

Article

First Report of *Streptococcus ruminantium* in Wildlife: Phenotypic Differences with a Spanish Domestic Ruminant Isolate

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Abstract: *Streptococcus ruminantium* is a recent reclassification of the former *Streptococcus suis* serovar 33. Although knowledge about *S. suis* is extensive, information on *S. ruminantium* host range and pathogenic potential is still scarce. This bacterium has been isolated from lesions in domestic ruminants, but there are no reports in wild animals. Here, we provide information on lesions associated with *S. ruminantium* in Pyrenean chamois (*Rupicapra pyrenaica*) and domestic sheep from NE Spain, as well as phenotypic biopatterns and antimicrobial resistance (AMR) of the isolates. Overall, lesions caused by *S. ruminantium* were similar to those caused by *S. suis*, excluding polyserositis. Heterogeneity of the phenotypic profiles was observed within the *S. ruminantium* strains by VITEK-2, resulting in only two tests common to all *S. ruminantium* isolates and different from *S. suis*: Alpha-Galactosidase and Methyl-B-D-Glucopyranoside, both positive for *S. suis* and negative for *S. ruminantium* strains. Isolates from Pyrenean chamois were susceptible to all antimicrobials tested, except danofloxacin, whereas the domestic sheep isolate was resistant to tetracycline. In conclusion, *S. ruminantium* can cause infection and be associated with pathology in both wild and domestic ruminants. Due to its phenotypic diversity, a specific PCR is optimal for identification in routine diagnosis.

Keywords: *Streptococcus ruminantium*; antimicrobial resistance; Pyrenean chamois

1. Introduction

Nowadays, novel bacteria are continually discovered or renamed due to the use of genomic information. Within the *Streptococcus* genus, four new species were proposed in 2020 [1]. *Streptococcus* comprises species that are naturally found in the microbiota of mucosae, especially in the upper respiratory tract, but can also cause disease in humans and animals [2].

Serovar 33 of *Streptococcus suis*, one of the most important swine bacterial pathogens worldwide [3], has recently been reclassified as a species nova, *Streptococcus ruminantium* [4]. With the development of a specific PCR for the identification of *S. ruminantium* by Okura

et al. [5], many *S. suis*-like strains, including serovar 33 and non-typeable *S. suis* strains, together with strains from other species, such as *Streptococcus oralis* and *Streptococcus mitis*, have been reclassified as *S. ruminantium* [5,6]. Despite capsule switching being demonstrated for *S. suis* among serotypes 2, 3, and 7 [7], it is unknown if *S. ruminantium* could be involved in this interchange with other *S. suis* serotypes. Thus far, all *S. ruminantium* isolates, except two that were recovered from a milk-feeding robot and a bulk tank milk [5,6], have been isolated from domestic ruminants (cattle, sheep, and goat), but none from wildlife species.

Due to its recent description, information about lesions associated with *S. ruminantium* is scarce. In cattle, *S. ruminantium* has been associated with endocarditis, arthritis, pneumonia, mastitis, and abscesses in the liver, lung, and tympanic cavity, whereas in sheep, it has been associated with pneumonia, endocarditis, and arthritis [5,6]. No clinical information is available on the only strain isolated from goats [5,6]. *S. ruminantium*, like its relative *S. suis*, has also been found as commensal in tonsils or oral cavities in ruminants [5,6]. Despite the lack of epidemiological information, *S. ruminantium* could be a colonizer of the upper respiratory tract of ruminants, similar to *S. suis* in piglets.

To prevent the development of bacterial infections, livestock is often treated prophylactically with antimicrobial agents. The widespread use of antimicrobials creates a selective environmental pressure, which favors selecting bacterial genes that confer antimicrobial resistance. In high-altitude alpine environments, where Pyrenean chamois (*Rupicapra pyrenaica*) lives, the presence of free-ranging livestock during summer seems to be the main source of AMR for wild animals [8]. Domestic sheep are closely monitored, and antimicrobials are applied when an infectious process is detected. Therefore, the closer the animals are to humans, the greater the exposure to antimicrobials and possibly to resistant bacteria [9].

The present study gives the first information about the lesions produced by the biochemical characterization and antimicrobial susceptibility of four clinical isolates from Pyrenean chamois and one from domestic sheep from Catalonia, Spain, classified as *S. ruminantium* by partial 16S rRNA gene sequence and specific PCR. These strains represent the first report of *S. ruminantium* in wildlife worldwide and the first report in domestic ruminants in Spain.

