

Enterococcus faecalis meningitis in a goat kid: Molecular detection and phylogenetic analysis of the *sprE* virulence gene

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Abstract

Enterococcus faecalis associated meningitis is uncommon in humans and has been rarely reported in animals. This report documents a case of meningitis due to *E. faecalis* in a goat kid and details its confirmation through microbiological, molecular, sequencing and phylogenetic approaches. In a goat kid with neurological signs, cerebrospinal fluid was collected, bacterial and molecular analysis was done. The isolate was subjected to cultural and biochemical tests, Polymerase chain reaction using genus- and species-specific primers (E1/E2 and FL1/FL2), sequencing of the serine protease (*sprE*) gene and phylogenetic analysis. PCR confirmed the isolate as *E. faecalis*. Sequence analysis of the *sprE* gene confirmed the isolate EF-CSF-VCRI/OND as *E. faecalis*. Phylogenetic analysis revealed that this isolate represented a distinct lineage with *E. faecalis*. Strain DQ985690.1 (Poland) also branched independently in the phylogenetic tree, exhibiting a divergence pattern similar to isolate EF-CSF-VCRI/OND. This study reported a rare case of *E. faecalis* meningitis in a goat kid. Molecular and phylogenetic analyses identified a distinct lineage, underscoring the need for further research on its clinical significance, epidemiological role and control strategies to reduce economic losses in goat farming.

Keywords: Ataxia, *Enterococcus faecalis*, Goat, Meningitis, *sprE* gene

Highlights

- First report of *Enterococcus faecalis* causing meningitis in a goat kid, a rare caprine neurological disease.
- Etiology confirmed by culture, biochemical tests, PCR with genus- and species-specific primers, and *sprE* sequencing.
- *sprE* gene sequence (EF-CSF-VCRI/OND) formed a distinct phylogenetic lineage divergent from known *E. faecalis* strains.
- Highlights the need for epidemiological surveillance and pathogenicity studies to manage *E. faecalis* in goat farming.

Enterococcus faecalis is a common Gram-positive coccus, usually considered as a commensal organism of the gut and urogenital tract. Nowadays, nosocomial infections associated with *Enterococcus* spp. is an emerging serious issue because of irrational use of antibiotics, immune preparations, and quickly acquiring virulence and multidrug resistance determinants via mobile elements (Raza et al., 2018).

However, recent human studies have documented *E. faecalis* can cause meningitis, and it was proposed that this organism may reach the brain through the haematogenous route (bacteraemia). Similarly, in humans, reported 0.3–4% of bacterial meningitis were caused by *E. faecalis* (Buchs, 1977; Iaria et al., 2005), and the mortality rate for enterococcal meningitis is

20% (Fiore et al., 2019). Based on the available literature, the reports of Enterococcal meningitis in animals are scarce. This present study report could be considered as an uncommon case of *E. faecalis* associated meningitis in a goat, contributing valuable insights into the occurrence and potential impact of this pathogen in atypical hosts.

The present case was recorded at the Veterinary Clinical Complex (VCC) of Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu, India. A four-month-old female goat kid was presented with a history of ataxia, wobbling gait and intermittent seizures. The cerebrospinal fluid (CSF) sample was collected at the lumbosacral space according to Schöb et al. (2024). The collected CSF was

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subjected to bacteriological examination for enterotoxaemia, mycoplasmosis, listeriosis, and enterococci. Further molecular analysis for Caprine Arthritis Encephalitis virus and *E. faecalis* was done by specific PCR, followed by gene sequencing were done to narrow down the etiology. The details of the laboratory analysis are as follows:

CSF sample was inoculated in the nutrient broth and incubated at 37°C and also at 4°C to rule out *Listeria monocytogenes*. After overnight incubation at 37°C, CSF was streaked onto 10% sheep blood agar and MacConkey agar plates and incubated under both aerobic and anaerobic conditions. Isolated colonies were examined by colony morphology and staining techniques. The analytical profile index (API) 20E strips, including 20 biochemical tests, were used for species-level identification as per the manufacturer's instructions (HiMedia). Further, the sample kept under 4°C was inoculated weekly once up to 12 weeks on blood agar plate to rule out *L. monocytogenes*. The Sample was also inoculated in PPLO broth to rule out *Mycoplasma* infection.

Nucleic acid was extracted from isolated bacterial colonies by using the spin column extraction method (QIAamp DNA Mini Kit, Qiagen). Using specific primer sets E1/E2 (Deasy et al., 2000) and FL1/FL2 (Jackson et al., 2004), PCR was employed to identify targets at both the genus and species levels. The PCR reaction mixture included 2 µL of DNA template, 1.5 µL each of forward and reverse primers (10 µM), 8 µL of Premix Taq (2x Taq master mix, Ampliqon) and 7 µL of nuclease-free water. The PCR protocol consisted of an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes. The amplicons were separated by electrophoresis on a 1.5% (w/v) agarose gel at 80 V for 45 minutes. Pathogenicity of

the isolate was confirmed by *sprE* gene-specific PCR. PCR product of *sprE* gene was sequenced by Sanger automated sequencer (Genurem BioSciences Pvt. Ltd, Tamil Nadu). The Primer sequences used in this study, along with their annealing temperatures and amplicon sizes are given in the Table 1.

