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Mucosal disease outbreak and possible sources of bovine viral diarrhea virus in herds from a beef farm of Buenos Aires province

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Aims

- To describe an outbreak of mucosal disease (MD) in a beef farm.
- To identify possible sources of bovine viral diarrhea virus (BVDV).
- To compare BVDV detection methods in suckling calves.
- To evaluate the phylogenetic relationship of BVDV in positive samples.

Fig. 1. Erosions and sharp ulcers in the muzzle, inner side of lips, hard palate, gingiva, and tongue.

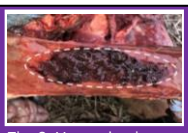

Fig. 2. Hemorrhagic necrosis of Peyer's patches.

Fig. 3. BVDV-Ag in ear notch (skin) samples from two steers.

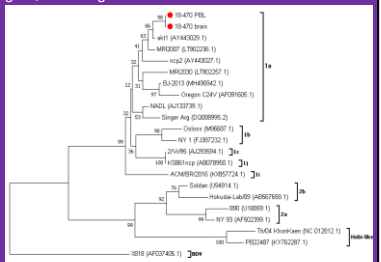

Fig. 4. Phylogram based on the partial nucleotide sequences of the 5´UTR fragment of BVDV strains. Sequences highlighted by red dots represent BVDV from samples of this study. Bootstrap values estimated with 1000 pseudoreplicates.

Materials and methods

- **Outbreak investigation** conducted in a beef farm in Buenos Aires province (2018). Steers and heifers died (30/205, 14.6%) over the course of few months. Necropsy of two steers showed erosions and ulcers in the digestive organs, atrophy and necrosis of Peyer´s patches (Fig. 1 and 2).
- **BVDV detection methods and samples.** A) **Necropsied cattle** were analyzed by immunochromatography test (ear notches; Fig. 3), multiplex RT-PCR (RT-mPCR, BVDV-1 and -2) (spleen), and virus isolation followed by immunofluorescence (VI+DIF) (organs).
  B) **Cattle** were evaluated by RT-mPCR using peripheric blood leucocytes (PBL) (n=393) and sera (n=63). Herds (H) and categories were as follows: H1) 147 suckling calves and 154 cows; H2) 61 suckling calves and 70 cows, 22 purchased while pregnant in 2018; H3) 13 cows not calved; H4) 11 bulls. H2 was handled separately. BVDV positive animals were also evaluated using different samples and methods.
- **Phylogenetic analysis.** The 5´UTR fragment (288 pb) was analyzed using the neighbor joining method. Nucleotide (nt) genetic distances were calculated using Kimura 2P model (MEGA v7.0.26 software).

Results

- Cytopathic BVDV was isolated from the brain, lung, and spleen of both steers. BVDV-1 was identified in spleen samples.
- Thirteen (13/456, 2.8%) suckling calves of H2 were positive for BVDV-1 in PBL. The detection of BVDV in these calves was compared using sera and other methods (Table 1); two serum samples were negative by VI+DIF.
- The 5´UTR sequences (100% nt identity) of BVDV-1 in positive samples showed a very close phylogenetic relationship and grouped in the subgenotype 1a (Fig. 4). The samples belonged to one steer (brain) suffering MD and one suckling calf (PBL).

Table 1. Detection of BVDV in suckling calves.

Calf id.	Sample	Nested RT-mPCR	Ag-ELISA	VI+DIF
36	serum	+	(-)	
	PBL	+		+
42	serum	+	(-)	
	PBL	+		+
45	serum	+	(-)	
	PBL	+		+
60	serum	+	NA	
	PBL	+		(-)
61	serum	+	(-)	
	PBL	+		+
72	serum	+	(-)	
	PBL	+		+
73	serum	(-)	NA	
	PBL	+		NA
80	serum	+	(-)	
	PBL	+		NA
82	serum	+	(-)	
	PBL	+		+
85	serum	+	(-)	
	PBL	+		+
86	serum	+	(-)	
	PBL	+		(-)
88	serum	(-)	NA	
	PBL	+		(-)
401	serum	+	(-)	
	PBL	+		+

Conclusions

- ✓ BVDV control programs in beef farms from Argentina should evaluate neonatal calves when testing for PI cattle.
- ✓ RT-PCR based methods and suitable samples are highly encouraged to avoid false negative results in suckling calves, mainly when certain methods (Ag-ELISA and VI+DIF) can be influenced by passive maternal antibodies.