equine suspected of having strangles. A cross-sectional study design was conducted from July to December 2022 in five districts of the central Gondar zone, Ethiopia. One-hundred sixty swab samples were taken from animals that had been clinically suspected. The *SeM* gene was detected using polymerase chain reaction, and the antimicrobial susceptibility test was performed using the Kirby-Bauer disc diffusion method. The binary logistic regression model was employed to test for statistical significance. In 31.87% (51/160) of the samples, *Streptococcus equi* species were isolated, and 31.37% (16/51) of these species carried the *SeM* gene. There was a significant amount of tetracycline (81.5%), erythromycin (81.5%), and vancomycin (75.5%) resistance among the 16 isolates. Strangles were more likely to be present in animals who shared feed containers (AOR = 7.59; 95% CI = 1.44–39.93), drank from the same water troughs (AOR = 7.74; 95% CI = 1.44–41.01), and spent the night together (AOR = 5.97; 95% CI 1.41–25.37). The findings of this study showed that the research areas harboured *Streptococcus equi subspecies equi*. Sharing feed containers and water troughs were potential sources of strangles infection; thus, these containers need to be cleaned regularly.

Keywords Antimicrobial susceptibility, Central Gondar zone: equines, SeM gene, Strangles, Streptococcus equi subspecies equi

Introduction

Ethiopia has a large equine population and gained a number of benefits from them; however, they are the most abused, mistreated, accorded low social status and the most neglected animals in Ethiopia [1]. Moreover, equines are often treated with less respect than other livestock species and suffer from infectious and noninfectious diseases that reduce the productivity of these animals [2, 3].

One of the diseases, strangles, is a highly infectious disease of equines caused by *Streptococcus equi subspecies equi*, resulting in significant economic losses to equine husbandry [4]. It affects the upper respiratory system of horses, donkeys, and mules of all ages and breeds [4, 5]. The disease is characterized by abrupt onset of fever followed by upper respiratory tract catarrh, as evidenced by mucopurulent nasal discharge and acute swelling with subsequent abscess formation in the submandibular and retropharyngeal lymph nodes [5–8]. Transmission of infection occurs when there is either direct or

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indirect transfer of *S equi* between affected and susceptible horses. Purulent discharges from animals with active and recovering strangles are an important and easily recognizable source of new *S equi* infections [6].

Strangles is misdiagnosed and complicated by other infections such as pneumonia, guttural pouch empyema and purpura hemorrhagic (a small hemorrhage in the skin) [4, 8]. The culture of nasal swabs, nasal washes, or pus aspirated from abscesses remains the "gold standard" for the detection of *S equi* [6, 7]. *S. equi subspecies equi* is a beta-hemolytic that is often identified using biochemical tests, such as catalase and oxidase negativity and the absence of trehalose, lactose, mannitol, or sorbitol fermentation [9]. Polymerase chain reaction (PCR) is designed to detect the DNA sequence of *SeM*, the gene for the antiphagocytic M protein of *S equi* [6]. The *SeM* gene is a significant virulence component that protects *S. equi subspecies equi* against phagocytosis and is only present in this strain, making it a viable candidate for research on strangles outbreaks [10].

The stage and severity of the disease usually determine the best method of action for strangles. Most strangles cases do not need any treatment; instead, care is given to encourage animals to get better on their own [7]. Penicillin is generally considered the drug of choice for the treatment of nonpneumococcal streptococcal disease, with alternative drugs used depending on ease of administration or the site of infection [6]. Some reports indicated that antibiotics used in the treatment of strangles failed due to the development of resistance in *Streptococcus species* [11]. The investigation of strangles outbreaks requires interviews with animal owners to get a complete history and assess the probable full extent of the disease problem [6].

According to the authors' knowledge and information, there have not been any confirmed reports of strangles in Ethiopia, despite field veterinarians' preliminary diagnosis of the disease. As a result, this study aimed to isolate *S. equi subspecies equi*, molecularly detect the *SeM* gene, determine its antibiotic susceptibility profile, and identify the risk factors for strangles in equines in the central Gondar zone.

Materials and methods

Study areas

The study was carried out in Gondar City, Gondar Zurya, Wegera, Dembya, and Chilga districts, all located in the central Gondar zone of the Amhara region of Ethiopia (Fig. 1). The zone is located in northern Ethiopia, at 12.3° to 13.38° N latitude and 35.05° to 38.3° E longitude, and has an altitude ranging from 900 to 2267 m above sea level (m.a.s.l.). The average annual temperature is between 19.5 and 26 °C, while the annual rainfall is between 880 and 1772 mm. The total population of equines in the central Gondar zone is 34,417 horses, 8,619 mules, and 437,989 donkeys.

Study animals

Animals (horses, donkeys, and mules) presented to the veterinary clinics manifesting clinical signs such as cough, sneezing, nasal discharge, fever, and swollen lymph nodes, which were suspected as strangles and included in the study [6, 7].

Study design and sampling technique

A cross-sectional study design was employed from July to December 2022. The study districts were purposefully selected based on the potential of the equine population, the inclusion of highland, midland and lowland agroecology, and the involvement of equines in the daily life of the community.

