



# Isolates from Diseased Cattle Suggest the Presence of Potential Novel *Streptococcus ruminantium* Serotypes

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## Introduction

*Streptococcus ruminantium*, previously classified as *Streptococcus suis* serotype 33, is frequently isolated from ruminants, particularly cattle. Due to historical misclassification, *S. ruminantium* isolates may have been inaccurately identified as *S. suis*, which complicates our understanding of their epidemiology and pathogenic potential.

In this study, we identified ten *S. ruminantium* isolates from diseased or dead cattle. Notably, while some isolates corresponded to the classical serotype 33, seven others harbored distinct capsular polysaccharide (*cps*) loci.

## Methods

Bacterial isolates were collected from diseased or dead cattle, each isolate from a different farm. Seven isolates were from calves, and all but one isolate from a rectal swab were cultured from lung or bronchoalveolar lavage. The other two isolates were also cultured from lungs, but from one bull and one heifer. The last isolate was from mastitic milk. The isolates were initially identified by MALDI-TOF as *S. suis* isolates. Whole genome sequences revealed their close relationship to *Streptococcus suis* serotype 33, which is now classified as *Streptococcus ruminantium*. A Neighbor-Joining phylogenetic tree was constructed from the core genome alignment of *S. suis* reference serotypes and our analyzed *S. ruminantium* isolates, and visualized using Jalview (Figure 1).

Clusters of *cps* genes were extracted and analyzed to investigate potential serotype diversity

## Results and discussion

The average nucleotide identity (ANI) among the analyzed isolates ranged from 98.35% to 98.80%. Analysis of the *cps* gene clusters revealed potentially novel capsular type among *S. ruminantium* strains. The *cps* cluster of five isolates was similar to the *cps* cluster of serotype 33. For the other isolates, the *cps* cluster was similar to each other but significantly different from the *cps* cluster of serotype 33, suggesting a potential new serotype of *S. ruminantium* (Table 3). This finding suggests a broader *cps* diversity than previously recognized, indicating the presence of additional serotypes. Detailed classification based on *cps* loci may thus contribute to a better understanding of the pathogenicity and zoonotic potential of *S. ruminantium*.

Table 1: Pangenome genes summary

Genes	Description	Number
Core genes	(99% <= strains <= 100%)	1458
Soft core genes	(95% <= strains < 99%)	0
Shell genes	(15% <= strains < 95%)	1887
Cloud genes	(0% <= strains < 15%)	3245
Total genes	(0% <= strains <= 100%)	6590

Table 2: Schematic representation of genes presence or absence between samples:

C10_filtered		2830
D8_filtered		4490
E10_filtered		2485
E9_filtered		2026
F10_2022_filtered		1934
F12_2022_filtered		1996
G2_2022_filtered		1964
G4_2022_filtered		1984
G5_2022_filtered		1840
G8_2022_filtered		1981
G9_2022_filtered		1915

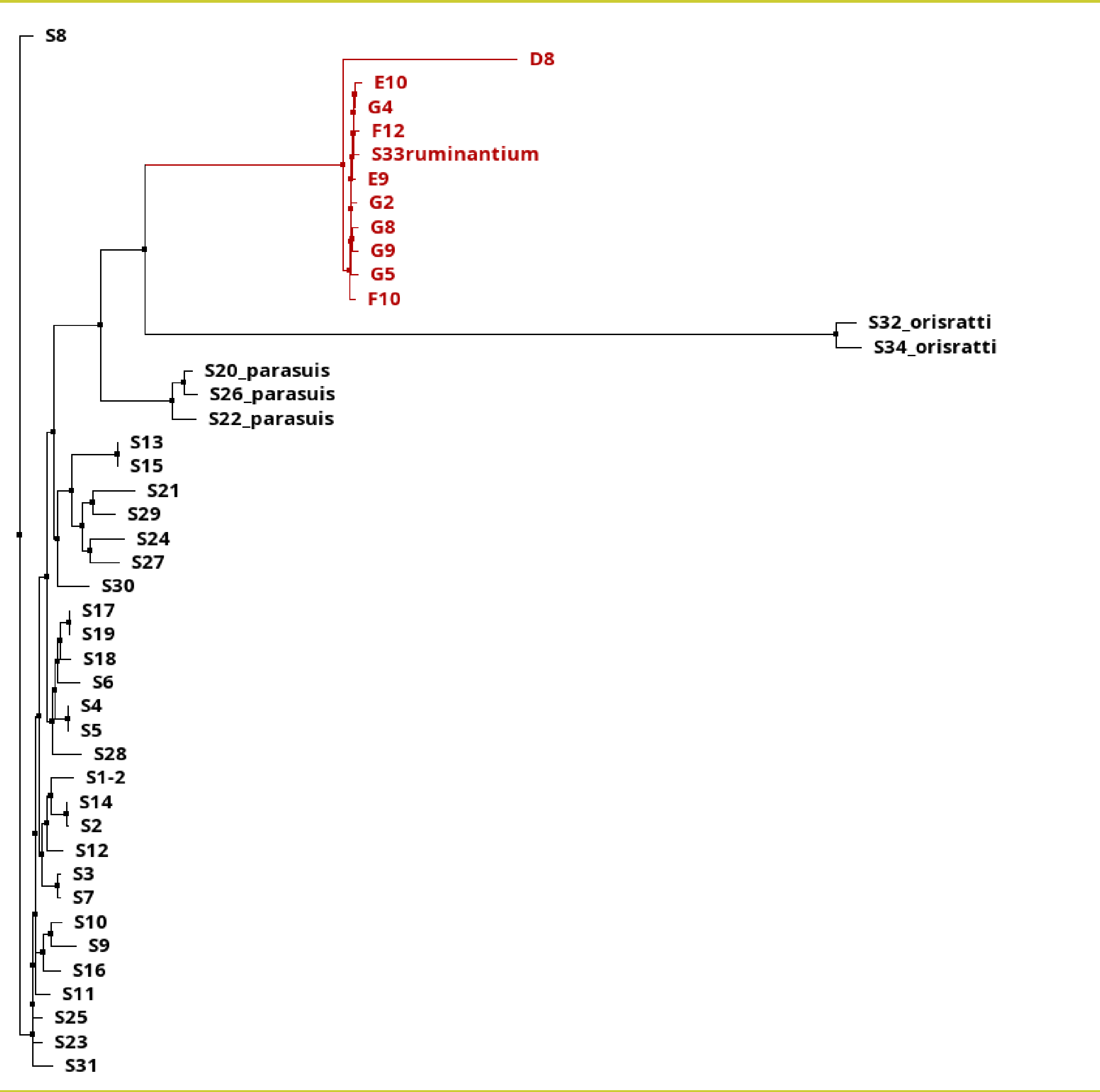
Table 3: Summary of genes in the *cps* locus. Genes were annotated using PROKKA.

