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Molecular Detection of *Streptococcus equi* subsp. *Zooepidemicus* and *equi* in Guinea Pig with Lymphadenitis by Targeting the *comB* and *seeI* Genes

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ABSTRACT

Despite the economic and sanitary relevance of cervical lymphadenitis in guinea pigs, little molecular information is available regarding the role of *Streptococcus* (*S.*) *equi* subspecies in cervical lymphadenitis in Peru. The present study aimed to identify the subspecies *S. equi* subsp. *zooepidemicus* (SEZ) and *S. equi* subsp. *equi* (SEE) in guinea pigs (*Cavia porcellus*), with and without cervical lymphadenitis, from the Amazonas and Cajamarca regions of Peru, using PCR amplification of the *sodA*, *comB*, and *seeI* genes. A total of 50 guinea pigs, aged 6-8 months, were evaluated, 30 from Cajamarca (mean weight 1210.1 grams; 22 Peruvian breed, 1 Inti, and 7 Brown) and 20 from Amazonas (mean weight 950 grams; 10 Peruvian breed and 10 Inti), comprising 30 animals with cervical lymphadenitis and 20 clinically healthy animals. The *sodA* gene was first employed as a preliminary diagnostic marker, which confirmed the presence of *S. equi* in all animals presenting lymphadenitis. Subsequent amplification of the *comB* gene confirmed the exclusive presence of SEZ in all clinical cases, with no detection in the 20 clinically healthy guinea pigs analyzed in both study regions. In contrast, the *seeI* gene, specific to SEE, indicated no amplification in any of the samples, indicating the absence of SEE in the study population. The prevalence of SEZ (positive *comB*) among guinea pigs with cervical lymphadenitis was 100% in Amazonas (10/10; 95% CI: 69.2-100%) and 100% in Cajamarca (20/20; 95% CI: 83.2-100%), with no significant difference between regions. The present findings established SEZ as the primary etiological agent of cervical lymphadenitis in guinea pigs from both regions, with no molecular evidence of SEE. The present study underscored the utility of *comB* and *seeI* as specific and reliable molecular markers for differentiating SEZ and SEE.

Keywords: *comB* gene, Guinea pig, *seeI* gene, *Streptococcus equi* subsp. *equi*, *Streptococcus equi* subsp. *zooepidemicus*

INTRODUCTION

Streptococcus (*S.*) *equi* subsp. *zooepidemicus* (SEZ) and *S. equi* subsp. *equi* (SEE) are Gram-positive cocci belonging to Lancefield group C (Bustos et al., 2017; Martínez et al., 2021). The SEZ is part of the upper respiratory tract microbiota of equids but can act as an opportunistic pathogen causing rhinitis, pneumonia, mastitis, and other infections in different hosts, including guinea pigs (Pelkonen et al., 2013; Quispe Avendaño, 2019). By contrast, SEE is responsible for strangles in horses, a transmissible condition that produces purulent swelling and abscess formation in the cervical and head lymph nodes (Alber et al., 2004; Bustos et al., 2017).

The biochemical differentiation of *S. equi* subspecies is generally based on sugar fermentation patterns. Typically, strains that ferment trehalose and lactose are classified as *zooepidemicus* subspecies, whereas those that ferment only salicin are identified as *equi* subspecies. However, atypical isolates exhibiting variable fermentation profiles have been documented, suggesting that these characteristics may not be uniformly exhibited across all strains (Abreu et al., 2016). However, due to the complexity of taxonomic differentiation between SEE and SEZ using conventional microbiological methods, molecular techniques such as polymerase chain reaction (PCR) targeting specific marker genes have been employed for more precise identification (Alber et al., 2004; Bustos et al., 2017). One of the most widely used molecular markers for preliminary detection of the genus *Streptococcus* and particularly *S. equi* is the *sodA* gene, which encodes a superoxide dismutase (Alber et al., 2004; 2005); however, *sodA* does not permit differentiation between the

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zooepidemicus and *equi* subspecies (Alber et al., 2004). Therefore, in the present study, *sodA* was employed as a preliminary diagnostic tool to confirm the presence of *S. equi* in guinea pigs with cervical lymphadenitis.

Moreover, several molecular markers have been investigated in *S. equi* subspecies, including *seeI* (Alber et al., 2004), as well as others such as *seem*, *szeL*, and *szeM* (Alber et al., 2005), which encode superantigenic proteins but are not consistently present across strains. Additional targets, such as *SzP* (Anzai et al., 2002) and *comB* (Morris et al., 2023), have also been explored for diagnostic purposes. In the present study, *seeI* and *comB* were selected, following initial screening with *sodA*, due to their high specificity for each subspecies. The *seeI* gene encodes an exotoxin present only in SEE (Alber et al., 2004), whereas the *comB* gene is part of the genetic competence system of SEZ (Morris et al., 2023), which allows the simultaneous detection of both subspecies by PCR.