Sample size and data collection methods

The sample size was all animals presented in each clinic suspected of strangles throughout the study period. Physical examination measurements such as body temperature, heart rate, and respiration rate were taken as part of the clinical examination (Supplementary 1). Moreover, equines, which showed cough, swollen lymph nodes with abscess formation, serous and mucopurulent nasal discharge, rupture of the mandibular lymph node, and enlargement of the submandibular lymph node, were clinically suspected to have strangles.

One hundred sixty nasal swab samples (75 horses, 59 donkeys, and 26 mules) were collected. Sterile cotton-tipped swabs moistened with peptone water broth (Microxpress Pvt. Ltd., India) were inserted into the nasal cavities of each equine, and the mucus surface was rubbed by rotating the swabs on both nostrils gently. The swabs were then placed back into labelled sterile universal tubes containing 5 ml of peptone water broth (Microxpress Pvt. Ltd., India). Labelled samples of nasal swabs were transported in an icebox to the Veterinary Microbiology Laboratory of the College of Veterinary Medicine and Animal Sciences, University of Gondar, for microbiological analysis. Questionnaires were used to gather information about strangles to assess the risk factors. Interviews with animal owners who had taken their animals (equines suspected with strangles) to veterinary clinics were conducted to evaluate the owners' ways of managing their animals, their current feeding and watering practices, their history of transportation, and the equines' most recent health state (Supplementary 1).

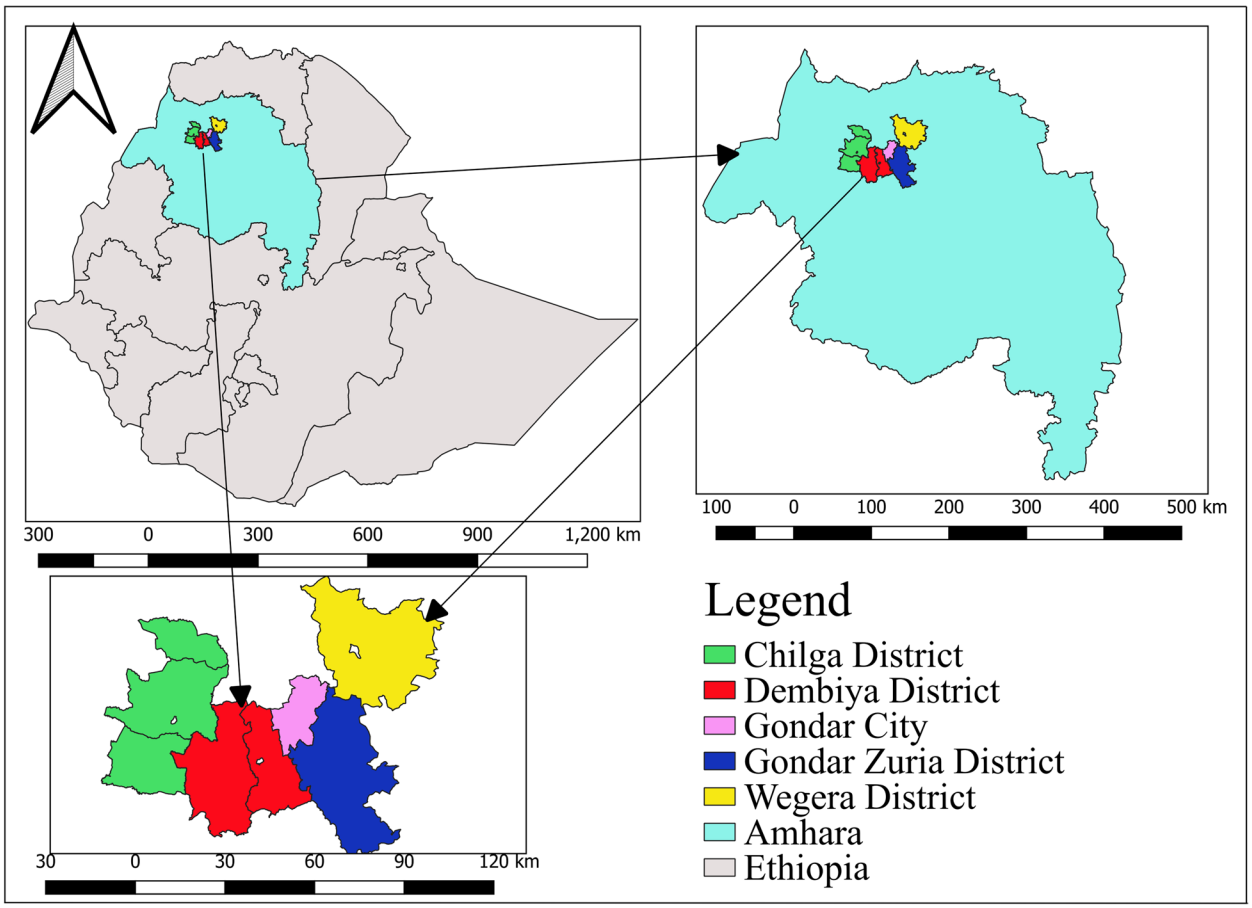

Fig. 1 Map of the study areas prepared using QGIS 3.30

Isolation and identification of *Streptococcusequi* species

Isolation and Identification of *Streptococcusequi* SpeciesCollected swap samples were cultured on 5% blood agar (Sisco Research Laboratories Pvt. Ltd., India) at 37 °C under aerobic conditions, and to differentiate the genus *Streptococcus*, the colony was transferred from blood agar to Edward medium (Oxoid Ltd., Basingstoke, Hants., England), which was supplemented with 5% sheep blood on Edward medium and incubated at 37 °C for 24 h. Under complete aerobic conditions, a honey-like colony was taken and subcultured onto 5% blood agar (Sisco Research Laboratories Pvt. Ltd., India) plates and incubated at 37 °C under aerobic conditions.

