Genetic diversity of bovine viral diarrhoea virus (BVDV) from Peru and Chile[1]

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ABSTRACT.- Ståhl K., Benito A., Felmer R., Zuñiga J., Reinhardt G., Rivera H., Baule C. & Moreno-López J. 2009. **Genetic diversity of bovine viral diarrhoea virus (BVDV) from Peru and Chile.** *Pesquisa Veterinária Brasileira 29(1):41-44.* Joint Virology Research and Development Division, National Veterinary Institute and Swedish University of Agricultural Sciences Uppsala, Sweden. E-mail: Karl.Stahl@bvf.slu.se

Twenty-five BVDV strains, detected in serum from persistently infected cattle from Peru (n=15) and Chile (n=10) were genetically characterized. The phylogenetic analysis based on the 5' UTR showed that all 25 strains belonged to genotype 1. Twenty-three of the strains could further be subdivided into subtype 1b, and two out of ten Chilean strains into subtype 1a. In conclusion, in total 23 out of 25 strains analyzed were of genotype 1, subtype 1b. This is the predominant BVDV subtype in many countries all over the world, including USA. The close homology with previously described strains reflects the influence of livestock trade on the diversity of BVDV circulating within and between countries and continents. Peru and Chile have imported large numbers of cattle from USA and Europe, mostly with insufficient or lacking health documentation.

INDEX TERMS: Bovine viral diarrhoea virus, BVDV, diversity, pestivirus, phylogenetic analysis.

RESUMO.- [**Diversidade genética de vírus da diarréia viral bovina (BVDV) do Peru e Chile.**] Um total de 25 isolados do vírus da diarréia viral bovina (BVDV), sendo 15 originarias do Peru e 10 do Chile foram sujeitas a caracterização genética. A árvore filogenética baseada na análise da região proximal não-codificante (5'UTR) do genoma viral demonstrou que as 25 estirpes pertencem ao genótipo 1 do vírus BVD. Vinte e três destas estirpes puderam adicionalmente ser subdivididas no subtipo 1b, enquanto duas das 10 estirpes isolados provenientes do Chile foram identificadas como pertencentes ao subtipo 1a. Em conclusão, 23 de um total de 25 isolados analisados pertencem ao genótipo 1, subtipo 1b. Este é o subtipo de BVDV predominante em muitos países do mundo, incluindo os EUA. A elevada similaridade genética com isolados descritos anteriormente em outras regiões do mundo realça o papel do comércio internacional de gado no estabelecimento de diversidade genética do vírus BVD. Tanto o Peru como o Chile têm historia de importação de grandes quantidades de gado dos Estados Unidos e da Europa, no entanto sem suficiente documentação comprovativa do estado sanitário no que concerne a esta virose.

TERMOS DE INDEXAÇAÞO: BVDV, diversidade, pestivírus, análise filogenética.

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INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is the denomination of a heterogeneous group of viruses in the family *Flaviviridae*, genus Pestivirus, which are economically important pathogens worldwide, and which primarily affects ruminants. The BVDV genome consists of a single stranded positive-sense RNA of approximately 12.3 kb, with one open reading frame flanked by 5' and 3' untranslated regions (UTR). Two genotypes of BVDV (BVDV-1, and -2) are recognized, and they constitute, together with border disease virus and classical swine fever virus, the four accepted species of the

genus. In addition, a tentative fifth species is represented by a virus isolated from a giraffe (Becher et al. 2003). BVDV-1 remains the dominant genotype and has been found worldwide. BVDV-2 is widespread in the United States and Canada, where it has been associated with outbreaks of severe acute BVDV or haemorrhagic syndrome (Pellerin et al. 1994), but has also been reported in South America, Asia and Europe (Flores et al. 2002, Park et al. 2004, Cranwell et al. 2005). The two genotypes can be further subdivided, and at least eleven subtypes of BVDV-1 and two of BVDV-2 have been identified (Vilcek *e*t al. 2001, Flores et al. 2002). The genetic typing of BVDV has most frequently been based on sequence comparison of partial sequences of the 5' UTR, Npro or E2 regions (Vilcek et al. *2*001, Becher et al. 2003). Analysis of the 5' UTR, a highly conserved region of the genome, has shown to be a reliable and reproducible method for genetic characterization of BVDV isolates (Ridpath 2005) even though the genetic resolution obtained is not as high as that obtained with more variable regions as Npro or E2. It is also the target region for most PCR-based diagnostics, and as such, a suitable target for direct sequencing from the PCR product.