In guinea pigs (*Cavia porcellus*), *SEZ* is the primary causative agent of cervical lymphadenitis, a disease characterized by abscesses in the neck lymph nodes, and causes significant economic losses for small farmers (Jara et al., 2023; Quispe Avendaño, 2019). Recent studies in Peru have reported a high frequency of *Streptococcus* spp. associated with cervical abscesses in guinea pigs, with prevalence values exceeding 90% in Cusco, Peru (Angulo-Tisoc et al., 2021), while Vargas-Rocha et al. (2023) highlighted that cervical lymphadenitis remains one of the primary infectious diseases affecting guinea pig farming in the country. Despite its impact, there is limited molecular information on SEZ in guinea pig farming in Peru. Therefore, the present study aimed to confirm the presence of *SEZ* and to determine whether *SEE* is present in guinea pigs with and without cervical lymphadenitis from two regions of Northern Peru (Amazonas and Cajamarca), using PCR amplification of *sodA*, *seeI*, and *comB* marker genes.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Institutional Research Ethics Committee of the Toribio Rodríguez de Mendoza National University of Amazonas, Peru (CIEI-Nº0050). All animal procedures were carried out in accordance with animal welfare principles.

Animal selection, treatment, and sampling

Fifty domestic guinea pigs aged 6-8 months, from the provinces of Luya (Amazonas region) and Cajamarca (Cajamarca region), were selected. The animals from Cajamarca (n = 30) were all females, with a mean weight of 1210.1 g, and included 22 of the Peruvian breed, one Inti, and seven Brown. The animals from Amazonas (n = 20) comprised 13 females and seven males, with a mean weight of 950 g, and included 10 of the Peruvian breed and 10 Inti. A specialist in small animal health, who graduated from the National University Toribio Rodriguez de Mendoza, Peru, identified guinea pigs with signs compatible with cervical lymphadenitis, such as external neck abscess, enlarged lymph nodes, low weight, and nasal discharge, according to diagnostic criteria reported by Quispe Avendaño (2019) and Vargas-Rocha et al. (2023).

A total of 50 guinea pigs were evaluated, comprising four groups, including 20 with cervical lymphadenitis and 10 clinically healthy from Cajamarca (Table 1) and 10 with cervical lymphadenitis and 10 clinically healthy from Amazonas (Table 2).

Each animal was considered a biological replicate, and all PCR assays were performed in duplicate to confirm reproducibility. All selected animals were transported to the laboratory for processing. The guinea pigs had been raised under traditional farming conditions, at ambient temperatures ranging from 8 to 23°C and relative humidity of 60-70%. Their diet consisted of elephant grass (*Cenchrus purpureus*), alfalfa (*Medicago sativa*), and commercial concentrate (MESAJU, Peru), provided twice daily (morning and afternoon). Each animal received a daily total equivalent to approximately 5% of its live weight in concentrate and 30% of its live weight in fresh forage, with water available *ad libitum*. Feeding management was based on local practices described by Andrade-Yucailla et al. (2016). None of the guinea pigs had been vaccinated. The animals were housed in pens made of bricks (34.5 cm wide × 192 cm long × 15.8 cm deep) and were between 6 and 8 months of age at the time of sampling. The study was conducted in three months. Each guinea pig was administered an intraperitoneal anesthetic of ketamine (40 mg/kg; Ambavet, Ecuador) with xylazine (5 mg/kg; Ambavet, Ecuador); afterward, euthanasia was performed by an overdose of T-61 (Intervet International GmbH, Germany) at 0.5-2 mL per animal, depending on size through intrapulmonary injection for small rodents, as recommended by the manufacturer and following guidelines for the humane sacrifice of rodents (CONCEA, 2016).

Table 1. General characteristics of the guinea pigs selected from Cajamarca, Peru, according to the breed, weight, and sex.

