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**Preliminary characterization of bovine parainfluenza 3 virus in clinical samples of respiratory disease from Spain**

*LAZARO GASPAR S. [1], BENITO A. [1], SERRANO J. [1], ARNAL J. [1]*

*[1] Exopol SL, San Mateo de Gallego, Spain*

Introduction
Bovine parainfluenza virus 3 (PI3V) is one of the most important agents involved in the bovine respiratory disease complex (BRDC). Its RNA genome of approximately 15Kbp size encodes six structural and three non-structural proteins. Three genotypes (PI3V-A, PI3V-B and PI3V-C) based on phylogenetic whole genome analysis have already been described (1). PI3V-A is further subdivided into 3 subgenotypes, namely A1, A2 and A3. Specific genes such as phosphoprotein (P) have also been identified as suitable for genotype identification (2). Although PI3V has been detected in 26% of BRDC outbreaks in Spain according to recent studies (3), information about local current circulating genotypes is unavailable.
Methods
Between 2018 and 2022, 168 clinical cases compatible with respiratory disease submitted to our laboratory resulted positive for PI3V by real-time PCR (EXOone PI3V oneMIX, Ref: PI3V, Exopol SL, Zaragoza, Spain). Twenty-seven of these cases, whose Cq values ranged from 20 to 30, derived from 15 different Spanish provinces and including lungs and bronchoalveolar lavages, were attempted for P gene sequencing. Two strains included in commercial MLV vaccines (INT2-2013 and RLB103) were also sequenced.
Sanger sequencing of the P gene was divided into three overlapping segments of 740bp, 800bp and 900bp. Sequence analysis was performed with Unipro UGENE bioinformatics v45.1 software. SDTv1.2 software was used for pairwise comparison. Multiple sequence alignment and phylogenetic analysis were conducted using MAFFT software and the Neighbour Joining method (bootstrap 1000) respectively. Reference sequences of the different genotypes (A1, A2, A3, B and C) retrieved from GenBank were included in the phylogenetic analysis.
Results
P gene from 18 clinical samples originated from 13 different Spanish provinces and two commercial vaccine strains was successfully sequenced. Phylogenetic analysis revealed that the 16 P gene sequences from clinical samples, as well as the two vaccine strains, were classified as PI3V-C genotype, while the remaining 2 P sequences were included in the A2 sequence cluster. Spanish PI3V-C sequences were grouped together and separated from those corresponding to C genotype obtained in Japan, China, Korea, and the USA.
Pairwise alignment of P gene sequences revealed a difference of 0.2-2.4 among requested samples identified as PI3V-C genotype. Genotype A samples showed 96.5% similarity between them.
Discussion
To our knowledge, this is the first report of the characterization of PI3V genotypes in cattle with respiratory disease in Spain. Even though the limited number of samples in this study, these preliminary findings suggest that genotype C could be more predominant in comparison to genotype A. In addition, both genotypes reported in this work are included in different commercial vaccines licensed in Spain.
Nevertheless, the great variability observed within A genotype (4) and the reduced immune cross-protection between genotypes suggested by several studies (4,5) highlight the importance of the genetic surveillance of the PI3V virus.
Further research increasing the number of samples would be necessary to provide a comprehensive understanding of the current PI3V situation in Spain.