2. Materials and Methods

2.1. Bacterial Identification and Genotyping

Five *S. ruminantium* isolates were included in the present study, four from Pyrenean chamois and one from domestic sheep. Information on hosts is included in Table 1.

Table 1. Main characteristics of the *Streptococcus ruminantium* isolates included in this study.

Isolate	Organ of Isolation	Host Species	Age and Sex	Cause of Death	Main Necropsy Findings	Place	Date
RP15178-A2	Lung lesion	Chamois (<i>Rupicapra pyrenaica</i>)	4 years, male	Seasonal controlled hunting	Infectious queratoconjunctivitis, diarrhea, and bacterial cranioventral suppurative bronchopneumonia	Freser-Setcases National Game Reserve (Queralbs, Catalonia, Spain)	December 2015
RP16030-M1	Lung lesion	Chamois (<i>Rupicapra pyrenaica</i>)	4 years, female	Seasonal controlled hunting	Old fibrous pleural adhesions from previous pneumonia and cysticercosis in peritoneum and diaphragm	Freser-Setcases National Game Reserve (Queralbs, Catalonia, Spain)	May 2016
CCGMV933	Lung lesion	Chamois (<i>Rupicapra pyrenaica</i>)	14 months, male	Severe pestivirus infection *	Cachexia, bacterial cranioventral suppurative bronchopneumonia, multifocal acute suppurative hepatitis, and nonsuppurative viral meningoencephalitis	Aigüestortes i Estany de Sant Maurici National Park (La Vall de Boí, Catalonia, Spain)	August 2020
CCGMV935	Heart valves vegetation	Chamois (<i>Rupicapra pyrenaica</i>)	5 months, male	Bacterial septicemia *	Bacterial suppurative endocarditis, meningoencephalitis, embolic pneumonia, hepatitis, nephritis, splenitis, lymphadenitis, myelitis, contagious ecthyma, verminous pneumonia, and bronchopneumonia	Freser-Setcases National Game Reserve (Queralbs, Catalonia, Spain)	October 2020
CCGMV928	Liver abscess	Domestic sheep (<i>Ovis aries</i>)	3 months, male	Slaughterhouse	Liver abscesses and cranioventral suppurative bronchopneumonia	Municipal slaughterhouse (Farm located in Catalonia, Spain)	September 2019

* Probable cause of death.

Bacterial isolates were recovered from the lesions after plating onto Columbia agar and chocolate agar (Biomérieux, Marcy l'Étoile, France) and incubation 24 h in a 5% CO₂ atmosphere at 37 °C.

DNA extraction was performed using Chelex based Instagene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. *S. ruminantium* identification was performed with the species-specific PCR described by Okura et al. [5], based on the 16S rRNA gene sequence. To discriminate from *S. suis*, the *recN*-PCR described by Ishida et al. [10] was also performed. *S. suis* reference strain P1/7 was used as the negative and positive control, respectively, in the described PCRs. To confirm that the five isolates were not epidemiologically associated and constituted different *S. ruminantium* strains, fingerprinting by ERIC-PCR was performed [11]. Table 2 shows the primers used in the PCRs.

Table 2. Primers used for bacteria identification.

PCR	Primers	Primer sequence	Reference
16S	358F 907R	CTACGGGAGGCAGCAGT CCGTCWATTCMTTGTGAGTT	Amplification and sequencing of the V2-V5 region of the 16S rRNA gene [12].
<i>recN-S. suis</i>	SSrecN-F SSrecN-R	CTACAAACAGCTCTCTTCT ACAACAGCCAATTCATGGCGTGATT	PCR based on the <i>S. suis</i> recombination/repair protein (<i>recN</i>) [10].
<i>S. ruminantium</i>	Forward Reverse	GCAAGTGGAACGCAACTTTTCA CTATGTATCGTTGCCTTGGTAG	PCR designed to discriminate <i>S. ruminantium</i> from <i>S. suis</i> . [5].
ERIC	ERIC-1F ERIC-2R	ATGTAAGCTCCTGGGGATTAC AAGTAAGTGACTGGGGTGAGCG	Enterobacterial repetitive intergenic consensus PCR [11].