Individual	Breed	Weight (g)	Sex
21C	Peru	1500	Female
22C	Peru	1000	Female
23C	Brown	1100	Female
24C	Peru	1400	Female
25C	Peru	1300	Female
26C	Peru	1500	Female
27C	Peru	1150	Female
28C	Peru	1500	Female
29C	Peru	1450	Female
30C	Brown	1550	Female
31C	Brown	1156	Female
32C	Brown	1157	Female
33C	Brown	1140	Female
34C	Peru	1150	Female
35C	Peru	1200	Female
36C	Peru	1050	Female
37C	Peru	1250	Female
38C	Peru	1300	Female
39C	Peru	1100	Female
40C	Peru	1550	Female
41C	Peru	1000	Female
42C	Peru	1100	Female
43C	Peru	950	Female
44C	Peru	1050	Female
45C	Peru	1250	Female
46C	Peru	1250	Female
47C	Brown	1150	Female
48C	Inti	1000	Female
49C	Brown	1050	Female
50C	Peru	1000	Female

Table 2. General characteristics of the guinea pigs selected from Amazonas, Peru, according to breed, weight, and sex

Individual	Breed	Weight (g)	Sex
1L	Peru	1500	Female
2L	Peru	1450	Female
3L	Peru	850	Male
4L	Peru	1200	Female
5L	Peru	650	Female
6L	Inti	1050	Female
7L	Inti	900	Female
8L	Inti	600	Female
9L	Inti	650	Female
10L	Inti	450	Male
11L	Inti	950	Male
12L	Inti	800	Female
13L	Inti	1050	Male
14L	Inti	1350	Male
15L	Inti	700	Female
16L	Peru	1200	Female
17L	Peru	800	Female
18L	Peru	1500	Female
19L	Peru	550	Male
20L	Peru	800	Male

Sample collection

Necropsies were conducted under sterile conditions, and the inflamed lymph nodes were collected from each guinea pig with cervical lymphadenitis. In the healthy guinea pigs, the corresponding healthy cervical lymph nodes were collected as control samples. No deaths occurred during the study. In total, samples were obtained from 50 guinea pigs; 20 with lymphadenitis and 10 clinically healthy animals from Cajamarca, and 10 with lymphadenitis and 10 clinically healthy animals from Amazonas. Each animal represented one independent sample. Sample collection and handling were performed under sterile conditions following general procedures for lymph node sampling in guinea pigs with lymphadenitis as described by Vargas-Rocha et al. (2023). The DNA extraction and downstream processing followed internal laboratory protocols routinely used for bacterial molecular analyses. The collection of cervical lymph nodes from guinea pigs with lymphadenitis and from clinically healthy animals was conducted within the microbiology laboratory inside a Class II biosafety cabinet (Biobase, USA). Upon excision, all tissues, including abscesses and/or lymph nodes, were initially rinsed with saline solution. Each sample was placed into a sterile 50 mL tube containing saline solution. For samples from animals with lymphadenitis, the tubes were treated with UV light for 15 minutes inside the biosafety cabinet to reduce excess purulent content. Approximately 50 mg of material was then collected from each sample using specific methods according to health status. In infected animals, purulent exudate was collected by sectioning the lymph node with a sterile scalpel, and in healthy guinea pigs, lymphatic tissue was homogenized into a fine powder using a mortar and pestle with liquid nitrogen. All resulting samples were transferred to 1.5 mL tubes and suspended in 160 µL of sterile PBS buffer (Thermo Fisher Scientific, USA). To prevent cross-contamination, all instruments were disinfected with 70% ethanol across samples. Samples were stored at 4°C until all animals were processed, with an average interval of approximately two hours between collection and genomic DNA extraction. This protocol minimized handling time and maintained the cold chain, thereby preventing DNA degradation and contamination. All samples were processed for extraction on the same day.

Target primers

Genomic DNA (gDNA) was then isolated using the ReliaPrep™ gDNA tissue miniprep system kit (Promega, USA), incorporating an additional lysozyme treatment (20 mg/mL) to enhance bacterial cell lysis, followed by the addition of 20 µL of proteinase K to ensure complete enzymatic digestion and release of genomic material. The resulting DNA was further purified with the genomic DNA clean and concentrator kit (Zymo Research Operations, USA). Quantification and purity assessment of gDNA was assessed with a NanoDrop One Spectrophotometer (Thermo Scientific, USA), and

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only samples with a 260/280 ratio between 1.8 and 2.0 and a minimum concentration of 20 ng/µL were used for PCR analyses.

As an initial step in the molecular analysis, a preliminary diagnostic PCR was performed using the *sodA* gene as a universal marker for detecting *S. equi* (Alber et al., 2004; Mir et al., 2013). Primers described by Alber et al. (2004) were used, which amplified a conserved fragment of ~235 bp (Table 3). This PCR was designed to confirm the presence of *S. equi*, without discriminating between subspecies. The PCR program was based on the thermal profile described by Alber et al. (2004) with slight modifications. The reactions started with an initial denaturation at 94°C for two minutes, followed by 35 cycles consisting of denaturation at 94°C for 10 seconds, annealing at 56°C for 10 seconds, and extension at 72°C for 30 seconds. A final elongation step was performed at 72°C for five minutes.