The isolated bacteria were subcultured onto MacConkey agar (HiMidia Laboratories Pvt. Ltd., India) to differentiate *Streptococcus* species from *Enterococcus* species, of which *Enterococcus* species can tolerate bile salt and can grow on MacConkey agar, but *Streptococcus* species cannot grow. Therefore, beta-hemolytic colonies were subcultured on fresh 5% blood agar, and the morphology of the *Streptococcus* species (shape, arrangement) was examined by Gram staining. The *Streptococcus equi* species isolates were identified by biochemical tests such as the catalase test, the vogues proskuer test (VP), the oxidative fermentative test (OF), and the carbohydrate fermentation test. Moreover, sugar fermentation tests were carried out for glucose (HiMidia laboratories Pvt., Ltd., India), maltose, sucrose, sorbitol, starch; mannitol (Blulux laboratories Pvt., Ltd., India), lactose (Carelabmed, India), and arabinose (Finkem laboratories Pvt., Ltd., South Africa). The general flowchart of *Streptococcus* species isolation technique is depicted in Fig. 2.

Polymerase chain reaction of the *SeM gene*

Polymerase chain reaction was carried out to detect the presence of the *SeM* gene, which encodes the M-like protein exclusively found in *S. equi subspecies equi.* Therefore, all suspected isolates of *S. equi* species were tested for the *SeM* gene at the Molecular Biology Laboratory, Institute of Biotechnology, University of Gondar. The *Streptococcus equi* species isolates were cultured on tryptone soy broth (Micro-Express, India) and incubated overnight at 37 °C. Then the genomic DNA of *S. equi* was extracted from overnight cultures using the conventional

