Prevalence of Border Disease virus in Basque dairy-sheep estimated by bulk-milk analysis

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INTRODUCTION

- Border disease virus (BDV) is the sheep pestivirus that can also infect cattle and pigs and be a major cause of reproductive failure and immunosupression.
- Pestivirus can be eradicated from large areas by implementing systematic non-vaccination control schemes based on identifying and eliminating persistently infected animals (PIs).
- PIs were infected in-utero, are immunotolerant, constantly eliminate virus and infect other in-contact animals which in contrast, overcome infection and develop antibodies.
- Bulk-tank milk (BTM) antibody analysis has been used in dairy-cattle herds as an initial screening test to identify infected herds however no similar studies have been done in dairy-sheep flocks and BDV prevalence remains by enlarge unknown.

MATERIAL & METHODS

Study design and population

- Bulk-tank milk (BTM) samples from all 154 Latxa dairy sheep flocks in the 3 Basque provinces (see Map) registered with the regional milk board, for BDV diagnosis and somatic cell count (SCC).
- Most flocks were at the start of the milking period when only 50% of sheep (and mostly ≥2nd lactation ewes) were being milked (Table 1).

Table 1. Study population

Province	No. of flocks	No. of ewes >1 yr-old	No. of ewes in milking	Median SCC (x10³)
Araba	53	21986	11695	461
Gipuzkoa	57	21097	10549	473
Bizkaia	44	10767	4002	550
All	154	53850	26246	473

Competitive-ELISA (Inst. Pourquier) analysis

- Samples deemed antibody-positive when antibody inhibition percentage (AIP) <80%
- Flock-seroprevalence estimated from AIP values: (kit's guidelines for bovine BTM) AIP≥80%, 46-79% and ≤45% corresponded to <10%, 10-30% and >30% seroprevalence.

RT-PCR and sequence analysis

- RT-PCR for 5'NCR sequences on somatic cells RNA from all samples.
- 7 agarose-purified amplicons sequenced and compared with GenBank sequences.
- Phylogenetic trees with similar aligned sequences using neighbor-joining and maximum parsimony and bootstrap analysis for stability assessment.

RESULTS

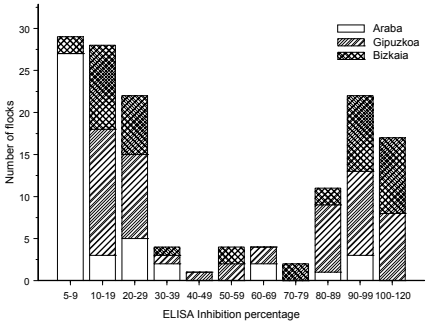


Fig. 1. Frequency of the ELISA AIP by province

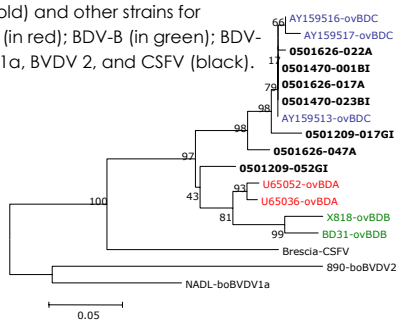
Table 2. Percentage of antibody and PCR+ve flocks and estimated flock seroprevalence:

Province	% ELISA-antibody positive flocks	Estimated flock seroprevalence (%) (% flocks)			% PCR positive flocks
		>30	10-30	<10	
Araba	93	89	4	8	17
Gipuzkoa	54	47	7	46	4
Bizkaia	55	46	9	46	7
All	68	61	7	33	9

Table 3. Estimates of a logistic model investigating presence of BDV antibodies in BTM, flock size, province and SCC.

Variable	Odds ratio	95%CI	P value
Flock size (ewes)*			
54-190	1.00	-	-
193-292	2.99	0.99, 9.02	0.052
296-363	4.65	1.43, 15.11	0.011
369-535	3.98	1.18, 13.37	0.026
536-1761	12.17	2.73, 54.16	0.001
Province			
Bizkaia	1.00	-	
Gipuzkoa	0.67	0.28, 1.63	0.379
Araba	5.85	1.67, 20.43	0.006

Fig. 2. Neighbor-joining phylogenetic tree of 5'NCR with study strains (bold) and other strains for comparison: BDV-A (in red); BDV-B (in green); BDV-C (blue) and BVDV 1a, BVDV 2, and CSFV (black).



- AIPs were concentrated in one (Araba) or two (Bizkaia and Gipuzkoa) narrow and separate distributions (AIP<30% and >79%) (Fig.1), allowing to clearly discriminate antibody- positive and -negative flocks and to estimate within flock seroprevalence (Table 2).
- Only 14 flocks were PCR-positive and AIP in these flocks were <20% in 13 flocks and 78% in 1 flock.
- Being an antibody positive flock was associated with increasing flock size and province but not to the SCC (Table 3)
- 6/7 BDV amplicons reliably clustered with BDV type C strains and the remaining was closer to BDV type A although branching with BDV types A and B was not so clearly supported by bootstrap analysis (Fig. 2).

SUMMARY & CONCLUSIONS

- First large-scale BDV-prevalence study in dairy-sheep and BMT analysis allowed screening most professional flocks in Basque Country for BDV antibodies and PCR-products fast and inexpensively.
- BDV antibody-results indicate most Basque flocks have had recent exposure to BDV, including 54-55% in the provinces of Bizkaia and Gipuzkoa and >90% in Araba.
- However, few BTM samples were PCR-positive suggesting few infected sheep were contributing to the BT when samples were taken and highlights the limitations of this approach to identify infection.
- Genotype analysis of a selection of BDV PCR amplicons provided further evidence that type C strains are typical of this region and the apparent wide presence of a single type suggests a common origin of infection and that differences in BDV-exposure between provinces are probably due to recent local events that facilitated infection such as use of communal pasture and sheep trading.
- Results advocate for systematic BDV control to reduce its financial and welfare impact on sheep and their potential role as a pestivirus reservoir for other species.

ACKNOWLEDGEMENTS: Funded by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) (grant RTA04-057) & the Department of Agriculture, Basque Government.