The identification of the isolates was further confirmed by partially sequencing the 16S rRNA gene, using the primers 358F and 907R (Table 2). The resulting sequences were compared to the GenBank database using the BLAST algorithm at NCBI.

2.2. Isolates Characterization

Biochemical characterization was performed using the VITEK-2 system with the compact Gram-positive card (Biomérieux) for the five *S. ruminantium* isolates and *S. suis* serovar 2 virulence reference strain P1/7.

Antimicrobial susceptibility testing to determine minimal inhibitory concentrations (MIC) was performed using Sensititre Vet Bovine BOPO7F Plate (Sensititre® Susceptibility plates, Thermo Fisher Scientific, West Sussex, UK) following manufacturer's instructions. The antimicrobials tested were: ampicillin (AMP, 0.25–16 µg/mL), ceftiofur (XNL, 0.25–8 µg/mL), clindamycin (CLI, 0.25–16 µg/mL), danofloxacin (DANO, 0.12–1 µg/mL), enrofloxacin (ENRO, 0.12–2 µg/mL), florfenicol (FFN, 0.25–8 µg/mL), gamithromycin (GAM, 1–8 µg/mL), gentamicin (GEN, 1–16 µg/mL), neomycin (NEO, 4–32 µg/mL), penicillin (PEN, 0.12–8 µg/mL), spectinomycin (SPE, 8–64 µg/mL), sulfadimethoxine (SDM, 256 µg/mL), tetracycline (TET, 0.5–8 µg/mL), tiamulin (TIA, 0.5–32 µg/mL), tildipirosin (TIP, 1–16 µg/mL), tilmicosin (TIL, 2–16 µg/mL), trimethoprim-sulfamethoxazole (SXT, 2/38 µg/mL), tulathromycin (TUL, 8–64 µg/mL) and tylosin tartrate (TYLT, 0.5–32 µg/mL). Plates were incubated at 37 °C for 24 h and the reading was performed manually.

When available, results were interpreted using VET01S and VET08 CLSI resistance breakpoints for cattle [13,14].

2.3. Histopathology

Histopathological examinations were performed for the cases from 2019 and 2020 (isolates CCGMV928, CCGMV933, and CCGMV935, Table 1). For histopathological examination, representative tissue samples were collected and preserved in 10% neutral buffered formalin. After 48 h of fixation, tissues were embedded in paraffin and routinely stained with hematoxylin-eosin and Gram staining.

3. Results

3.1. Specific PCR Is a Suitable Method for Identification of *S. ruminantium*

All samples presented colonies with alpha-hemolysis and macroscopically compatible with *Streptococcus* spp. after 24 h of incubation on Columbia agar.

The sequence from the 16s rRNA gene fragment from all the isolates showed the highest homology to *S. ruminantium* in NCBI (Table S1).

All isolates were negative for the *recN*-PCR, specific for *S. suis*, and presented a PCR product of approximately 240 bp in the *S. ruminantium*-PCR, described by Okura et al. [5] as the specific PCR product. Different fingerprinting profiles were observed in the ERIC-PCR (Figure S1), confirming that the five isolates may be considered different strains.

3.2. *S. ruminantium* Isolates Presented Phenotypic Differences

Forty-three different biochemical reactions were performed using the VITEK-2 instrument. Bacterial identification of each isolate using the phenotypic biopatterns is shown in Table S2. *S. ruminantium* strains showed a heterogeneous pattern, with only 65.1% of the tests (28 out of 43, Table S2) with identical results for all the five strains. These results led to a different species identification among the *S. ruminantium* isolates, but all were identified within the *Streptococcus* genus. Moreover, all strains except CCGMV933 were identified as possible *S. suis* (50–95% identification, Table S2). The strains did not share an identical biochemical profile. Only two tests were common to all the *S. ruminantium* isolates and different to the *S. suis* reference strain P1/7: Alpha-Galactosidase and Methyl-B-D-Glucopyranoside, both positive for *S. suis* and negative for *S. ruminantium*.

All the strains were susceptible to ampicillin, ceftiofur, clindamycin, gamithromycin, penicillin, sulfadimethoxine, trimethoprim-sulfamethoxazole, and tulathromycin, as no growth was observed at the lowest concentration of antimicrobial tested (Table 3). The strain CCGMV928, isolated from domestic sheep, was resistant to tetracycline since it grew at all concentrations tested. This strain also showed the highest MIC value for gentamicin, which could be considered as intermediate susceptibility. In the case of danofloxacin, only a susceptible breakpoint has been established (≤ 0.25 $\mu\text{g}/\text{mL}$), and therefore only strains CCGMV935 and CCGMV928 can be considered susceptible. The strains showed similar patterns for the remaining antimicrobials tested, and according to the guidelines described in material and methods, they could be considered susceptible.