S. ruminantium - reference strain (C10)			E10			G2, G9			G5			F12		
bp	gene	product	bp	gene	product	bp	gene	product	bp	gene	product	bp	gene	product
1110	gff	UDP-galactopyranose mutase	1110	gff	UDP-galactopyranose mutase	1110	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase
1392		hypothetical protein	1392		hypothetical protein	1392		hypothetical protein	1158		hypothetical protein	1158		hypothetical protein
1053	rfbB	dTDP-glucose 4,6-dehydratase	1053	rfbB	dTDP-glucose 4,6-dehydratase	1053	rfbB	dTDP-glucose 4,6-dehydratase	1062	rfbB	dTDP-glucose 4,6-dehydratase	1062	rfbB	dTDP-glucose 4,6-dehydratase
720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
975		hypothetical protein	975		hypothetical protein	975		hypothetical protein	975		hypothetical protein	975		hypothetical protein
1311		hypothetical protein	228		hypothetical protein			* Serotyping seq. present, not annotated by PROKKA						
921		hypothetical protein	216	tarI	Ribitol-5-phosphate cytidyltransferase	216	tarI	Ribitol-5-phosphate cytidyltransferase	1074		hypothetical protein	1074		hypothetical protein
948		hypothetical protein	1293		hypothetical protein	1293		hypothetical protein	918		hypothetical protein	918		hypothetical protein
816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase	924		hypothetical protein	924		hypothetical protein	954		hypothetical protein	954		hypothetical protein
603	pggC_1	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase	816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase
948		hypothetical protein	603	pggC	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase	603	pggC	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase	603	pggC	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase	603	pggC	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase
717		hypothetical protein	717		hypothetical protein	717		hypothetical protein	717		hypothetical protein	717		hypothetical protein
1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase
732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB_1	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB
696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD_1	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD
690	ywqC_1	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC
1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_2	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_2	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU
744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA

S. ruminantium - reference strain (C10)			D8, E9, F10, G8			G4		
bp	gene	product	bp	gene	product	bp	gene	product
1110	gff	UDP-galactopyranose mutase	1107	gff	UDP-galactopyranose mutase	1107	gff	UDP-galactopyranose mutase
1392		hypothetical protein	696	cugP	UTP-glucose-1-phosphate uridylyltransferase	696	cugP	UTP-glucose-1-phosphate uridylyltransferase
1053	rfbB	dTDP-glucose 4,6-dehydratase	918		hypothetical protein	918		hypothetical protein
720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	1548	thiK	Thiamine kinase	1548	thiK	Thiamine kinase
975		hypothetical protein	846		hypothetical protein	846		hypothetical protein
1311		hypothetical protein	840		hypothetical protein	840		hypothetical protein
921		hypothetical protein	1479	murJ_1	lipid II flippase MurJ	1479	murJ_1	lipid II flippase MurJ
948		hypothetical protein	1299		hypothetical protein	1299		hypothetical protein
816	wbbD	UDP-Gal-alpha-D-GlcNAc-diphosphoundecaprenol beta-1,3-galactosyltransferase	1131		hypothetical protein	1131		hypothetical protein
603	pggC_1	Undecaprenyl phosphate N,N'-diacetylbaicillosamine 1-phosphate transferase				1113	epsF	Putative glycosyltransferase EpsF
717		hypothetical protein	993		Capsular polysaccharide phosphotransferase cps12A	993		Capsular polysaccharide phosphotransferase cps12A
1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1098	mnmA	UDP-N-acetylglucosamine 2-epimerase	1098	mnmA	UDP-N-acetylglucosamine 2-epimerase
732	cpsB	Tyrosine-protein phosphatase CpsB	999		hypothetical protein	759		hypothetical protein
696	cpsD	Tyrosine-protein kinase CpsD	642		hypothetical protein	642		hypothetical protein
690	ywqC_1	putative capsular polysaccharide biosynthesis protein YwqC	1149	msmA	D-inositol-3-phosphate glycosyltransferase	1149	msmA	D-inositol-3-phosphate glycosyltransferase
1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1176		hypothetical protein	1176		hypothetical protein
744	yaaA	Peroxide stress resistance protein YaaA	1377		hypothetical protein	1377		hypothetical protein
			732	cpsB_1	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB
			696	cpsD_1	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD
			690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC
			1446	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1446	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU
			744	yaaA_1	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA

Serotype-specific PCR fragment location highlighted in blue

Figure 1: Core genome alignment phylogenetic tree – Neighbor-joining method. *S. ruminantium* in red; *S. suis* in black.



## Acknowledgements

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