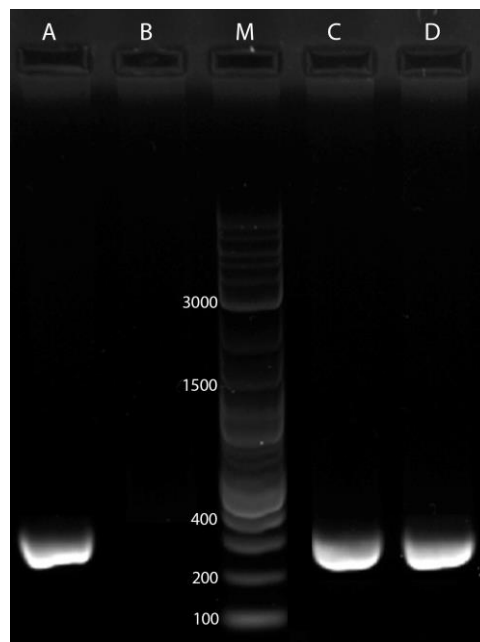


**Figure S1. FISH analysis using an X-specific probe (CH82-201N14) in three healthy control dogs:** (A) *Brutus's* father, (B) *Bufalo*, and (B) *Tauro*. All three dogs exhibit a normal karyotype (78,XY), as indicated by the probe signal appearing on only one chromosome. Arrows point to the Y chromosomes in each panel.



**Figure S2. 1% agarose gel electrophoresis shows the PCR results performed with canine-specific SRY primers. Lanes are loaded as follows:** *Brutus's* father (A), *Brutus* (B), 2-log DNA marker (M; band sizes indicated in bp), *Bufalo* (C), and *Tauro* (D). The presence of the band in *Brutus's* father, *Bufalo*, and *Tauro*, at the expected size (271 bp), indicates the presence of the SRY gene, while its absence in *Brutus* confirms the SRY-negative status.