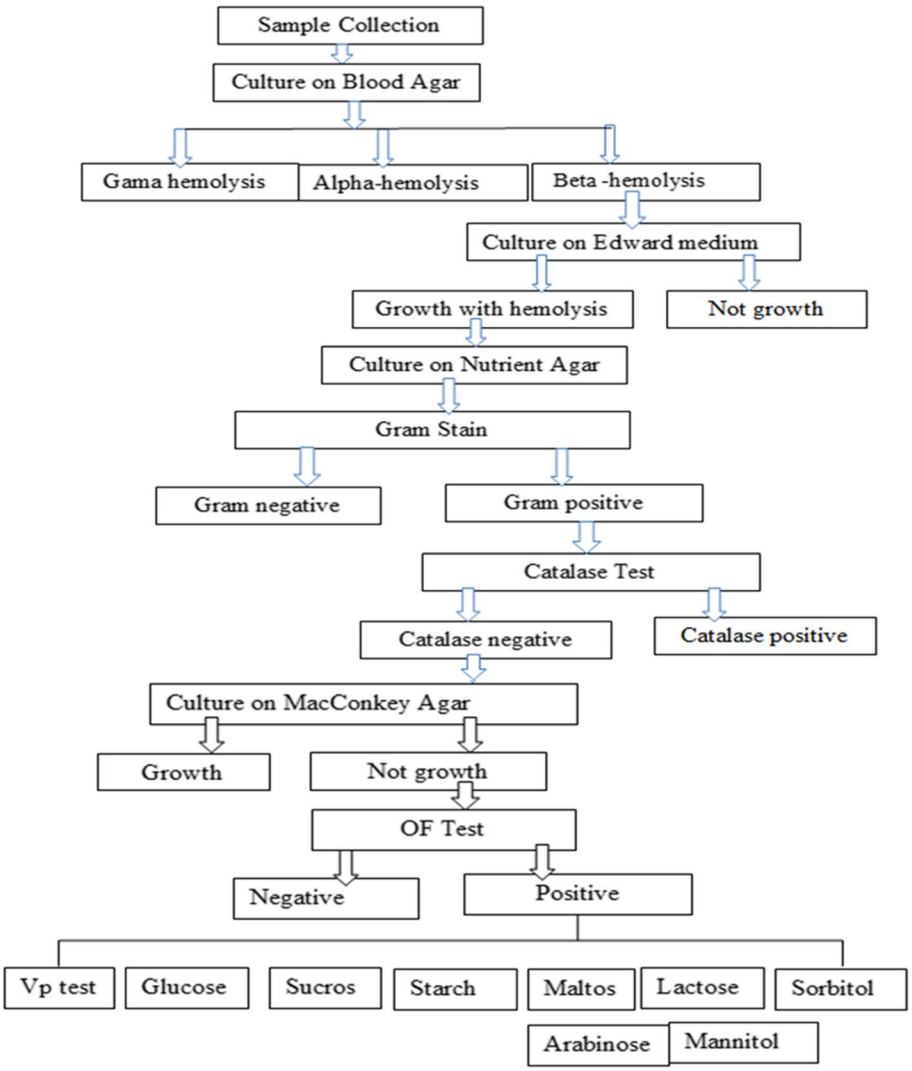

Fig. 2 General flow chart of the *Streptococcus* isolation technique

boiling method [12]. After centrifuging 1.5 ml of 24-hour cultured broth at 12,000 rpm for 5 min, the supernatant was discarded, and the pellets were washed twice with sterile water. The pellets were then mixed with 50 µl of lysozyme (20 mg/ml) (Himedia India), homogenized using a vortex, and then incubated at 37 °C for 5 min before being heated in a boiling water bath at 56 °C for another 5 min. The debris was then separated using a vortex and centrifuged for 5 min at 12,000 rpm. The supernatant was then used as the DNA template for PCR and kept at -20 °C until needed. A NanoDrop was used to check the purity of the extracted DNA. The absorbance at 260 and 280 nm giving a ratio between 1.7 and 1.9 ng/µl was considered pure DNA and used for PCR amplification.

Amplification was carried out in a thermal cycler (TC-412, Germany) and the PCR protocol was performed as described by Kelly (2006). The 541 bp *SeM* gene was amplified using forward primers (5'- CAGAAAACT AAGTGCCGGTG −3') and reverse primers (5'-ATT CGGTAAGAGCTTGACGC-3), initial denaturation at 95 °C for 5 min, denaturation at 90 °C for 30 s, annealing at 52 °C for 40 s, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The reaction volume used was 20 µL containing 4 µL of the reaction mix (Blulux, India), 2 µL of the extracted DNA template, 0.5 µL of each primer, and 13 µL of nuclease-free water. The primers used in this study were procured from Proligo Pty., Ltd., Australia.

Visualization of the *SeM* gene through agarose gel electrophoresis

The PCR amplification products were analyzed by electrophoresis in a 2% agarose gel and stained with 3 µL of

ethidium bromide (Blulux, India). Twelve microliters of the PCR product along with 3 µl of gel loading dye (Medox, India) was loaded into the wells and enough running buffer, Tris-acetate EDTA (TAE 1x) (Medox, India), was added to cover the surface of the gel. Then, electrophoresis was performed at 100 volts for 40 min. The product sizes were determined by comparison with the relative mobility of the 100 bp DNA standard ladder (Mumbai, India). After sufficient migration, the gels were taken to the gel documentation system machine (Biobase, China), and the results were visualized and recorded.

Antibiogram profile of *Streptococcus equi subspecies equi*

The antimicrobial susceptibility test results of *S. equi subspecies equi* were determined using the standard antimicrobial susceptibility guidelines described in the Clinical Laboratory Standard Institute [13]. The in vitro susceptibility test was performed on a molecularly confirmed isolate of *S. equi ssp. equi* and disc diffusion susceptibility tests were used. Six antibiotics used to treat equine strangles including penicillin G (10 U), amoxicillin (30 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (1.25 µg), erythromycin (30 µg), and vancomycin (30 µg) were tested.

Colonies of the same morphologic type were mixed into 5 ml sterile saline water and vortexed. The turbidity was compared to 0.5 McFarland standards and inoculated onto Muller-Hinton agar (Oxoid Ltd., Basingstoke, England). After 15 min, antibiotic discs were placed into inoculated Muller-Hinton agar (Oxoid Ltd., Basingstoke, England) and aerobically incubated for 24 h at 37 °C. The zone of inhibition was measured using a ruler. According to the CLSI (2021) standard, individual antibiotic inhibition was classified as susceptible, intermediate, or resistant [13] (Supplementary 2).

Data management and analysis

All collected data were coded and entered into a Microsoft Excel 2016 spreadsheet, while analysis was performed using Stata software version 16. Moreover, to summarize the data, descriptive statistics were used, and the strength of the association between potential risk factors (explanatory variables) and the presence of strangles infection (the response variable) was determined by binary logistic regression. All variables in the univariable analysis with a P value ≤ 0.2 were included in the multivariable analysis. For all tests, P values < 0.05 were considered statistically significant.

Results

Clinical examination

During the clinical examination, coughing, febrile body temperature, congested mucous membranes, unilateral or bilateral mucopurulent nasal discharge (Fig. 3), moist cough, extension of the head and neck to relieve pain, enlargement of the submandibular lymph node and lymph node with abscess were observed.

Bacteriological identification

Morphological identification of the isolates showed small, circular, translucent, and glistening mucoid colonies with beta-hemolysis on blood agar and small, honey-like colonies with complete hemolysis on Edward medium. It revealed Gram-positive bacteria with coccus-shaped and arranged in chains or pairs under the microscope (Fig. 4). In addition, the isolates were fermented with glucose, maltose, sucrose, and starch but not sorbitol, lactose, mannitol, and arabinose (Fig. 5) (Supplementary 3).

Out of 160 samples, 51 (31.87%) isolates phenotypically resembled *Streptococcus equi species*; 20 (39.21%), 19 (37.25%), and 12 (23.52%) of the *Streptococcus equi species* isolates were from horses, donkeys, and mules, respectively. The highest percentage of *Streptococcus equi species* was isolated from Wegera 36.95% (17/46), followed by Dembeya 36.67% (11/30), Chilga 34.21% (13/38) and Gondar Zuriya 28.51% (8/28), while an 11.2% (2/18) lower percentage was isolated from Gondar city (Table 1).