Upon obtaining a positive confirmation with the *sodA* marker, specific amplification was performed for the molecular identification of SEZ and SEE, using the *comB* and *seeI* genes as targets, respectively. Specific primers according to Morris et al. (2023) were used for the *comB* primer (expected product ~450 bp) and a *seeI* primer (expected product 520 bp) according to Alber et al. (2004; Table 4). Each PCR assay was prepared in a final volume of 25 µL, which included 4 µL of genomic DNA template (≈100 ng), 12.5 µL of GoTaq® Green Master Mix 2X (Promega, USA), 1 µL of each primer (forward and reverse, 10 µM stock, final concentration 0.4 µM each), and 6.5 µL of nuclease-free water with standard ramp rates. Amplifications were performed in a thermocycler (Applied Biosystems SimpliAmp, USA). For the *comB* marker, the program started with denaturation at 95°C for two minutes, followed by 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 15 seconds, and ended with a final extension at 72°C for five minutes. For the *seeI* marker, the program began with denaturation at 94°C for two minutes, then 35 cycles of 94°C for 10 seconds, 56°C for 10 seconds, and 72°C for 30 seconds, concluding with an extension at 72°C for five minutes. Each PCR run included a tube with no extracted gDNA and molecular-grade water as a negative control to rule out contamination. As positive controls, Kwik-Stik™ reference strains, including *Streptococcus equi* subsp. *equi* ATCC 33398 and *S. equi* subsp. *zooepidemicus* ATCC 700400, derived from the American type culture collection (ATCC, USA), were used. To minimize the risk of contamination, all PCR procedures were conducted following a unidirectional workflow, with physically separated areas designated for pre- and post-PCR operations. Reagent preparation was performed inside a Class II biosafety cabinet (Biobase, USA) using dedicated pipettes and aerosol-resistant filter tips. All reagents were aliquoted to prevent repeated freeze–thaw cycles. Work surfaces were routinely disinfected with sodium hypochlorite 10%, followed by ethanol 70% and UV light exposure before each use.

Table 3. Primers for amplification of the *sodA* gene for *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi subsp. equi*

Target gene	Primer	Sequence (5´-3´)	PCR product size (bp)	Reference
sodA	sodA equi/zooep-F	CAGCATTCCTGCTGACATTCGTCAGG	235	Alber et al., 2004
	sodA equi/zooep-R	CTGACCAGCCTTATTATTCAACCAGCC		

Table 4. Primers for amplification of the *comB* gene for *Streptococcus equi* subsp. *zooepidemicus* and the *seeI* gene for *Streptococcus equi* subsp. *equi*

Target gene	Primer	Sequence	Expected product (pb)	Reference
comB	comB_F	GCTGGTTCAGGAGTGCAACA	450	Morris et al., 2023
	comB_R	TTCGTGGAAAATCGTGCTGT		
seeI	seeI-F	GAAGGTCCGCCATTTTCAGGTAGTTTG	520	Alber et al., 2004
	seeI-R	GCATACTCTCTCTGTCACCATGTCCTG		

Data analysis

The PCR products were analyzed by electrophoresis on agarose gels 2% in TAE buffer (Promega, USA), running at 100 V for 50 minutes. A 100 bp molecular weight marker (Promega and Novagen, USA) was used to compare and confirm the expected size of the amplified bands. The gels were visualized under UV transillumination and documented using a digital photodocumentation system (BIO-RAD, USA). For descriptive statistical analysis, the prevalence of SEZ (positive *comB*) in guinea pigs with cervical lymphadenitis was calculated for each region and overall. Exact binomial (Clopper-Pearson) 95% confidence intervals were estimated for each prevalence value, and Fisher's exact test at p = 1.00

was used to compare the proportions between regions (Amazonas compared to Cajamarca), which is appropriate for small and unequal sample sizes.

RESULTS

A total of 50 DNA samples from guinea pigs with cervical lymphadenitis and clinically healthy controls from the Amazonas and Cajamarca regions, Peru, were analyzed by conventional PCR. As the first step of the molecular analysis, amplification of the *sodA* was performed (Table 3). In Luya (Amazonas), 10 clinically healthy guinea pigs and 10 with cervical lymphadenitis were analyzed, and positive amplification of the *sodA* gene was observed in all the clinical cases (Figure 1). Meanwhile, in Cajamarca, 10 healthy guinea pigs and 20 with lymphadenitis were evaluated, and *sodA* gene amplification was detected in all clinical cases (Figure 2).