Several studies have demonstrated biologically significant differences in antigenic determinants between the two genotypes (Ridpath 2005) and also between subtypes within BVDV-1 (Fulton et al. 2003) and indicate that these differences may contribute to BVDV vaccine failure (Zimmer et al. 2002, Fulton et al. 2005). Genetic diversity of BVDV isolates is therefore not only of academic interest, but has practical implications for surveillance and vaccine design. To date there are reports on genetic diversity of BVDV from many countries on all continents, and from South America a few studies have been reported from Argentina, Brazil and recently also from Chile (Jones et al. 2001, Flores et al. 2002, Pizarro-Lucero et al. 2006). In this report we describe for the first time the genetic diversity of BVDV strains collected in Peru. In addition, we include a number of strains collected in Chile.

MATERIALS AND METHODS

All samples originated from persistently infected animals identified during previous research projects focusing on BVDV epidemiology. Peruvian samples (n=15) originated from farms in major dairy areas in central (n=1) and southern parts (n=14) of the country (Ståhl et al. 2002, 2006, 2008), and Chilean samples (n=10) from dairy and beef herds in the southern regions IX (Región de la Araucanía) and X (Región de los Lagos) (Felmer, unpublished data).

For sequencing, RNA was extracted directly from serum using a GenoM-48 extraction robot (Genovision AS, Norway) and the extraction kit MagAttract Virus Mini M48 (Qiagen Gmbh, Germany) according to the standard protocol. A 296-nucleotide (nt) segment of the 5´ UTR was amplified in a one-step RT-PCR, as previously described using the primers OPES 13A and OPES 14A (Ståhl et al. 2005). The generated product was sequenced according to standard protocols using the same primers. A 237-nt fragment of the 5´UTR, corresponding to position 135-371 of BVDV SD-1 (GenBank accession no. M96751), was used for further analyses. The nucleotide sequences were assembled and proofread using the SeqMan II and EditSeq programmes in the DNASTAR programme package

(DNASTAR Inc., Madison, WI, USA), then aligned and compared by the Clustal W method of the MegAlign programme from the same package. Reference strains and previously described field isolates representing different subtypes of BVDV-1 were obtained from the GenBank and included in the alignments. Phylogenetic trees were constructed using neighbour-joining (Saitou & Nei 1987), estimating evolutionary distances with the Kimura two-parameter method (Kimura 1980). The robustness of the trees was evaluated by bootstrap resampling of 1000 replicates (Felsenstein 1985). All phylogenetic analyses were performed using PAUP*, version 4.0 b10 (Swofford 2003).

RESULTS AND DISCUSSION

All sera identified as positive by the BVDV-antigen ELISA were confirmed as positive by the RT-PCR. The target region was recognised by the primer pair OPES 13A/14A and was amplified and sequenced from all samples. The phylogenetic analysis based on the 5' UTR showed that all 25 strains belonged to genotype 1 (Fig.1). Twenty-three of the strains
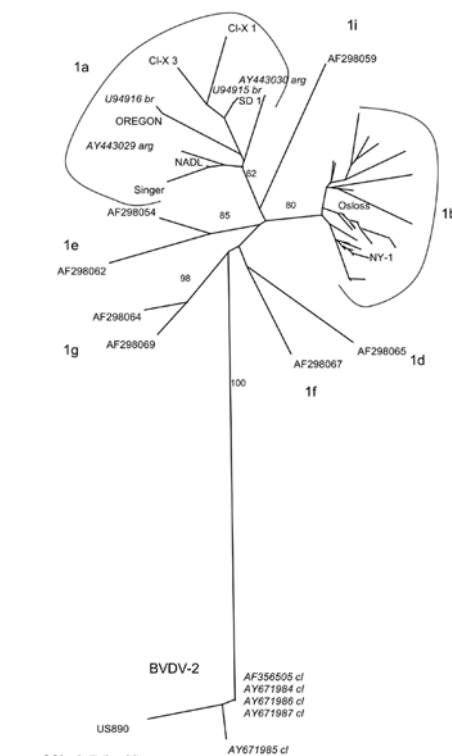