Table 3. Minimal Inhibitory Concentrations ($\mu\text{g}/\text{mL}$) obtained using Sensititre Vet Bovine BOPO7F Plate.

Antimicrobial	RP15178-A2	RP16030-M1	CCGMV933	CCGMV935	CCGMV928
Ampicillin	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25
Ceftiofur	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25
Clindamycin	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25
Danofloxacin	0.50 *	0.50 *	1 *	≤ 0.12	0.25
Enrofloxacin	0.50	0.50	1	≤ 0.12	0.25
Florfenicol	2	1	2	1	1
Gamithromycin	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1
Gentamicin	4	4	4	4	8
Neomycin	16	8	16	16	16
Penicillin	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12
Spectinomycin	16	16	16	16	16
Sulfadimethoxine	≤ 256	≤ 256	≤ 256	≤ 256	≤ 256
Tetracycline	1	1	1	≤ 0.5	>8 *

Table 3. Cont.

Antimicrobial	RP15178-A2	RP16030-M1	CCGMV933	CCGMV935	CCGMV928
Tiamulin	4	4	8	16	16
Tildipirosin	16	8	8	16	16
Tilmicosin	8	8	8	16	8
Trimethoprim-sulfamethoxazole	≤2/38	≤2/38	≤2/38	≤2/38	≤2/38
Tulathromycin	≤8	≤8	≤8	≤8	≤8
Tylosin tartrate	1	≤0.5	≤0.5	≤0.5	≤0.5

* Resistant.

3.3. Lesions Associated with *S. ruminantium* Isolation

The Pyrenean chamois from which the CCGMV933 strain was isolated was found with severe depression and poor body condition and died during transportation to the Faculty of Veterinary Medicine of the Universitat Autònoma de Barcelona. At the post-mortem and microscopic examinations, the main findings were nonsuppurative encephalitis consistent with a viral infection, mild to moderate and chronic suppurative bacterial cranioventral bronchopneumonia, and acute multifocal suppurative hepatitis. *S. ruminantium* was isolated from the lung, along with *Pasteurella multocida*. Pestivirus infection (Border disease virus) was confirmed through PCR in spleen and brain tissues following previously published methods [15].

The Pyrenean chamois of the CCGMV935 strain was found dead with poor body condition and mild alopecic and hyperkeratotic lesions around the coronary band of the hooves in all four legs. At necropsy, irregular yellow vegetations covered with clotted blood were observed on the left atrioventricular valve, aortic, and right atrioventricular (Figure 1A). Histological examinations showed valve leaflets expanded by fibroconnective tissue, fibrin, inflammatory cells, and numerous coccus-shaped Gram-positive bacteria (Figure 1B,C). Other findings were microscopic multifocal/embolic septic fibrin thrombi and necrosis in the brain, lungs, liver, kidney (infarcts and embolic glomerulitis), spleen and bone marrow, and multifocal fibrinoid arteritis consistent with septicemia. In all these tissues, Gram-positive cocci were identified within the lesions. There were lesions of a preexistent suppurative cranioventral bacterial bronchopneumonia, from where numerous colonies of *Mannheimia haemolytica* were isolated, and caudal verminous pneumonia was also present. Skin lesions were highly consistent with mild chronic lesions of contagious ecthyma (parapoxvirus infection, endemic in Pyrenean chamois).

In the case of the domestic sheep, where *S. ruminantium* CCGMV928 was isolated from, multiple white to yellowish nodular suppurative lesions of 0.5 to 2 cm in diameter were detected in the liver (Figure 2A,B). These lesions were randomly distributed on the surface and throughout the parenchyma. Adjacent lymph nodes appeared to increase in size but without evident macroscopic lesions. Additionally, there were consolidation and reddening of cranio-ventral lung lobes consistent with suppurative bronchopneumonia and multifocal areas of grey to whitish discoloration and consolidation in caudo-dorsal lobes consistent with verminous pneumonia. At histology, multiple extensive abscesses were detected, distorting the hepatic parenchymal architecture. These were characterized by a large necrotic core with abundant cellular debris and eosinophilic amorphous material with multifocal areas of mineralization (Figure 2C). Numerous Gram-positive cocci were observed mixed with the necrotic material (Figure 2D). The periphery of this core was lined by numerous viable and degenerated neutrophils, surrounded by a thick band of fibroconnective tissue (capsule), highly infiltrated by lymphocytes, plasma cells, histiocytes with lesser neutrophils, and scattered eosinophils. Occasionally, remnants of biliary epithelium could be seen surrounding these abscesses (Figure 2B). The vast majority of the portal