Molecular detection of the *SeM* gene

Out of the 51 *S. equi* subspecies *equi* isolates, 16 (31.37%) or 10% (16/160) of the total samples had the *SeM* gene, confirming that it is *Streptococcus equi* ssp. *equi* (Fig. 6) (Supplementary 4). The highest percentage of isolates harbouring the *SeM* gene, 35% (7/20), were identified from horses, followed by 31.6% (6/19) from donkeys and 25% (3/12) from mules. In terms of the district, 38.46%

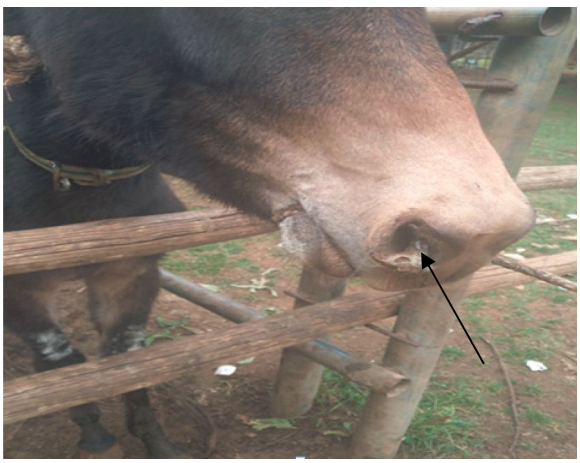

Fig. 3 Mucopurulent nasal discharge observed (photo taken from Gedebye Veterinary Clinic, Wegera District)

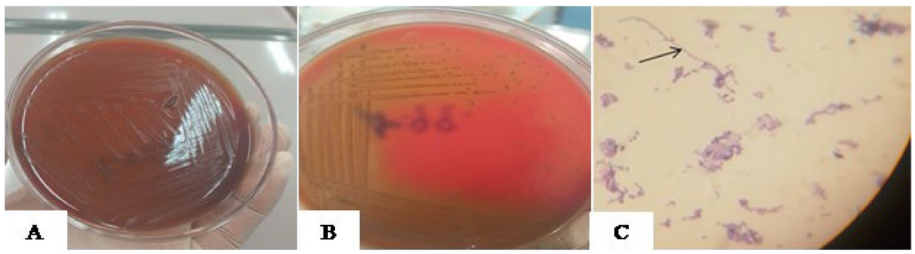

Fig. 4 Bacterial isolate **A** in blood agar mucoid colonies, **B** in Edwards agar small colonies with complete hemolysis, **C** Gram-positive cocci arranged in chains with characteristics of *Streptococcus* species

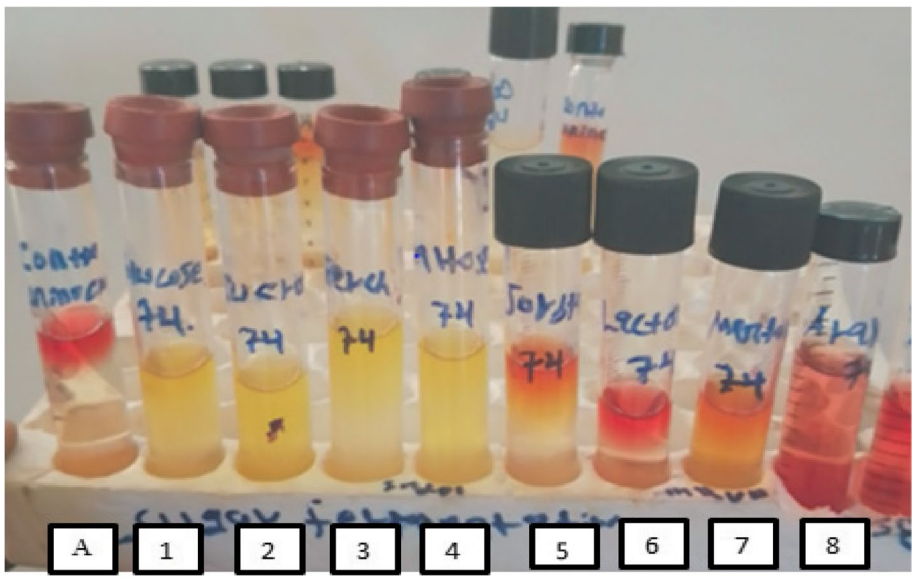

Fig. 5 Carbohydrate fermentation test results for *Streptococcus* isolates. "A" is the uninoculated negative control, 1 = glucose, 2 = sucrose, 3 = starch, 4 = maltose, 5 = sorbitol, 6 = lactose, 7 = mannitol, and 8 = arabinose. *S. equi* was fermentative for the first four sugars (1–4), but nonfermentative or negative for the remaining four sugars (5–8)

(5/13), 35.29% (6/17), 27.27% (3/11) and 25% (2/8) of isolates had the *SeM* gene from the Chilga, Wegera, Dembiya and Gondar Zuriya districts, respectively. None of the isolates from Gondar City harboured the gene (Table 1).