Fig.1. Unrooted phylogram based on a 237 nt fragment of the 5´NCR from 15 Peruvian and 10 Chilean BVDV isolates. Sequences from reference strains (NADL, SD-1, Singer, Osloss, NY-1 and US890; accession numbers. NC_00146, M96751, L32875, M96687, L32879 and Z79772, respectively), previously described field isolates representing 7 of 11 described subtypes (Vilcek et al. 2001) and strains from Argentina (arg), Brazil (br) and Chile (cl) were obtained from the GenBank and included for comparison. Two out of ten Chilean strains (Cl-X1 and Cl-X3) clustered within the BVDV-1a subtype, whereas all Peruvian and the remaining eight Chilean strains clustered within the BVDV-1b subtype (for detail see Fig. 2). Values from bootstrap resampling of 1000 replicates are indicated for nodes with values above 60%.

could further be subdivided into subtype 1b, and two out of ten Chilean strains into subtype 1a. Three major clusters supported by bootstrap values above 60% could be distinguished among strains within subtype 1b, and a blast was run on representatives from each cluster to find matching sequences in the GenBank (Fig.2). Three Peruvian strains clustered with reference strain NY-1, and showed strong homology with previously described strains from Chile, US, Japan and Spain (range: 95.3-99.6%). Chilean strains clustered with Peruvian strain Pe-Man1-03, from
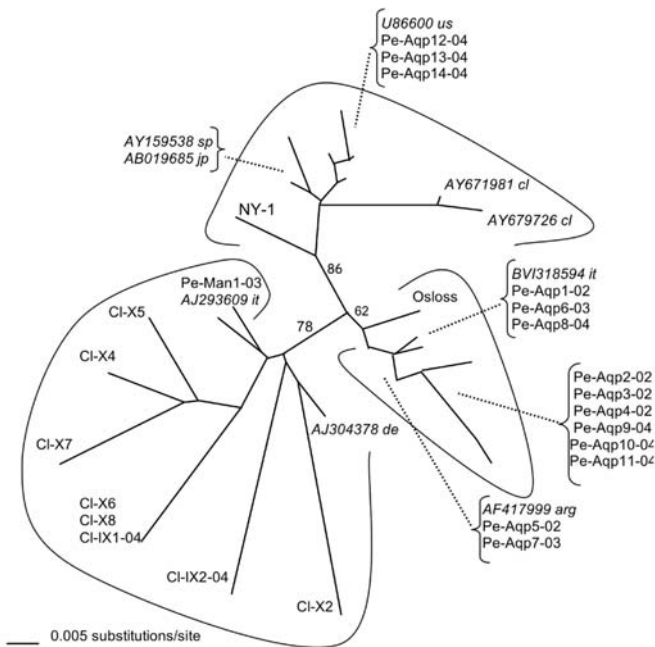

Fig.2. Unrooted phylogram based on a 237 nt fragment of the 5´NCR from 15 Peruvian and 8 Chilean BVDV isolates classified as BVDV-1b. Sequences from prototype BVDV-1b strains (Osloss and NY-1; accession nos. M96687 and L32879, respectively) and previously described field isolates (in italics) from Chile (cl), Argentina (arg), Italy (it), Germany (de), Spain (sp), Japan (jp) and USA (us), were obtained from the GenBank and included for comparison. Values from bootstrap resampling of 1000 replicates are indicated for nodes with values above 60%. Peruvian and Chilean strains have been labelled according to region of origin, number of isolate and year of isolation (if available).

central Peru, and previously described strains from Italy and Germany (homology range: 92-100%). The third cluster was formed by 11 of the Peruvian strains, reference strain Osloss and strains from Italy, and Argentina (homology range: 95.8-100%).