The prevalence of SEZ (positive *comB*) among guinea pigs with cervical lymphadenitis was 100% in Amazonas (10/10; 95% CI: 69.2-100%) and 100% in Cajamarca (20/20; 95% CI: 83.2-100%), with no significant difference between regions (Fisher's exact test, p = 1.00).

The amplification of the *comB* gene produced a specific amplicon of ~450 bp in all guinea pigs with cervical lymphadenitis sampled from both regions, whereas none of the healthy guinea pigs exhibited amplification of the *comB* gene (Figures 3 and 4). Notably, in the province of Luya (Amazonas), Peru, *comB* was found in all 10 guinea pigs with lymphadenitis and in none of the 10 healthy guinea pigs (Figure 3). Similarly, in the Cajamarca province, a positive *comB* amplification was observed in 20 out of 20 guinea pigs exhibiting lymphadenitis. In contrast, no positive results were observed in 10 healthy guinea pigs (Figure 4). The present results confirmed the presence of SEZ in all animals that presented cervical lymphadenitis, and the absence of SEZ in the clinically healthy guinea pigs examined. Additionally, the PCR tests for the *seeI* marker were negative in 100% of the samples. No amplification of *seeI* was detected in any guinea pig. The molecular detection by *seeI*/*comB* PCR demonstrated that the cases of cervical lymphadenitis in guinea pigs from the provinces of Luya and Cajamarca, Peru, were associated with SEZ, with no evidence of the involvement of SEE in these populations (Figures 5 and 6).

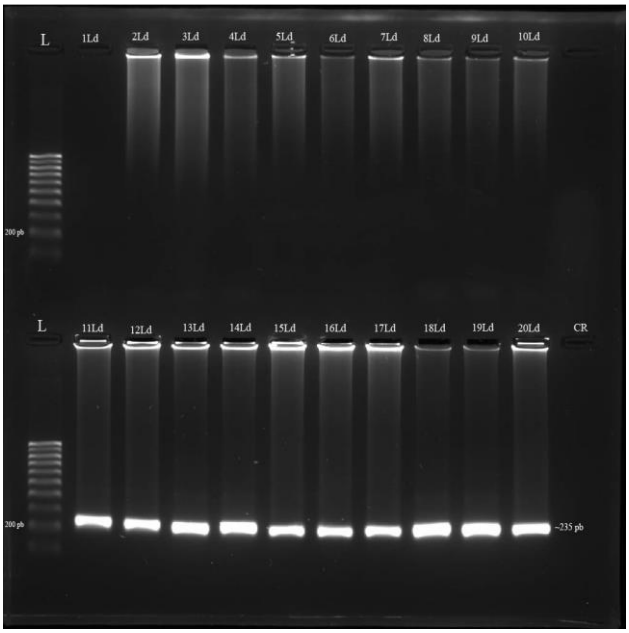

Figure 1. The PCR-amplified product of the *sodA* gene (~235 bp) for *Streptococcus equi* in guinea pigs aged 6-8 months with and without lymphadenitis from Amazonas, Peru. Lane L: 100 bp ladder, Lanes 1Ld-10Ld: Samples from healthy guinea pigs (negative for *S. equi*), Lanes 11Ld-20Ld: Samples from diseased guinea pigs (positive for *S. equi* at ~235 bp). Lane CR: Reagent control.

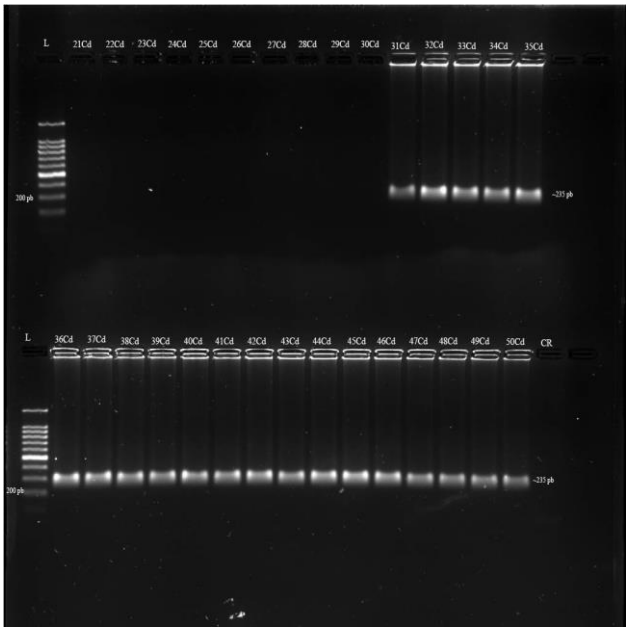

Figure 2. PCR-amplified product of the *sodA* gene (~235 bp) for *Streptococcus equi* in guinea pigs aged 6-8 months with and without lymphadenitis from Cajamarca, Peru. Lane L: 100 bp ladder, Lanes 21Cd-30Cd: Samples from healthy guinea pigs (negative for *S. equi*), Lanes 31Cc-50Cc: Samples from diseased guinea pigs (positive for *S. equi* at ~235 bp). Lane CR: Reagent control.