spaces were markedly expanded by abundant lymphocytes, plasma cells, some histiocytes, and numerous eosinophils.

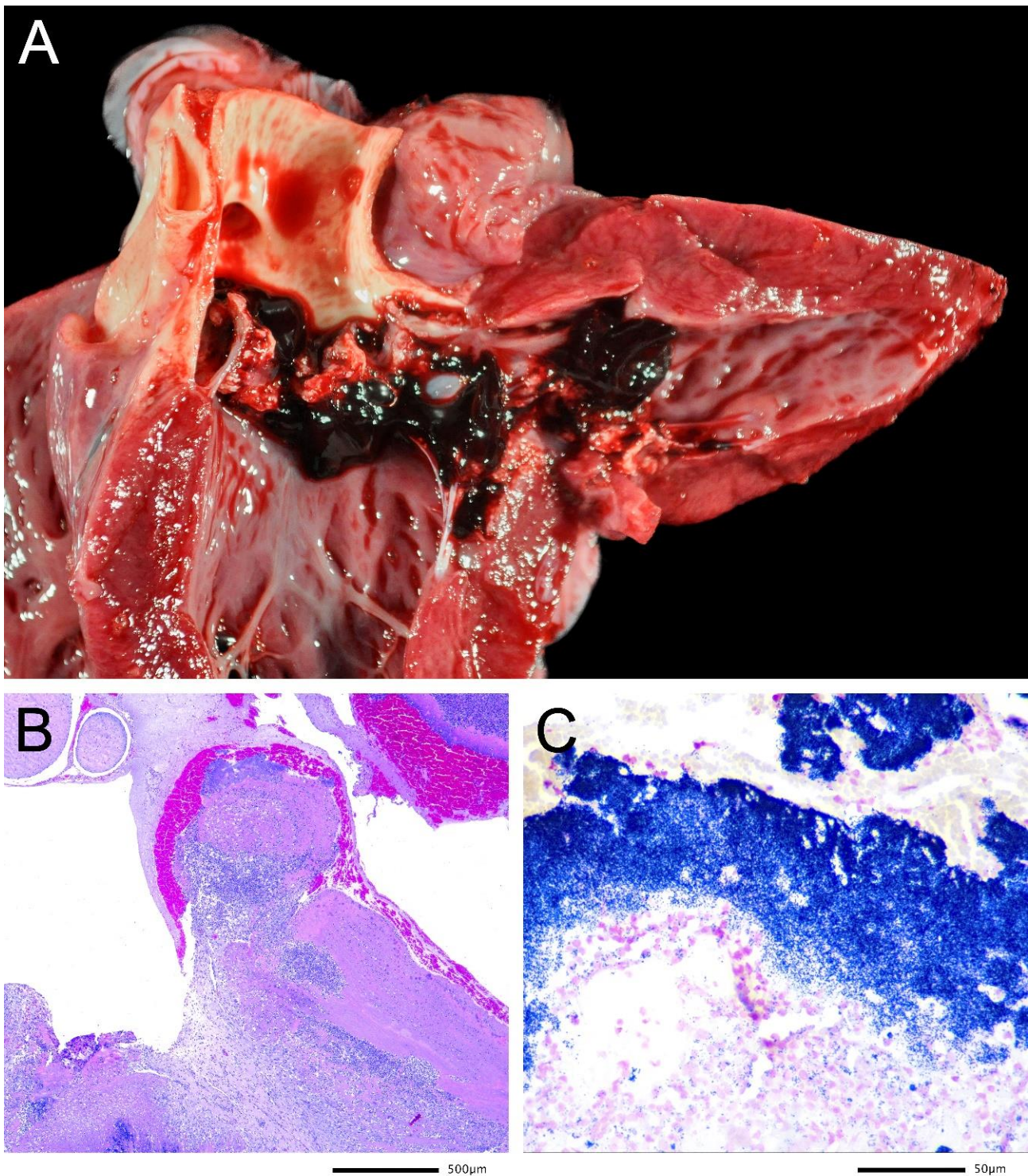


Figure 1. Pyrenean chamois heart from which *Streptococcus ruminantium* was isolated, strain CCGMV935. (A) Vegetative endocarditis of the left atrioventricular valve in a chamois (*Rupicapra pyrenaica*) caused by *S. ruminantium*. (B) H&E staining shows that the valve leaflet is expanded by fibrin, inflammatory cells, and numerous bacterial colonies. (C) Gram staining showing coccus-shaped Gram-positive (dark blue) bacteria in the vegetation.