Antibiogram profile

The antimicrobial susceptibility profile of *S. equi* subspecies *equi* isolates indicated that tetracycline 30 µg, erythromycin 30 µg, and vancomycin 30 µg were more resistant, with percentages of 81.5%, 81.5%, and 75.5%, respectively. However, susceptibility was shown for penicillin G 10 U and amoxicillin 30 mg (56.25%). Slightly intermediate susceptibility was observed against amoxicillin 30 µg and penicillin G 10U with a percentage of 25% for both antibiotics (Fig. 7). More than 75% (12/16) of the isolates were resistant to more than three antibiotics, of which 18.75% (3/16) were resistant to three antimicrobials, 43.75% (7/16) were resistant to four antimicrobials and 12.5% (2/16) were resistant to five antimicrobials (Table 2) (Supplementary 5).

Risk factor analysis for the occurrence of *Streptococcus equi ssp. equi*

In the univariable logistic regression analysis, the history of travel, pull cart activities, the practice of sharing feed containers, drinking from water troughs, and spending nights together were statically significant ($P < 0.05$). Equines that spent the night together were five and more times (AOR) = 5.97; 95% CI = 1.41–25.37) more likely to have strangles than those that did not spend the night together. Similarly, equines that drank from shared water troughs were seven or more times (AOR = 7.74; 95% CI = 1.44–41.01) more likely to have strangles than equines that did not drink from water

Table 1 Proportion of *Streptococcus equi* species and *SeM* gene in terms of districts and species of animals

Districts	Equines spices	*S. equi* (%)	*SeM* gene (%)	Sub total	
				S. equi (%)	SeM gene (%)
Gondar city (*n* = 18)	Horse (*n* = 10)	2(20)	0(0)	2(11.11)	0(0)
	Donkey (*n* = 5)	0(0)	0(0)		
	Mule (*n* = 3)	0(0)	0(0)		
Gondar zurya (*n* = 28)	Horse (*n* = 13)	3(23.07)	1(33.33)	8(28.51)	2(25)
	Donkey (*n* = 11)	3(27.27)	1(33.33)		
	Mule (*n* = 4)	2(50)	0(0)		
Dembeya (*n* = 30)	Horse (*n* = 6)	2(33.33)	0(0)	11(36.67)	3(27.27)
	Donkey (*n* = 13)	5(38.46)	1(20)		
	Mule (*n* = 11)	4(36.36)	2(50)		
Chilga (*n* = 38)	Horse (*n* = 25)	6(24)	3(42.86)	13(34.21)	5(38.46)
	Donkey (*n* = 10)	4(40)	2(50)		
	Mule (*n* = 3)	3(100)	0(0)		
Wegera (*n* = 46)	Horse (*n* = 21)	7(33.33)	3(42.85)	17(36.95)	6(35.29)
	Donkey (*n* = 20)	7(35)	2(28.57)		
	Mule(*n* = 5)	3(60)	1(33.33)		
Total (*n* = 160)	Horse (*n* = 75)	20(26.67)	7(35)	51(31.87)	16(31.37)
	Donkey (*n* = 59)	19(32.20)	6(31.6)		
	Mule (*n* = 26)	12(46.15)	3(25)		

The percentage of *S. equi* was calculated from the samples collected; however, the percentage of the *SeM* gene was calculated from *S. equi*-positive samples. n is the number of samples collected

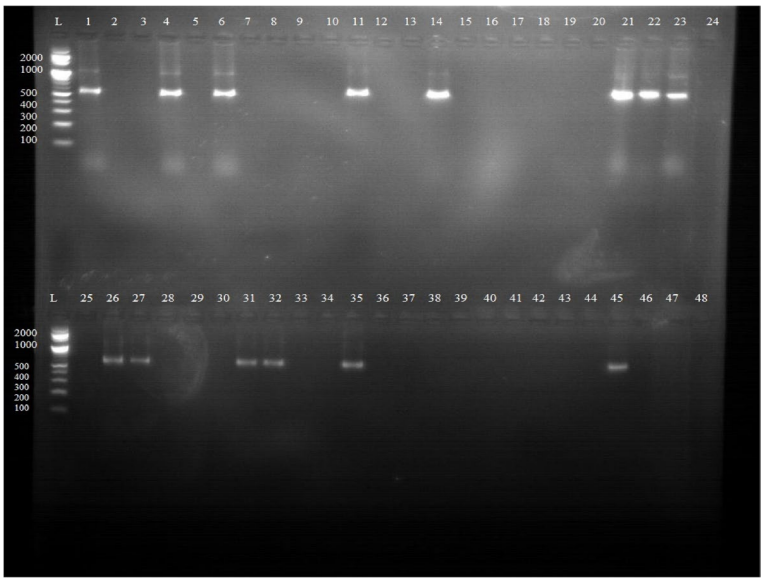

Fig. 6 Representative gel document of the *SeM* gene. L = 100 bp Ladder, Lane1 = positive control, Lane 2 = negative control, Lanes 4,6,11,14,21,22,23,26,27,31,32,35 and 45 were positive for *SeM* gene with 541 bp

troughs. Moreover, equines that shared feed containers were seven or more times (AOR = 7.59; 95% CI = 1.44–39.93) more likely to cause strangles than those that did not share a feeding container (Table 3).