This is the first study of the genetic diversity of BVDV from Peru. Despite the limited number of strains included in this study, and despite the fact that most of the strains originated from one major dairy region in the southern part of the country, important conclusions can be drawn from the results. Several studies have addressed the question of the geographical distribution of BVDV genotypes or subtypes within a country, and in these a low regional variation has been found (Tajima et al. 2001, Hurtado et al. 2003, Mishra et al. 2004, Stalder et

al. 2005). This lack of variation is probably the result of uncontrolled livestock trade within a country and an efficient spread of prevalent strains. Thus, we may expect that our results are valid also for the other major dairy regions in Peru. According to the phylogenetic analysis all 15 strains were of genotype 1, subtype 1b. This is the predominant BVDV subtype in many countries all over the world, including USA (Tajima & Dubovi 2005). In spite of this, most vaccines on the market are produced with prototype BVDV-1a strains, sometimes in combination with a BVDV-2 strain (Ridpath 2005). Four BVDV vaccines are currently available in Peru; all are based on inactivated BVDV-1a strains, in one vaccine in combination with an inactivated BVDV-2 strain[7] (L. Olivera, SENASA, personal communi-cation). Three of the vaccines claim foetal protection and prevention of persistently infected calves. To date, a few studies have been published demonstrating foetal protection through vaccination with inactivated BVDV vaccines (Brownlie et al. 1995, Patel et al. 2002). This protection, however, has been against homologous subtypes of BVDV. Therefore, the efficacy of these vaccines to protect foetuses against infection under field conditions have been questioned (van Oirschot et al. 1999, O'Rourke 2002), and field observations, where PI calves have been born in vaccinated herds, support this concern (Van Campen et al. 2000, Gaede et al. 2004, Graham et al. 2004).

A recent study of BVDV diversity from Chile demonstrated an unusually high percentage of genotype 2 (51.5%) among 33 BVDV field isolates collected in the central part of the country (Pizarro-Lucero et al. 2006). Based on this finding the authors concluded that BVDV-2 possibly had circulated for a long time in the country. According to our result all Chilean strains (n=10) included in our analysis were of genotype 1. This discrepancy may be due to the limited number of strains analyzed or to regional variation within the country. Such a regional variation, however, would not support the conclusion that BVDV-2 has circulated for a long time in the country. The close sequence homology reported between the BVDV-2 isolates (98.2-100%, Fig.1) also indicates a more recent introduction, and probably a common origin.

The phylogram based on the analysis of the subtype 1b strains (Fig.2) is interesting, and the close homology with previously described strains reflects the influence of livestock trade on the diversity of BVDV circulating within and between countries and continents. Peru and Chile have imported large numbers of cattle from USA and Europe, mostly with insufficient or lacking health documentation. Persistently infected (PI) animals are the main source of virus transmission within and between herds, and given an approximate prevalence of PI animals of 2% (including dams carrying PI foetuses) in any BVDV

[7]Cattle Master and Cattle Master Gold, Pfizer Animal Health, Pfizer Inc, New York, USA. http://www.cattlemastergold.com/docs/CattleMastGOLDFetal.pdf

Hiprabovis-3, HIPRA, Girona, Spain. http://www.hipra.com/docs/pdf/42810_eng.pdf

Bovilis Qarat, Intervet/Schering-Plough Animal Health, Boxmeer, The Netherlands.

infected population, the risk (P) of introducing BVDV when buying 100 cattle with unknown BVDV status is $P=1-0.98^{100}=87\%$ (Houe 1999). Consequently, the risk of introducing the infection through trade is high, particularly considering that there are no regulations in international livestock trade regarding BVDV.

In conclusion, the results indicate that BVDV strains circulating in Peru are of genotype 1, and that BVDV 1b is the predominant subtype. Whether these results are representative for the entire country or not needs to be confirmed through further studies. The results further indicate, in contrast to previous studies (Pizarro-Lucero et al. 2006), that BVDV 1 is the predominant genotype circulating in the bovine population in the Southern regions IX and X of Chile. The discrepancy probably being due in part to the limited number of strains included in our study, but possibly also to regional variations within the country.

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