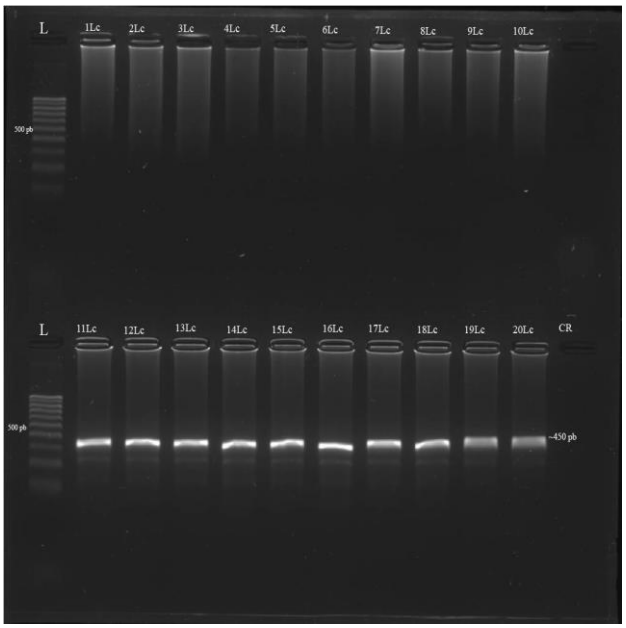

Figure 3. The PCR-amplified product of the *comB* gene (~450 bp) for *Streptococcus equi* subsp. *zooepidemicus* in guinea pigs aged 6-8 months without and with lymphadenitis from Amazonas, Peru. Lane L: 100 bp ladder (Novagen), Lanes 1Lc-10Lc: Samples from healthy guinea pigs (negative for *S. equi* subsp. *zooepidemicus*), Lanes 11Lc-20Lc: Samples from diseased guinea pigs (positive for *S. equi* subsp. *Zooepidemicus* at ~450 bp). Lane CR: Reagent control.

Figure 5. The PCR-amplified product of the *seeI* gene (~520 bp) for *Streptococcus equi* subsp. *equi* in guinea pigs aged 6-8 months with and without lymphadenitis in Luya, Peru. Lane L: 100 bp ladder (Novagen), Lanes 1Ls-10Ls: Samples from healthy guinea pigs (negative for *S. equi* subsp. *equi*), Lanes 11Ls-20Ls: Samples from diseased guinea pigs (negative for *S. equi* subsp. *equi*), Lane CR: reagent control.

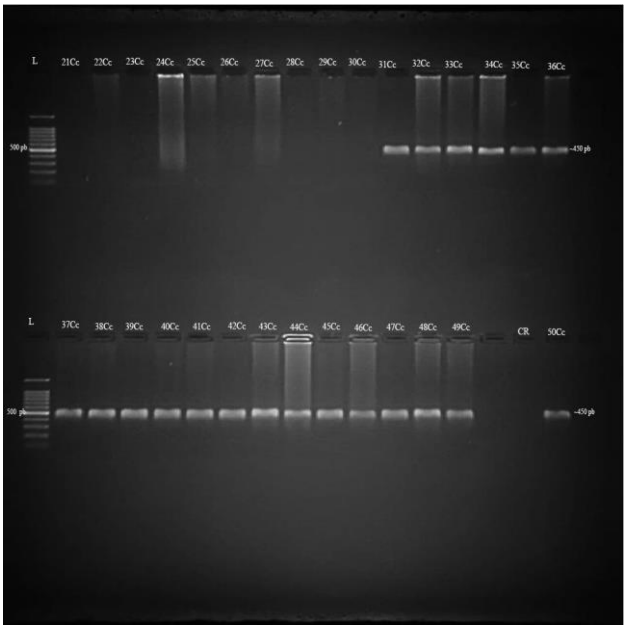

Figure 4. The PCR-amplified product of the *comB* gene (~450 bp) for *Streptococcus equi* subsp. *zooepidemicus* in guinea pigs aged 6-8 months without and with lymphadenitis from Cajamarca, Peru. Lane L: 100 bp ladder (Promega), Lanes 21Cc-30Cc: Samples from healthy guinea pigs (negative for *S. equi* subsp. *zooepidemicus*), Lanes 31Cc-50Cc: Samples from diseased guinea pigs (positive for *S. Equi* subsp. *Zooepidemicus* at ~450 bp), Lane CR: Reagent control.