Discussion

Strangles is a highly infectious respiratory disease of equines; clinical signs and laboratory diagnosis are the methods for confirming the infection. Clinical signs, such as mucopurulent nasal discharge and lymph node

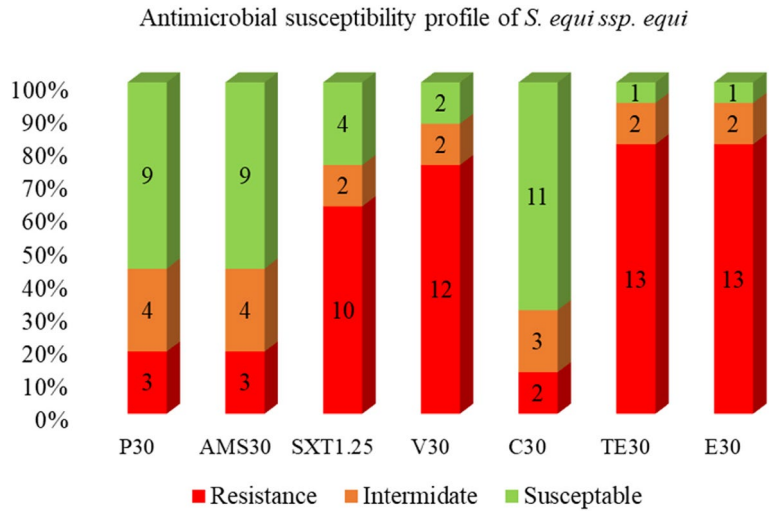

Fig. 7 Antimicrobial susceptibility profile of *S. equi ssp. equi* isolates. P30 = Penicillin 10 U, AMS30 = amoxicillin 30 µg, SXT1.25 = Sulfamethoxazole trimethoprim 1.25 µg, V30 = Vancomycin 30 µg, C30 = Chloramphenicol 30 µg, TE30 = Tetracycline 30 µg, E30 = Erythromycin 30 µg

Table 2 Multidrug resistance profile of *Streptococcus equi subspecies equi*

MDR	Drugs	Number of isolates	Percent	Total
Three	AMS30, TE30, E30	1	6.25%	18.75%
	SXT1.25, V30, E30,	1	6.25%	
	V30, TE30, E30,	1	6.25%	
Four	SXT1.25, V30, TE30, E30	7	43.75%	43.75%
Five	P30, SXT1.25, V30, TE30, E30,	2	12.50%	12.5%
Total		12	75%	75%

MDR Multidrug resistance, *P30* Penicillin G 10 U, *AMS30* Amoxicillin 30 µg, *SXT1.25* Sulfamethoxazole trimethoprim 1.25 µg, *V30* Vancomycin 30 µg, *TE30* Tetracycline 30 µg, *E30* Erythromycin 30 µg

swelling, are highly indicative of the disease; this finding is consistent with [14]. The isolation rate of *S. equi species* in the current study was 31.88%, which is lower than the 66.37% of Streptococci recovered from Arabian foals, and horses in Cairo, Egypt [15]. Several reasons could suggest much lower results; one of the reasons might be the sampling method: that sampling was done only from the nasopharyngeal tract and not from the guttural pouch, where this pathogen resides even after the disappearance of clinical symptoms of the disease, thereby resulting in negative results. This is in agreement with the statements described by Jaramillo et al. (2022), who described guttural pouch specimens as more sensitive for the detection of *S. equi subspecies equi* than nasopharyngeal tract swabs or nasopharyngeal washes taken from the same equine at the same time [16]. The other reason for less recovery through the culture technique could be the rarer bacteria present in the mucosal epithelium and the rapid desiccation of the bacteria outside the host.

In the current study, 31.37% (16/51) of *streptococcus* isolates revealed *S. equi* subspecies *equi*, which is lower than the report of Mohammed (2018). Out of 150 *Streptococcus equi* isolates, 124 (82.67%) revealed *S.equi subspecies equi* [15]. The lower result in the current study might be because the other *Streptococcus* species might be other subspecies. On the other hand, 10% (16/160) of *Streptococcus equi subspecies equi* were lower than the 16.21% (30/185), 14.48% (21/145), and 20.83% (35/168), reported by Mahmood (2014) from Baghdad city Iraq, Ivens et al. (2011) from the UK, and Patty and Cursons (2014) from New Zealand, respectively [17–19]. This difference is attributable to the sampling technique, sample type, media used, and culturing method. The reason for this high percentage is due to the methodology they used and the sampling sites they used, which included four sampling sites, nasal swabs, abscess swabs, guttural pouch wash, and nasopharyngeal wash. Although brain heart infusion broth was used for the enrichment of the sample, Columbia blood agar with the addition of a selective antibiotic mix was used as a selective medium. In the current study, *S. equi subspecies equi* was isolated using only nasal swabs, with no enrichment or selective antibiotics. The current study reports more isolates of *S. equi subspecies equi* than the study by Arafa et al. (2021), which was published in Egypt and reported 8 (5%) *S. equi subspecies equi* isolates out of 159 samples confirmed by PCR; this may be because they used a variety of genes (the *sodA* gene and the *seel* gene) for PCR confirmation [20]. Additionally, the other lower report was reported in Northern India in 2013 by Mir et al. (2013), which was 4 (2.83%) out of 141 swap samples [21].

