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Short communication

Genetic characterization of *Neospora caninum* from aborted bovine foetuses in Aguascalientes, Mexico

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ABSTRACT

The cyst-forming protozoan parasite *Neospora caninum* is one of the main causes of bovine abortion worldwide and is of great economic importance in the cattle industry. Recent studies have revealed extensive genetic variation among *N. caninum* isolates based on multilocus microsatellite genotyping. Currently, the most extensive study reported is based on the *N. caninum* genotyping of 96 samples from four countries on two continents (Spain, Argentina, Germany and Scotland) that demonstrate different clusters of multilocus genotypes (MLGs) implicated in cattle abortions as well as the population sub-substructuring of *N. caninum*, which is partially associated with the geographical origin. The aim of this study was to genotype *N. caninum* from aborted bovine foetuses that originated from Mexico within the region of Aguascalientes and to investigate their genetic diversity. Parasite DNA was detected in 27 out of the 63 analysed foetuses recovered from 10 different herds. Complete or nearly complete profiles based on 9 microsatellite markers were obtained from 11 samples. Diverse *N. caninum* MLGs were implicated in the occurrence of abortion in each herd. All of the Mexican MLGs differed from the MLGs previously determined for the Argentinean, Spanish, German and Scottish *N. caninum* populations. The Mexican MLGs failed to cluster by eBURST analyses. The MLG relationships using PCoA showed a close genetic relationship between the Spanish population and a portion of the Mexican population, but a more distant genetic relationship with the Argentinean genotypes. These results demonstrate the genetic diversity of *N. caninum* in the studied areas that differed from other populations of *N. caninum* around the world.

1. Introduction

Neospora caninum is an obligate intracellular parasite that can infect a wide variety of domestic and wild animals; however, it is particularly important in cattle because it can cause abortions, neonatal death and stillbirths (Dubey and Schares, 2011). *N. caninum* is distributed worldwide and is currently associated with important economic losses within the cattle industry (Reichel et al., 2013). Several studies have revealed the presence of great genetic diversity within *N. caninum* based on the analysis of microsatellite markers (MSs) (Al-Qassab et al., 2009; Regidor-Cerrillo et al., 2013). These molecular markers have demonstrated a suitable genetic discriminatory method for differentiating *N. caninum* at the isolate level and investigating the intra-species diversity. Recently, *N. caninum* genotyping based on 7 MSs was performed to investigate the genetic diversity, geographic distribution and genetic relationships among populations of *N. caninum* in samples collected from clinical cases in the field from different geographical regions of Europe (Spain, Germany and Scotland) as well as from Argentina (Regidor-Cerrillo et al., 2013). Genotyping with these markers demonstrated high levels of genetic diversity within the parasite populations from all of the different countries as well as population sub-structuring, which was partially associated with the geographical origin (Regidor-Cerrillo et al., 2013). Interestingly, the closest genetic relationship was observed between two *N. caninum* populations from Spain and Argentina. Nevertheless, the population of *N. caninum* that has been studied is limited to few geographical areas. The aim of this study was to evaluate the presence of *N. caninum* infection in 63 new cases of aborted cattle foetuses

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Table 1
Multilocus microsatellite genotypes obtained from samples of foetal tissues included in this study.

County	Herd	Sample ID[a]	Microsatellite genotype								
			[b]MS4	MS5	MS6A	MS6B	MS7[c]	MS8	MS10[c]	MS12	MS21
			GC-*(AT)n*-ACATTT-$(AT)_2$-AC	CG-*(TA)n*-TGTA-GG	GC-*(TA)n*-AC	CC-*(AT)n*-GT	ATAA-*(TA)n*	AC-*(AT)n*-GG	*(ACT)x-(AGA)y-(TGA)z*	GC-*(GT)n*-GC	TG-$(TACA)_3$-TACC-*(TACA)n*-TT
Aguasca-lientes	1	MEX-11-1	13	19	15	12	9.1[d]	15	6.14.9	16	6
	2	MEX-11-2	10	9	14	12	12	13	6.23.9	15	6
San Francisco	3	MEX-10-3	14	15	16	12	9.1	14	6.13.9	15	
	4	MEX-10-4	10		17	12	12			15	6
		MEX-10-5	10	9			12	13		15	6
	5	MEX-10-6		10	13	12	11	15	6.14.9	16	
		MEX-10-7						13			6
		MEX-10-8	14	15	15	12	9.1	13	6.28.9	16	6
		MEX-10-9	10			12	12		6.26.10		
		MEX-10-10	14	15	15	12	9.1	13	6.28.9	16	6
		MEX-11-11				12					
Jesús María	6	MEX-11-12	12		13	12	11	19	6.17.7	15	6
		MEX-10-13	9	9	15	13	12	15	6.20.9	15	6
		MEX-11-14	14		13	12	9.1	13	6.15.9	16	6
Calvillo	10	MEX-11-15	10	9	15	12	12	13	6.22.9	15	
El Llano	12	MEX-10-16					11				
	13	MEX-12-17	13	14			9.1	14	6.14.9	16	
		MEX-12-18		9	13	11			6.15.9		
		MEX-12−19[e]					9.1		6.17.8	15	6
		MEX-12-20[e]		9	14	12		13	6.17.8		
		MEX-12-21	10	9	17	13	12	14	6.23.9	15	6
		MEX-12-22	10	9	17	13	12	14	6.24.9	15	6
Pabellón	14	MEX-10-23		10				14	7.17.10	15	6

[a] Sample identification in this study: MEX (Mexico) – year of sample collection-n° sample.

[b] Alleles for each microsatellite are shown by the expected repetitive motive sequences according to fragment size analysis. Repeated motif sequences are indicated by italics in the MS sequence. The allele polymorphism of MS markers is expressed as the number of repeats (n and x.y.z for MS10) according to the allele assignment described by Regidor-Cerrillo et al. (2013). The size of the 6-FAM-labelled PCR products for all of the MSs was determined using a 48-capillary 3730 DNA analyser (Applied Biosystems, Foster City, CA) with Gene Scan-500 (LIZ) Size Standards (Applied Biosystems) at the Unidad Genómica del Parque Científico de Madrid and the GeneMapper1 V 3.5 Software (Applied Biosystems) as previously described (Regidor-Cerrillo et al., 2013).

[c] The MS7 and MS10 amplifications were also performed with non-labelled reverse primers for sequencing with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and a 3730 DNA analyser (Applied Biosystems) at the Unidad Genómica del Parque Científico de Madrid. The sequences were analysed using BioEdit Sequence Alignment Editor V7.0.1 (Copyright_ 1997–2004 Tom Hall, Ibis Therapeutics, Carlsbad, CA, USA).

[d] Alleles with a single nucleotide polymorphism in the microsatellite sequence show a nucleotide change of A by T at − 2 bp from the TA repetitive motif (AT-*TA*-$(TA)_n$-GG), which results in an additional TA repeat for the allele identified as 9.1.

[e] Two aborted foetuses were twins.