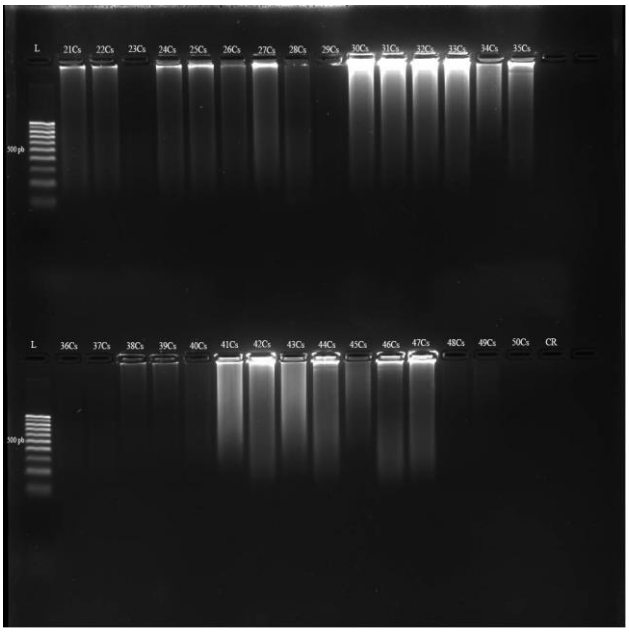

Figure 6. The PCR-amplified product of the *seeI* gene (~520 bp) for *Streptococcus equi* subsp. *equi* in guinea pigs aged 6-8 months with and without lymphadenitis in Cajamarca, Peru. Lane L: 100 bp ladder (Novagen), Lanes 21Cs-50Cs: Samples from healthy and diseased guinea pigs (negative for *S. equi* subsp. *equi*), Lane CR: Reagent control.

DISCUSSION

The detection of SEZ in guinea pigs with cervical lymphadenitis during the present study indicated its prevalence in the regions of Amazonas and Cajamarca in Peru. The SEZ was recognized as the leading cause of lymphadenitis in guinea pigs (Harkness et al., 2002). Additionally, severe outbreaks of cervical abscesses in Peruvian guinea pig farms have been attributed to SEZ (Jara et al., 2023). From a health and production perspective, early detection is crucial because SEZ is

an emerging zoonotic pathogen that can lead to severe infections in farm animals and humans (Jara et al., 2023). Since intensive guinea pig farming is an important economic activity in the Andean region of South America (Jara et al., 2023), an SEZ outbreak can result in substantial production losses due to mortality, growth retardation, or the culling of affected animals. The SEZ outbreaks are particularly relevant in some areas of the Amazonas and Cajamarca regions in Peru, where both large-scale and intensive production methods are employed, and where guinea pig farming serves as a primary source of livelihood income. Moreover, the zoonotic capability of SEZ poses risks to people who handle these animals, and human infection cases attributed to strains originating from guinea pigs have been documented (SHIC, 2021).

In the present study, SEZ was identified in guinea pigs with lymphadenitis from the provinces of Luya (Amazonas region) and Cajamarca, indicating that SEZ was present across different geographic regions of Northern Peru. In the Amazonas region, no previous studies have investigated lymphadenitis or the presence of SEZ, highlighting the importance of the present findings. In the case of Cajamarca, the presence of SEZ contrasted with the findings of Vargas-Rocha et al. (2023), who identified *Trueperella pyogenes* as the causative agent of cervical lymphadenitis in guinea pigs (*Cavia porcellus*) in Cajamarca, Peru, an uncommon pathogen associated with cervical abscesses in this species.

On the other hand, SEE was not detected in any of the sampled guinea pigs, which was consistent with its expected ecology, as SEE is a highly contagious pathogen of equids and is not part of the normal microbiota in rodents (SHIC, 2021). Therefore, the absence of SEE in guinea pigs, including those with cervical abscesses, indicated that SEE was not involved in the observed pathology. In addition, no evidence suggested that SEE was silently circulating in the facilities evaluated. Previous studies have not recorded the presence of SEE in guinea pigs, whereas SEZ has consistently been isolated as the causative agent of lymphadenitis in guinea pigs (Harkness et al., 2002; Jara et al., 2023), with documented outbreaks in guinea pig farms in the southern Peruvian Andes. This finding (absence of SEE in guinea pigs) during the present study confirmed that cervical abscesses in guinea pigs are almost always caused by SEZ rather than SEE. Therefore, the present findings reduce concerns about guinea pigs acting as a potential source of SEE or an equine-related zoonosis. According to previous reports, SEZ can act as an opportunistic pathogen in non-equine hosts, possibly entering through small oral lesions, whereas SEE requires horses as its natural host to persist and spread (SHIC, 2021). This distinction is consistent with the present findings, since SEZ was detected in guinea pigs with cervical abscesses, while SEE was absent in all samples.