Table 3 Factors associated with the occurrence of *S. equi subspecies equi*

Risk Factors	Negative (*n* = 144)	*S. equi subspp equi* (*n* = 16)	COR (95%CI)	AOR (95% CI)
Age				
Young	46	9	0.35(0.11–1.10)	0.35(0.07–1.61)
Adult	73	5	0.40(0.08–2.04)	1.85(0.19–17.61)
Old	25	2	Reference.	Reference.
History of travel				
Yes	40	12	11.27(3.04–41.64) *	3.05(0.71–13.03)
No	104	4	Reference	Reference
Pull cart				
Yes	84	13	8.15(2.21–29.93) *	0.86(0.11–6.65)
No	60	3	Reference	Reference
Share feeding container				
Yes	39	14	5.92(1.93–18.13) *	7.59(1.44–39.93) *
No	105	2	Reference	Reference
Drink from water troughs				
Yes	34	14	2.64(4.90 -10.66) *	7.74(1.46–41.01) *
No	110	2	Reference	Reference
Spending night together				
Yes	38	13	12(3.26–44.75) *	5.97(1.40 -25.37) *
No	106	3	Reference	Reference

AOR Adjusted odds ratio, *COR* Crude odds ratio, *CI* Confidence interval.

* *P*- value less than 0.05

The results of the multivariable logistic regression showed that sharing feed containers, sleeping together at night, and taking part in crowd events such as feeding time were all significant contributors to the detection of *S. equi subspecies equi*. *S. equi subspecies equi* were more likely detected from equines that shared a feed container, a water trough and spent together at night. These factors favor an outbreak of strangles because they are essential for transmission since direct contact occurs from animal to animal; this finding is in agreement with [22]. Moreover, indirect transmission occurs through the sharing of contaminated housing, water sources, and feeding utensils, these statements are in covenant with [6].

The antibiotic susceptibility profile of the isolated *S. equi subspecies equi* revealed substantial resistance to erythromycin (30 µg) and vancomycin (30 µg), which is in line with Seady et al. (2018), who reported 85% and 70% resistance to erythromycin and vancomycin, respectively [23]. Another resistance antibiotic was shown for tetracycline (30 µg), which is in line with Badier (2009), who reported 83.3% resistance against tetracycline [24]. The intermediate resistance against tetracycline is in agreement with Arafa et al. (2021), who reported 12.5% [20]. Moreover, the results of the antimicrobial susceptibility tests conducted in this study indicated that the sulfamethoxazole-trimethoprim resistance rate is consistent with that reported by [25], Lexington, United States.

The resistance of penicillin G in the current study was slightly lower than that reported by Arafa et al. (2021), who reported 50% of resistance to penicillin G. A lower percentage of resistance of *S. equi subspecies equi* to penicillin G was reported in Cambridge, England, in 2020 by [26]. The lower resistance of penicillin G in the current study may be due to the appropriate use of dose and dosage by the field veterinarians to treat strangles or may be due to the verity of the resistance gene.

The current study limitations were, samples were not taken from guttural poach which results in lower positive samples, number of samples were small due to sampling technique and sequencing were not performed due to budget constraints.

Conclusion

Strangles is an infectious respiratory disease of equines caused by *S. equi subspecies equi.* In this study, the *SeM* gene (the virulence gene of *S. equi subspecies equi*) was detected in clinically suspected equines in the central Gondar zone. The findings revealed that the possible sources of strangles infection were sharing contaminated objects, such as feed containers and drinking troughs and

through direct contact by spending nights together. More than 75% of the isolates were multidrug resistant. However, most isolates were susceptible to penicillin G 30 µg, and amoxicillin 30 µg. Therefore, feed containers and water troughs should be cleaned, litter and manure from the stable should be removed, and then buried, diseased equines should be treated and quarantined, veterinarians should create awareness among animal owners, and the treatment should be applied with the proper dosage of drugs. Moreover, the disease has an economic importance in the equine population, studies in large study areas should be conducted to formulate the disease prevention and control practice.

Abbreviations

AOR	Adjusted odds ratio
CLSI	Clinical Laboratory Standard Institute
EDTA	Ethylene diamine tetra acetic acid
PCR	Polymerase Chain Reaction
SeM	M-like protein of S. equi subspecies equi

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04236-z.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions
D.B. participated in writing the proposal, laboratory work and preparation of the manuscript. S.L.A. and B.D. contributed to methodology, supervision and manuscript edition. B.T. participated data analysis and manuscript edition. All authors read and approved the final manuscript for publication.

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Availability of data and materials
Data are available and shared from the first and corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate
Ethical clearance was obtained from the University of Gondar, College of Veterinary Medicine and Animal Sciences Institutional Ethical Review Board (IRB) with reference number CVMAS.sc-04/2022 and all experimental protocols were approved. Animals were handled and treated humanely in a way that minimized discomfort, distress, or pain during sample collection and study animals were not subjected to invasive procedures as only a nasal swab was collected from each animal. A Brief explanation of the objective of the work was provided to the owners, and informed consent was obtained. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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