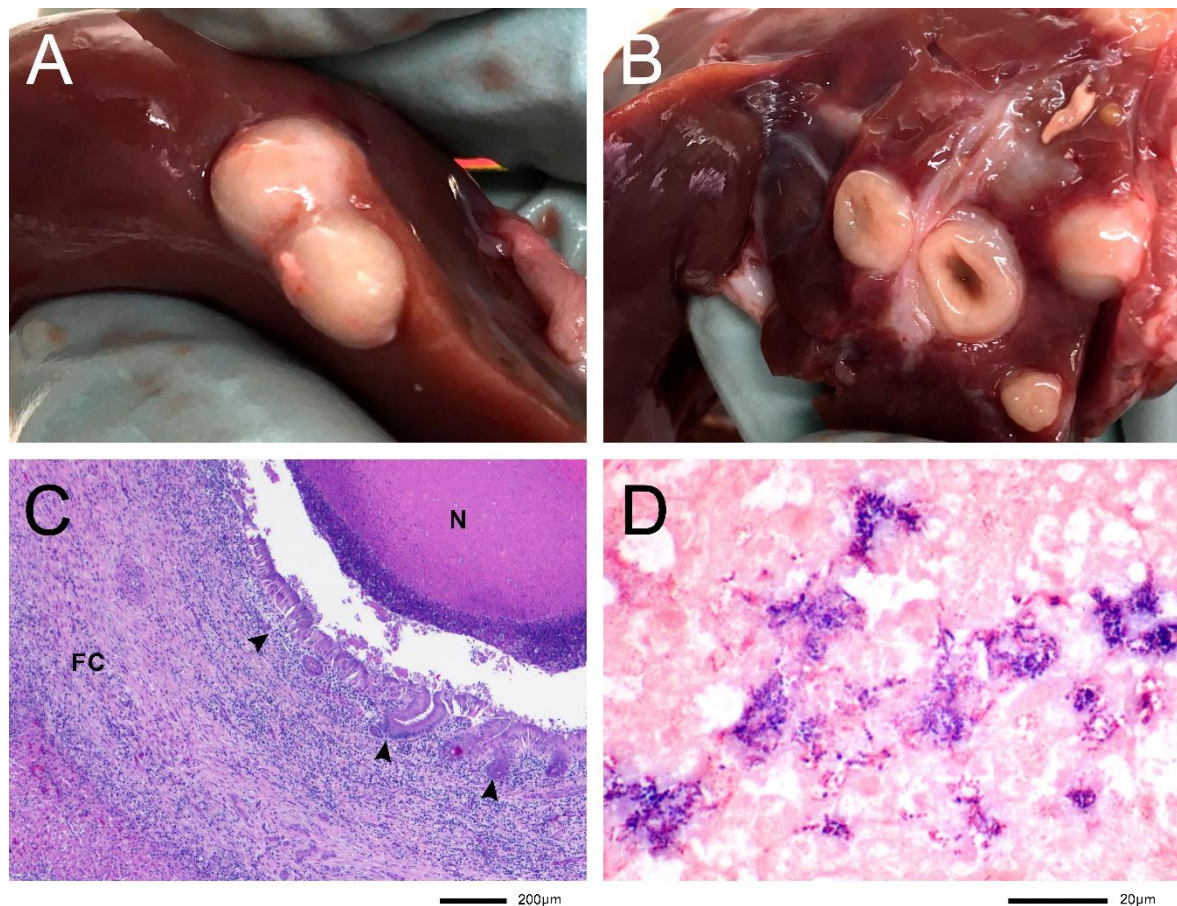


Figure 2. Sheep liver from which *Streptococcus ruminantium* was isolated, strain CCGMV928. (A) White nodular lesions on a liver surface measuring 0.5 to 2 cm in diameter. (B) Detail of a sectioned intraparenchymal nodule. (C) H&E staining showing a necrotic core (N) surrounded by a suppurative inflammatory infiltrate and a fibrous capsule (FC). Notice the remains of the biliary epithelium (arrowheads). (D) Gram staining. Showing multiple coccus-shaped Gram-positive bacteria within the necrotic core.