Furthermore, the absence of SEZ in the clinically healthy guinea pigs analyzed in both regions indicated that, under the conditions studied, asymptomatic animals were not serving as silent carriers. The absence of SEZ in healthy guinea pigs is relevant because SEZ can be part of the commensal microbiota of the upper respiratory tract of different domestic animals, such as equines, swine, cattle, and dogs, and under certain conditions, SEZ may act as an opportunistic pathogen (Anzai et al., 2002). The current findings are consistent with those of Anzai et al. (2002), indicating that in guinea pigs, SEZ likely functions more as a pathogen than as part of normal microbiota. Although subclinical carriage of SEZ was not detected during the present study, its intermittent presence cannot be entirely excluded.

A notable aspect of the present study was indicating how the *comB* and *seeI* genes can effectively differentiate SEZ and SEE. The *equi* subspecies possesses exotoxin genes that are not found in *S. zooepidemicus*, a characteristic that has been previously exploited to specifically detect SEE by PCR in cases of strangles (Alber et al., 2004). In fact, amplification of the *seeI* gene has been successfully used to identify *S. equi* in equine samples, demonstrating greater sensitivity than traditional culture and enabling the detection of asymptomatic carriers following outbreaks of the disease (Alber et al., 2004). Meanwhile, the *comB* gene has emerged as a specific molecular target of SEZ, proposed in recently developed diagnostics to detect SEZ (Morris et al., 2023). The PCR assays targeting *comB* and *seeI* effectively differentiate between SEZ and SEE in the samples during the present study, which was essential considering their very close genetic relationship (98% DNA homology; SHIC, 2021). This molecular approach provided a rapid and reliable diagnosis, overcoming the limitations of conventional culture and biochemical identification methods, which can be slow or inconclusive when phenotypic traits overlap, as previously reported for detecting *S. equi* (Weese et al., 2023). Therefore, using specific PCR methods not only confirmed the pathogen responsible for cervical lymphadenitis in guinea pigs but also offered critical epidemiological insights to distinguish infections caused by SEZ from a potential introduction of SEE, aiding decision-making in animal health and guinea pig production in the Amazonas and Cajamarca regions of Peru (Jara et al., 2023).

The current study acknowledged several limitations. Firstly, its cross-sectional design restricts the ability to establish temporal or causal relationships between SEZ detection and disease onset (Setia, 2016). Additionally, while PCR is a sensitive method, it possesses an inherent detection limit and may produce false-negative results when the pathogen load is minimal. Therefore, negative findings in apparently healthy animals may not conclusively exclude the presence of low-level carriage.

CONCLUSION

Cervical lymphadenitis in guinea pigs from the Amazonas and Cajamarca regions in Peru was found to be exclusively associated with *Streptococcus equi* subsp. *zooepidemicus*. No evidence of *S. equi* subsp. *equi* was detected in the animals evaluated, which excludes the participation of *S. equi* subsp. *equi* in cervical lymphadenitis in guinea pigs. The *comB* (450 bp) and *seeI* (520 bp) genes proved to be effective molecular targets for differentiating *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi*. The use of these markers enabled a rapid and specific PCR-based diagnosis, overcoming the limitations of conventional culture or biochemical tests, and providing valuable epidemiological and health information for guinea pig farming in Peru. It would therefore be advisable to conduct studies with more sensitive molecular techniques, such as qPCR and longitudinal approaches, to achieve a more precise characterization of the health status of guinea pig populations.

DECLARATIONS

Authors' contributions

Diana Lizeth Arista Vargas was responsible for the conception and design of the study, acquired and analyzed the data, interpreted the findings, and drafted and critically revised the article. Jakson Jacob Socrates Chuquimia del Solar contributed to the study's design and the interpretation of the results, and critically revised the manuscript. Jhorsan David Mauri Pablo and Elthon Thomas Hinojosa Enciso were involved in analyzing the data, drafting the article, and critically revising its intellectual content. Jorge Luis Maicelo Quintana participated in the design of the study and critically revised the manuscript. Marco Rainer Lopez Lapa contributed to the conception of the study and critically revised the article. All authors have read and approved the final edition of the manuscript.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirmed that they have not assisted AI in conducting and preparing the present study.

Availability of data and materials

The data to support the present study's findings are available upon reasonable request to the corresponding author.

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Competing interests

The authors declared no conflict of interest.

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