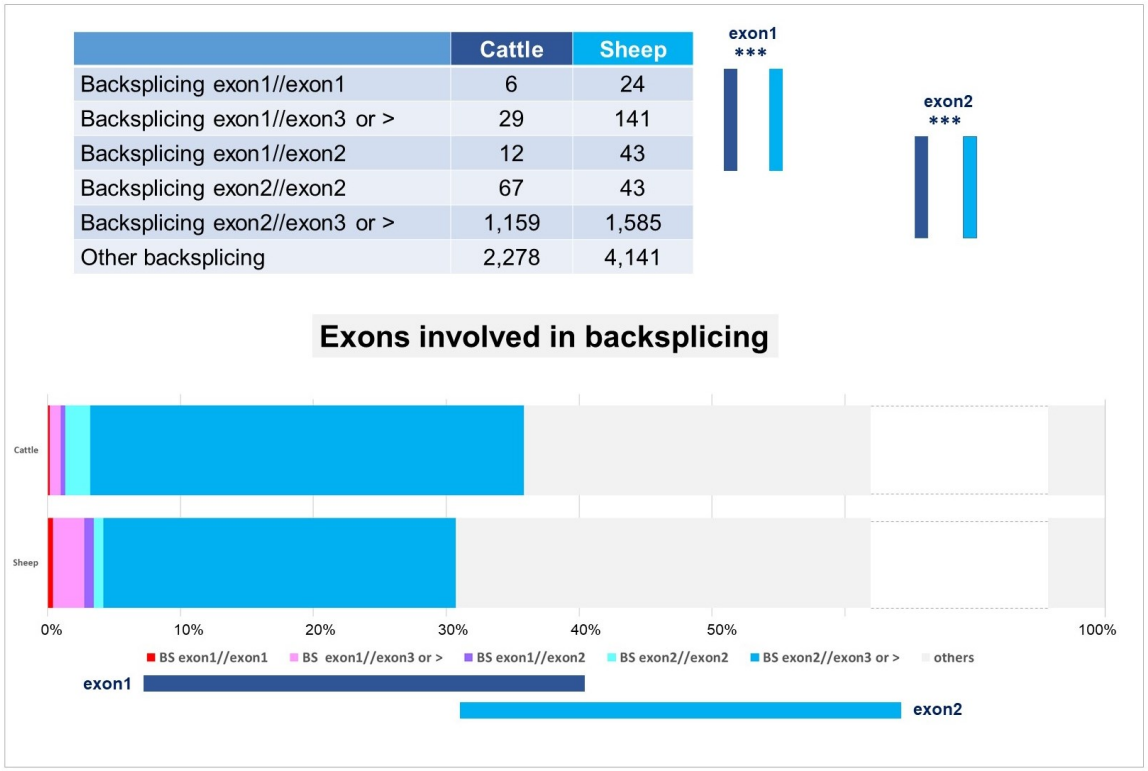
Complementary Analyses-3 (CA3)

Comparisons of sheep and cattle exonic circRNAs in terms of exon contents

It is unlikely for a true exon 1 (non-coding exon) to participate in a reproductible backsplicing. In contrast, exon2 is frequently involved, likely due to the common presence of a large intron between exons1 and 2. In a recent study, CIRCexplorer2 identified 5,977 and 3,551 exonic circRNAs in sheep and cattle samples, respectively (Maria Alonso Garcia et al., manuscript in preparation). This tool is designed to identify exonic circRNAs by detecting the two exons participating in the backsplicing event, providing a robust approach for circRNA characterization (detection and annotation).



	Cattle	Sheep
Backsplicing exon1//exon1	6	24
Backsplicing exon1//exon3 or >	29	141
Backsplicing exon1//exon2	12	43
Backsplicing exon2//exon2	67	43
Backsplicing exon2//exon3 or >	1,159	1,585
Other backsplicing	2,278	4,141

Figure C: Comparisons of sheep and cattle exonic circRNAs in terms of exon contents

The data observed from these ovine and bovine exonic circRNAs are compared in a table (top left) and shown in 100% bar charts. The difference between these two species is statistically significant for the involvement of exons identified as first and second (Pearson's Chi-squared test with Yates' continuity correction, at least the exon1 was involved in the backsplicing: p-value = 4.36 e-10, exon2 p-value = 4.17 e-12).
This shows that circRNA characterization conditions (reference genome + annotation) are more reliable for bovine data than for ovine data.
For this analysis, we used the reference annotation of sheep genome (Oar_rambouillet_v1.0 Ensembl release v.110) and the reference annotation of the cattle genome (Bos_taurus.ARS-UCD1.2 Ensembl release v.110).

Among sheep exonic circRNAs, 3.48% involved exon1 in backsplicing (208/5,977), compared to only 1.32% in cattle (47/3,551). This discrepancy is statistically significant (see figure), suggesting that exon misidentification as exon1 is likely more prevalent in the ovine than in the bovine genome. It would be reasonable to expect this difference to be mirrored by a lower involvement of exon2 in ovine circRNAs compared to bovine circRNAs. Indeed, a significant difference was observed, with a higher number of bovine exonic circRNAs involving exon2 (see figure C). These findings indicate that the conditions for circRNA characterization in relation to the reference genome (assembly and annotation) are more robust in cattle than in sheep.