4. Discussion

The presence and associated pathology of the new pathogen *S. ruminantium*, previously classified as *S. suis* serovar 33, has been assessed for the first time in wildlife. The diverse ERIC-PCR profiles and biochemical characteristics among strains isolated in Pyrenean chamois in different years suggest a high *S. ruminantium* diversity in NE Spain.

Wildlife ecosystems, such as the alpine mountains where the Pyrenean chamois live, could be a reservoir of resistance genes as well as resistant microorganisms [16,17]. In order to monitor the health of an ecosystem in the face of man-enhanced hazards, wildlife inhabiting these ecosystems has the potential to serve as sentinels [18]. Despite the major concern that AMR represents for human health and the environment, knowledge of the bacteria associated with disease and their AMR profiles is relatively sparse in wildlife, and considerable knowledge gaps remain [9,19,20]. The *S. ruminantium* isolated from domestic sheep was the only strain resistant to tetracycline (grew in the presence of concentrations of up to 8 µg/mL). Even though it was not possible to obtain information on the use of tetracyclines in the farm of origin, this antimicrobial belongs to the class of veterinary antimicrobials with the largest amount of sales, expressed in mg/PCU, in the UE in the 2005–2018 period (30.7% in 2018) [21]. Consequently, the presence of tetracycline-resistant strains is common in food-producing animals [22,23]. Moreover, within the streptococci

of bovine or ovine origin, tetracycline was reported as the antibiotic with the highest prevalence of resistance [24].

Due to limited bibliography, little is known about AMR in *S. ruminantium*. Okura et al. (2019) found genes that can confer resistance to different antimicrobials in 13 *S. ruminantium* isolates, including aminoglycosides, macrolides, phenicols, streptomycin, streptothricin, and tetracyclines, as well as high MIC values for chloramphenicol, erythromycin, kanamycin, streptomycin, and tetracycline in some of their tested strains. In agreement with our results, Gottschalk et al. reported that *S. ruminantium* strains were susceptible to penicillin, ampicillin, ceftiofur, enrofloxacin, trimethoprim-sulfamethoxazole, and florfenicol but highly resistant to chlortetracycline and oxytetracycline [6]. Thus, the possible role of *S. ruminantium* as a reservoir of AMR genes for other streptococci should be studied.

AMR profiles of bacteria from wild mammals are influenced by the proximity to human activities, being the use of antimicrobials in agriculture is one of the main sources of AMR [25]. Pyrenean chamois may be a good indicator of the environmental contamination of alpine ecosystems with resistance genes or microorganisms, as demonstrated for respiratory pathogens such as *Pasteurella multocida* or *Mannheimia haemolytica* [17]. In the present study, due to the low number of Pyrenean chamois strains, this species' suitability for studying resistances in *S. ruminantium* cannot be stated, and further research on this bacterium is needed to confirm these observations.

