Detection of *Demodex* mites in the skin of ferrets (*Mustela putorius*) with a PCR technique

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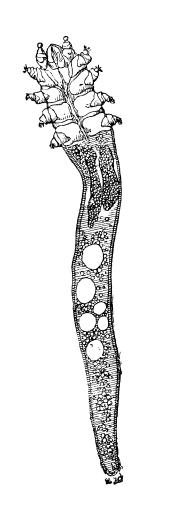
Demodicosis is a very uncommon disease in ferrets, which manifests itself mainly in individuals with a weakened immune system. In fact, in the medical literature there are only two published cases in ferrets treated with topical otic glucocorticoids, one ferret with lymphoma and three geriatric ferrets with chronic otitis.

The most commonly used *Demodex* detection methods are acetate strip, deep scrapping and PCR.

The quantitative PCR in real time method is a variant of PCR that allows the amount of DNA present in a sample to be quantified in real time during the amplification process. This technique, which has a high sensitivity, has been used in several species to detect *Demodex* DNA in the skin.

OBJECTIVES

- Detecting *Demodex* DNA in ferret skin using a qPCR technique.
- Identify the species of *Demodex*, to know if it is a species of *Demodex* already described in other mammals or one specific to the ferret.



HYPOTESIS

The hypothesis postulates that *Demodex* mites exist on the skin of ferrets similarly to their presence in other mammalian species.

The qPCR can be useful to detect *Demodex* DNA in the skin of ferrets and to identify the species.

RESULTS

Animal	Area	168 rDNA qPCR	18S rDNA qPCR
Tula	Face, dorsum and forelimb	Negative	Negative
Lola	Face, dorsum and forelimb	Negative	Negative
Smeagol	Face, dorsum and forelimb	Negative	Negative
Miyuki	Face, dorsum and forelimb	Negative	Negative
Gala	Face, dorsum and forelimb	Negative	Negative
Furia	Face, dorsum and forelimb	Negative	Negative
Spyro	Face, dorsum and forelimb	Negative	Negative
Yoshi	Face, dorsum and forelimb	Negative	Negative
Australian ferret	Individuals in a gaze	Positive	Positive

Figure 1. Table with samples and qPCR rt results.

sample from Australia amplified at a CT of 28 and with a Tm of 73.97. The amplified fragment was sequenced and compared to the database it showed a similarity of 90% with the sequence of Demodex cati.

18S rDNA: the sample from Australia was positive and when analyzing the sequence obtained in the database, a 100% similarity with Demodex musculi is obtained.

Score 427 bits(231)	Expect 6e-115	Identities 297/330(90%)	Gaps 2/330(0%)	Strand Plus/Plus
		YAGCGAAATCATTTGtt	-	GTATGAGTGGATTAA
Query 9 Sbjct 1			TTTTTATTGGAAACTT	
Query 69	TAGAA-Atttt	ttATGTAAGCTGTGTT	AGTGAATTTAATTTTT	CTATGAAAAATTTGA
Sbjct 61	T-GAAGATTTTT	TTATGAATGTTTTAATT	GGTGAATTTAATTTT	CCATGAAAAATTGGA
Query 128	AGTTTTCCTAAA	GACGAGAAGACCCCGA	ATCTTTATTTTGGAAG	AAGGTGTTTCAATTT
Sbjct 120	ATTATTTCTAAA	GACGAGAAGACCCCGA	ATCTTTATTTTAGAAA	AAGGTATTTTAATTT
Query 188	TTATTTGGGGGA	AAGTTTTATTGTTATAT	TTTAATTTTATTATGTT	GAACTTATAAAGGTT
Sbjct 180	TTATTTGGGGGA	AAGTTTTATTTTTATAT	TTATTTTTATTATGAG	GAACTTTTGTAGGTT
Query 248	GATTGGATAGAT	ACTTTGGGGATAACAGG	ATTATTTTTCTTTGAA	GTTCTTATTTTAGGG
Sbjct 240	GATTGGATAGAT	ACTTCGGGGTTAACAG	SATTATTTTTCTTTGAA	ĠŦŦĊŦŦĂŦŦŦŦĠĠĠĠ
Query 308	GGAGATTATTAC	CTCGATGTTGGCTTTTG	SA 337	
Sbjct 300	AGAGATTATTAC	CTCGATGTTGGCTTTTC	SÁ 329	
Score 837 bits(453)	Expect 0.0	Identities 453/453(100%)	Gaps 0/453(0%)	Plus/Plus
Query 1	GGCAGCAGGCACG	CAAATTACCCACTCTCA	GAACGAGGAGGTAGTGA	CGAAAAATAACAA
Sbjct 407	GGCAGCAGGCACG	CAAATTACCCACTCTCA	GAACGAGGAGGTAGTGA	CGAAAAATAACAA
Query 61	TACGGGACTATTA	ATAGGCCCCGTAATTGG	AATGAGTACAATATAAA	TCCTTTAACGAGG
Sbjct 467	TACGGGACTATT	ATAGGCCCCGTAATTGC	SAATGAGTACAATATAAA	TCCTTTAACGAGG
Query 121		111111111111111111	GCCGCGGTAATTCCAGC	
Sbjct 527			AĞCCĞCĞĞTĂĂTTCCAĞC	
Query 181			GTTGTATCTCAGTTCAT	
Sbjct 587			AGTTGTATCTCAGTTCAT	
Query 241			ACATTGAACCGATTTGTT	
Sbjct 647			ACATTGAACCGATTTGTT	
Query 301			PACTTTGAAAAAATTAGA	
Sbjct 707			TACTTTGAAAAAATTAGA	
Query 361			FAATGGAATAAGATCTAG 	
Sbjct 767				
Query 421 Sbjct 827		CTCAGTTAATGATTAAA 		
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Figure 2. Comparison of the sequences of the amplified fragments with the Gene Bank published sequences.

CONCLUSIONS

- 1.It has been possible to amplify *Demodex* DNA in the ferret with demodicosis resulting in a unique sequence that didn't correlated with any known species.
- 2. It's worth pointing out that no amplification of *Demodex* DNA was observed in samples from healthy ferrets. This result does not exclude the presence of these mites on the skin of ferrets, but can be attributed to technical factors and the low population of mites on healthy skin.