Universitat Autònoma de Barcelona

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FACULTAT DE VETERINÀRIA

MOLECULAR BASIS OF HEMOPHILIA A AND B IN TWO DOGS

OBJECTIVES

(1) Describe the clinical presentation of two animals affected by hemophilia; **(2)** To develop the technique to be able to sequence the F8 and F9 gene, through the sequencing in Sanger; **(3)** To develop all the findings of the molecular study that allow us to identify and know the cause of the deficiency of the factor that presents the studied animals.

CLINICAL CASE

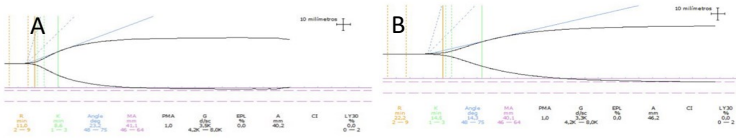
Lian Case 1, Shows Hemophilia A. A six years old male dog, Bearded Collie dog breed, breeder origin.

Happy Case 2, A seven months old male dog, Border Collie dog breed, particular origin.

They show severe hemorrhages with the change of dentition and spontaneous hemorrhagic episodes.

Table 1. Analytical values of the two dogs.

	PT (6-10.8)	aPTT (10-13.5)	%FVIII (>80%)	%FIX (>80%)
Case 1 Lian	7.6	45,4	23 %	109 %
Case 2 Happy	8	38	144.62 %	32.86 %



	R (2-9)	K (1-3)	Angle (48-75)	MA (46-64)	G (4,2K-8K)
Case 1 - Lian	11 min	8,6 min	23,2 deg	41,1 mm	3,5K d/sc
Case 2 - Happy	22,2 min	14,5 min	14,3 deg	40,1 mm	3,3K d/sc

Figure 1. Thromboelastography of the two animals. Image A corresponds to Lian and image B to Happy. Both images show that the animals are hypocoagulable.

CASE 1 - LIAN

After to get ready the technique to perform all the *F8* gen sequencing is determined that Lian does not show any detectable mutation by sequencing.

It is likely the origin of the mutation is some kind of rearrangement, reversal, duplication or intronic mutation.

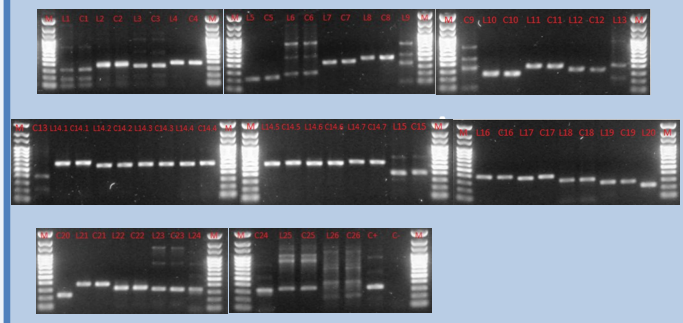


Figure 2 Amplification display of the entire *F8* gene on Lian . Every exon shows first Lian's amplification and followed by a positive control. Exon 7 appears divided into seven PCR given its length. PCR Are repeated for exon 1, 2, 6, 9, 13, 15, 24, 25 and 26.

Inversion of intron 22:

Described several times in dogs

Cause 40-50% of hemophilia A severe in dogs

CASE 2 - HAPPY

PCR amplification shows that exon 5 has a band of between 500 and 600 bp. When expected it should be 320 bp.

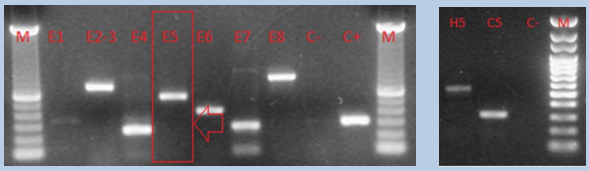


Figure 3. The first image corresponds to all the *F9* in Happy, in the second image we can see Happy's exon 5 and a positive control.

In aligning the sequence it is determined that Happy shows a mutation caused by a big insertion flanked by a 14bp AAGAATGGCA-GATG generating a codon STOP. TGC (Cys) > TGA (Stop).

RNA happens to have 452 aminoacids to 134 aminoacids.

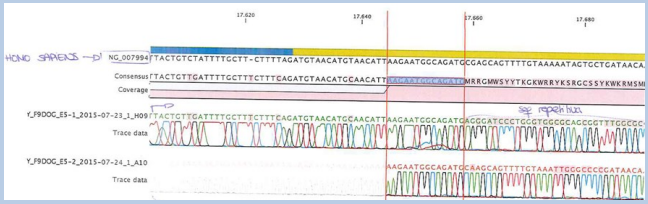


Figure 4. Image of sequencing where it is observed the insertion of 14 bp and the repetitive sequence. The image shows the alignment with the reference sequence, and thanks for that we can see a repetitive region obtained with the primer E5-1 'forward' and E5-2 'reverse'.

CONCLUSIONS

The cases described reach the veterinarian due to the presence of spontaneous bleeding, after finding the altered APPT, and looking at the value of FVIII and FIX they present leads us to diagnose the animals with hemophilia A and B respectively.

In the molecular study that has been carried out, the technique for sequencing both the *F8* gene and the *F9* gene in dogs has been developed, knowing that Lian does not present any mutation detectable through sequencing, while in the second case, it has been possible to describe the cause that causes the low levels of FIX, a mutation in exon 5 that causes a STOP codon caused by an insertion. *F9* c.401insSINE_Cf (nucleotide), p.Cys134Ter (amino acid).

Main references: **(1)** Brooks, M. B., Gu, W., Barnas, J. L., Ray, J., & Ray, K. (2003). A Line 1 insertion in the Factor IX gene segregates with mild hemophilia B in dogs. *Mammalian genome : official journal of the International Mammalian Genome Society*, *14*(11), 788–795. **(2)** Vidal, F., Farssac, E., Altisent, C., Puig, L., & Gallardo, D. (2001). Rapid hemophilia A molecular diagnosis by a simple DNA sequencing procedure: identification of 14 novel mutations. *Thrombosis and haemostasis*, *85*(4), 580–583. **(3)** Vidal Perez, F., & Gallardo Sainz, D. (2010). Bases Moleculares de la Hemofilia. [Internet]. Hemobase. [Consulted May 1st 2021]. Available at: http://www.hemobase.com/Molecular_Hemofilia/Index.htm