The nucleotide sequence obtained from the bacterial isolate EF-CSF-VCRI/OND using the Serine protease sequence-specific forward primer was subjected to nucleotide BLAST analysis using the NCBI BLAST web tool, which identified the organism as *E. faecalis*. EF-CSF-VCRI/OND using Serine protease sequence was submitted to NCBI (Accession number PV389473.1). The phylogenetic tree was constructed using the Neighbour Joining method (distance-based approach) in MEGA software version 11.0.13 with the similarity sequences selected from the NCBI database.

In the present study, the goat kid showed signs of ataxia, wobbling gait and intermittent seizures, which were suggestive of CNS affection. To narrow down the potential causes, mycoplasma and listeria infection were ruled out through cultural examination, which failed to identify any causative agents. Additionally, Caprine Arthritis Encephalitis (CAE) virus was excluded as the cause by performing PCR targeting the *gag* gene region of the CAE virus, which returned negative results (Karapýnar et al., 2016).

On blood agar, a small, moist, convex, semi-translucent and white to grey colony was observed (Fig. 1) from the cerebrospinal fluid sample. Growth was also noted on MacConkey agar, and upon Gram staining, the isolated bacteria appeared as Gram-positive cocci (Fig. 2), which is characteristic of *Enterococcus faecalis*. The identification of the strain was further confirmed using the Analytical Profile Index (API) 20E strips, with results provided in Table 2. This comprehensive approach confirmed the identity of the pathogen as *E. faecalis*, associated with the encephalitis in the goat.

Table 1. PCR primers used for the identification of *E. faecalis* isolates

Primer	Nucleotide sequence (5' - 3')	Amplicon size	Annealing temperature (°C)
E1/E2 (Genus specific)	F: TCAACCGGGGAGGGT R: ATTACTAGCGATTCCGG	733 bp	50
FL1/FL2 (Species specific)	F: ACTTATGTGACTAACTTAACC R: TAATGGTGAATCTTGTTTGG	360 bp	55
<i>sprE</i> gene (Expression of the <i>sprE</i> gene contributes to the production of diseases)	F: CTGCCGAAAGTCATTCACGAC R: TTGTTTGCCGACAGTCACGA	359 bp	55



Fig. 1. Non hemolytic colonies of *Enterococcus faecalis* on blood agar

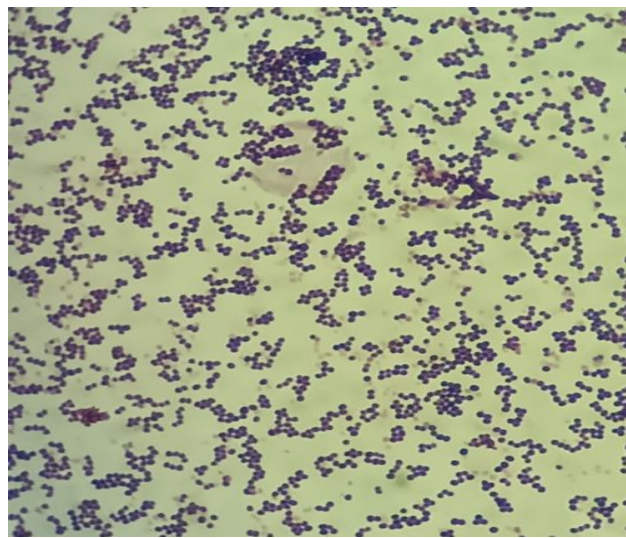


Fig. 2. *Enterococcus faecalis* in Gram staining

Table 2. Results of analytical profile index (API) 20E strips of *Enterococcus faecalis*

Sl. No.	Test	Result
1.	ONPG	Positive
2.	Esculin hydrolysis	Positive
3.	Lysine Utilization	Negative
4.	Ornithine Utilization	Negative
5.	Urease	Positive
6.	Phenylalanine deamination	Negative
7.	VogesProskauer	Negative
8.	Methyl red	Negative
9.	Indole	Negative
10.	PYR	Positive
11.	Beta Glucuronidase	Negative
12.	Alpha Galactosidase	Negative
13.	Beta xylosidase	Negative
Sugar fermentation test		
14.	Sucrose, Trehalose, Maltose, Lactose and Mannose	Positive
15.	Sorbitol, Cellobiose, Melibiose, Salicin, Raffinose	Negative

Enterococcus faecalis has been identified as a causative agent of encephalitis in lambs on several large-scale sheep farms in Xinjiang, China. Those infected lambs also exhibited neurological symptoms (Chai et al., 2019). Several studies have documented that enterococci were significant contributors to nosocomial infections, including UTI, post-surgical infections, bacteremia and endocarditis (Murray, 1990; Moellering, 1992; National Nosocomial Infections Surveillance [NNIS] System, 1999), but limited reports in neurological forms. According to Durand et al. (1993), enterococcal meningitis is an uncommon disease and

limited reports in animals. Since the present case was an uncommon form of *E. faecalis* associated meningitis in goat kid, further genus and species-level confirmation of the isolate was achieved through sequence-specific PCR using the E1/E2 primer pair (733 bp) and FL1/FL2 primer pair (360 bp), as shown in Fig. 3.