Overall, lesions caused by *S. ruminantium* reported in the literature are similar to those caused by *S. suis*. However, ruminants did not present polyserositis, which is a typical clinical sign in *S. suis* infection in piglets [3,26,27]. The lesions observed in the Pyrenean chamois in our study, both pneumonic and heart lesions, are consistent with previously described lesions for *S. ruminantium* in livestock [5,6]. Additionally, the liver abscesses produced by the CCGMV928 strain in sheep coincide with the described pathology in cattle [5]. The biliary epithelium surrounding the abscesses suggests an ascendant infection as a plausible origin in this sheep case. Unfortunately, it has not been possible to obtain additional data on the incidence of these lesions from the farm of origin. This particular case was submitted to the slaughterhouse support service (SESC) as a suspected tuberculosis case. Still, liver abscesses are a common finding rarely submitted for further investigations. Thus, it is probable that *S. ruminantium* cases are underreported. In the Pyrenean chamois, the origins of the *S. ruminantium* infections were not clear. The most chronic lesion for the CCGMV935 animal was the valve vegetation, evidenced by a severe proliferation of fibrous tissue. Despite the severity of the lesions from which *S. ruminantium* was isolated in these cases, *S. ruminantium* does not seem to be a pathogen with high clinical prevalence, according to the low number of global reports in livestock. However, misidentification is likely contributing to this low reporting. The epidemiological information recovered in the present study suggests that *S. ruminantium* may be the cause of secondary infections produced by a weakened immune system by other pathogens or stressors. As a result, it may be considered an opportunistic pathogen. In this sense, three Pyrenean chamois had significant viral (pestivirus or parapoxvirus infection) and/or bacterial concomitant infections. Pestivirus in chamois is commonly a chronic infection that has particularly been associated with immunosuppression and opportunistic secondary infections [28]. On the other hand, *Pasteurellaceae* are primary agents for pneumonia and were isolated in all pneumonic lesions from which *S. ruminantium* was also isolated. Altogether, it suggests that *S. ruminantium* may also be an opportunistic pathogen for chamois. In the domestic sheep case, despite no parasitic infections being recorded by the slaughterhouse veterinary authorities, numerous eosinophils were observed in the portal spaces, suggesting that a parasitic component was also present.

In the case of the closely related *S. suis*, higher susceptibility to infection has been demonstrated in piglets previously infected with porcine reproductive and respiratory syndrome virus [29–31], swine influenza virus [32], or porcine circovirus type 2 [33], but also in piglets inoculated with *S. suis* and pseudorabies virus [34].

The proper identification of *S. ruminantium* is key to improving this pathogen's scientific knowledge. Our results indicate that phenotypic characterization is insufficient to distinguish *S. ruminantium* from *S. suis*, and it could lead to a misidentification problem. In our opinion, the best approach for the identification of *S. ruminantium* is the specific PCR described by Okura et al. [5], which we recommend as a first step for the identification of *Streptococcus*-like isolates from ruminants. Isolates that are negative for this PCR should also be tested using the *recN*-PCR for *S. suis* as suggested by Gottschalk et al. [6] since there are other species described that can lead to a misidentification, as is the case of *S. rupicaprae* in chamois, negative for both PCR (data not shown). The identification of *S. ruminantium* using VITEK-2 is not recommended due to the high heterogeneity shown by the strains of this study. The 16S rRNA gene sequencing is also useful for identifying isolates negative to *S. ruminantium* or *S. suis* PCRs. Still, it is necessary to keep databases up to date because new species are continuously described, as was the case of *S. ruminantium*.

In the present study, *S. ruminantium* was isolated from pneumonic and heart lesions in Pyrenean chamois for the first time and from a liver lesion in sheep, which was previously reported. Identification of *S. ruminantium* was facilitated due to the use of a specific PCR, useful for the correct identification of this bacterium. However, phenotypic/biochemical profiles led to the misidentification of the isolates. AMR of this pathogen is an issue that should be further studied when more strains are available, both from clinical and non-clinical isolates. In our case, the isolates from wildlife showed a low antimicrobial resistance. However, it is necessary to establish veterinary-specific breakpoints for antimicrobials and improve testing methods since the information available is not fully applicable, and results can lead to controversy [35]. To conclude, more research, such as the sequencing of clinical isolates and isolates recovered from healthy animals, is needed to further assess the host range and pathogenic potential of *S. ruminantium* in livestock and wildlife.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/microbiolres13010008/s1>, Table S1: 16S rRNA query coverage and percent identity at NCBI for *S. ruminantium* strains. Table S2: Biochemical characterization by Vitek 2 GP (Biomérieux) for Gram-positive cocci of *S. ruminantium* isolates from domestic and wild ruminants in Spain and reference *S. suis* serovar 2 strain P1/7. Figure S1: ERIC-PCR of the Pyrenean chamois isolates. Columns 1 and 7, loading buffer. 2 strain RP15178-A2, 3 strain RP16030-M1, 4 strain CCGMV933, 5 strain CCGMV935, 6 negative control (water).

Author Contributions: C.N.-I., V.A., and M.L.A. designed the study, performed the bacterial isolation and identification, and drafted the manuscript. R.V., X.F.A., and E.V. collected field samples and participated in the pathological diagnosis. E.P. and R.V. performed the histopathology. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study may be available on request from the corresponding author.

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