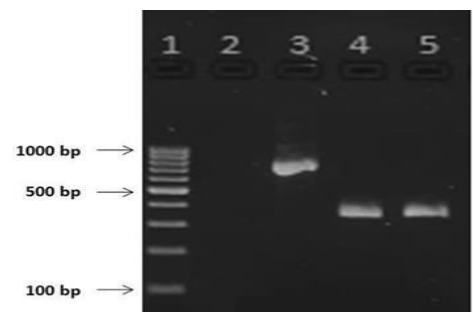


Fig. 3. Agarose gel electrophoresis of PCR amplified product of Genus specific, species specific and virulence genes of *Enterococcus faecalis*

These PCR results confirmed the identification of *Enterococcus faecalis* at both the genus and species levels. Additionally, the virulence potential or disease-causing capacity of the isolate was assessed by amplifying the *sprE* gene, a marker associated with virulence, using the specific PCR primer pair (357 bp),

as indicated in Fig. 3. This confirmed the presence of the *sprE* gene, further validating the pathogenicity of the isolate.

The phylogenetic tree represents the bootstrap consensus tree with replicates, with the bootstrap values indicated on the branches. The branch with higher values indicates stronger support for the relationships. The phylogenetic tree reveals a complex relationship between the analyzed *E. faecalis* strains. The phylogenetic tree results show two primary clusters as Cluster A and Cluster B. Isolate EF-CSF-VCRI/OND branching off independently from the main Cluster A. This suggests that the isolate EF-CSF-VCRI/OND represents a distinct heredity within the *E. faecalis* in its serine protease gene. Strain DQ985690.1 (Poland) branched independently, similar

the distinct phylogenetic clustering highlight its pathogenic potential and genetic diversity. These findings underscore the need to include *E. faecalis* in the differential diagnosis of neurological disorders in goats, and also findings emphasize the importance of veterinary surveillance and molecular diagnostics in managing rare and emerging enterococcal infections in animals.

Conflict of interest: The authors declare that they have no conflicts of interest.

Author's contribution: PS: Analysis, interpretation of the results and manuscript preparation; MS: Sample collection and manuscript preparation; PCP, TLR: Assisted in the manuscript preparation.

Data availability statement: The data used to support the findings of this study are included within the article.

Ethical approval: Ethical committee approval is not required for this study.

Use of artificial intelligence tools: During the preparation of this work, the author did not use any AI tools or services. The content was written and reviewed entirely by the authors, who take full responsibility for the publication.

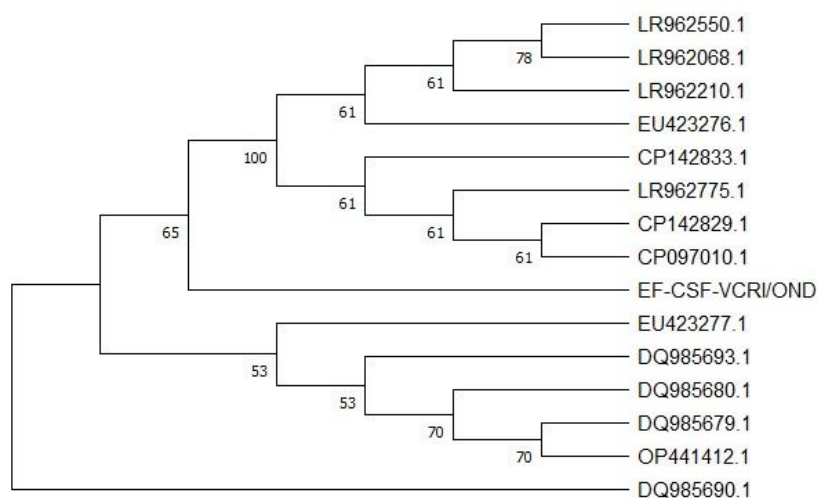


Fig. 4. Phylogenetic tree created using Neighbour Joining method in MEGA software version 11.0.13

to the isolate EF-CSF-VCRI/OND (Fig. 4).

This study documented a rare case of *Enterococcus faecalis* associated meningitis in a goat kid, confirmed through bacterial culture and biochemical and molecular characterization. The detection of the virulence-associated *sprE* gene and

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