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Molecular characterisation of the coding region for the p125 from homologous BVDV biotypes

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Summary. We amplified and sequenced the p125 coding regions of a 'homologous' pair of BVDV biotypes, Pe515 cytopathogenic and non-cytopathogenic. The sequences were aligned with the published sequences of Osloss, NADL and the HCV Alfort strains, but no insertions of host sequence were observed in that region.

Key words: BVDV, HCV, pestivirus, PCR

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Bovine viral diarrhoea virus (BVDV) is a positive stranded RNA virus, currently classified in the Togaviridae as a member of the Pestivirus genus, together with border disease virus (BDV) of sheep and hog cholera virus (HCV) [14]. Two forms of BVD virus are usually isolated from outbreaks of mucosal disease and these are differentiated principally by their different growth characteristics in vitro. The non-cytopathogenic (ncp) BVDV biotype induces little cytopathic effect on tissue culture cells, whilst the cytopathogenic (cp) biotype causes vacuolation in the cytoplasm and ensuing cell death.

Analysis of virus encoded proteins in cells infected with cp and ncp viruses also reveals that the viruses differ in their processing of a 125 kDa protein (Fig. 1). In cp biotypes the p125 is cleaved into 80 kDa and 54 kDa subunits. This cleavage and consequently the 80 kDa protein, is not observed in cells infected with ncp virus preparations. This difference is consistently observed in all of the 'homologous' pairs that have been studied to date [12, 6].

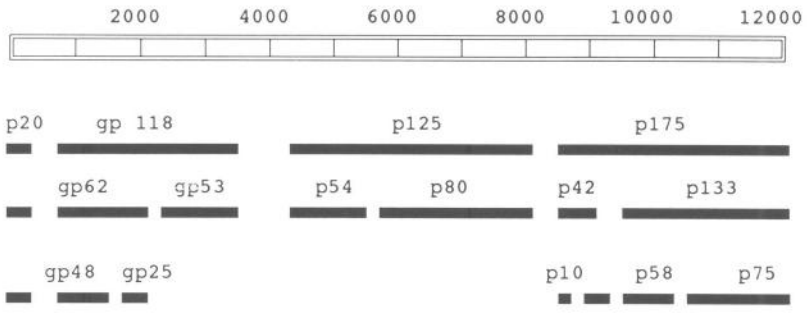

Fig. 1

The complete nucleotide sequences of two cp strains of BVDV [13, 4] and one ncp HCV isolate [9] have recently been published and confirmed the size of the BVDV and HCV genomes to be approximately 12.5 kb. When these sequences are aligned (Fig. 1) NADL is found to have an insertion of 90 amino acids while Osloss has an insertion of 76 amino acids, both of these occurring in the predicted coding region for the p54. The NADL insertion has 99% homology with a host cellular mRNA coding for a protein of unknown function [8] and the Osloss insertion corresponds to an ubiquitin-like protein [3, 10]. No comparable insertions were observed in the ncp HCV sequence [9]. Since these insertions only occur in cp isolates it has been suggested that this uptake of cellular sequences may be associated with the mutation of ncp to cp virus [10]. We decided to sequence the p125 coding region of the Pe515 'homologous' viruses to identify any differences between the two isolates.

BVDV RNA was prepared using a total RNA extraction method for Pe515ncp and a viral RNA technique for Pe515cp. The total RNA protocol was a modification of the method of Chomczynski et al. [2] where RNA is isolated by a single extraction with an acid guanidinium thiocyanate-phenol-chloroform mixture. The viral RNA method was Chang's modification [1] of Maniatis' method [7] and involves extraction of RNA from the cytoplasm of virus-infected cells using SDS/proteinase K followed by phenol/chloroform extraction. First strand cDNA synthesis was performed on both RNA preparations using MuLV reverse transcriptase (Gibco BRL) in a total volume of 100 µl. 10 µl aliquots of the reverse transcription cDNA product were amplified using specific primers (Fig. 2) with Taq DNA polymerase (Cetus corp). The cDNA was denatured at 94°C for 1 minute and the primers annealed at 50°C for 1.5 minutes. Primer extension was at 72°C for

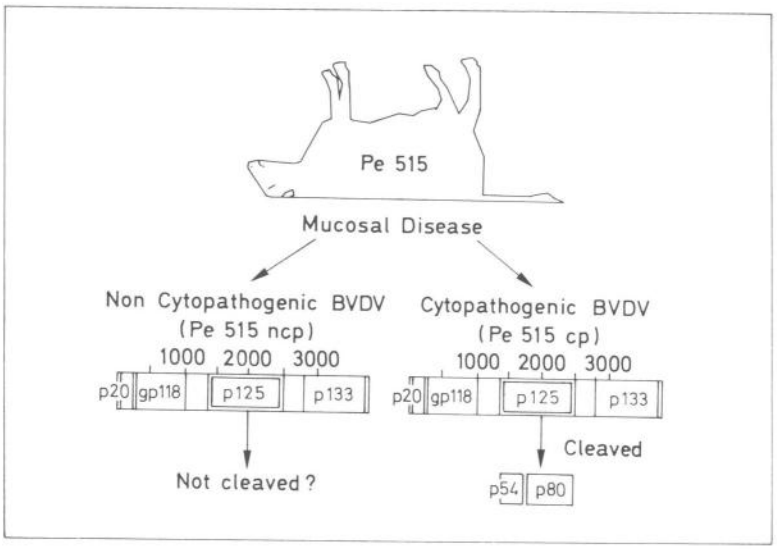

Fig. 2

2.5 minutes and the amplification cycle was performed 30 times with a final incubation at 72°C for 7 minutes.

The PCR products were analyzed by electrophoresis on a 0.8% agarose gel and were visualised with ethidium bromide under UV light. Fragments were excised from the gel and purified using the Geneclean kit. They were then either cloned into pUC9 for plasmid sequencing or sequenced directly using the amplification primers and Sequenase (Applied Biosystems). Using several combinations of primers, cDNAs corresponding to the entire p125 region of both biotypes were amplified and sequenced. As the sequence data was obtained it was aligned to the corresponding regions of the Osloss and NADL BVDV isolates and the Alfort HCV strain (Fig. 3).

The p54 region was successfully amplified in both viruses using primers that were complementary to the Pe515 cDNA. The p80 region, however, was produced in two overlapping segments using primers made from regions of good homology between the NADL and Osloss sequences. Apart from reducing the annealing temperature of the primers to 40°C in the latter case, fragments were amplified and sequenced under the same conditions. There was some difficulty in amplifying fragments of greater than 1 kb from the Pe515ncp cDNA made from the total RNA preparation. Smaller size sections were successfully amplified, indicating that this was not due to primer:template mismatch but perhaps was a problem with the quality of the RNA and, therefore, the cDNA. We amplified and sequenced the region coding for the p125 in the Pe515 'homologous' pair of viruses. The degree of sequence homology observed at both the DNA and amino acid level is particularly striking: the Pe515 viruses were 99.6% homologous with respect to each other and 97% homologous with NADL, excluding the known

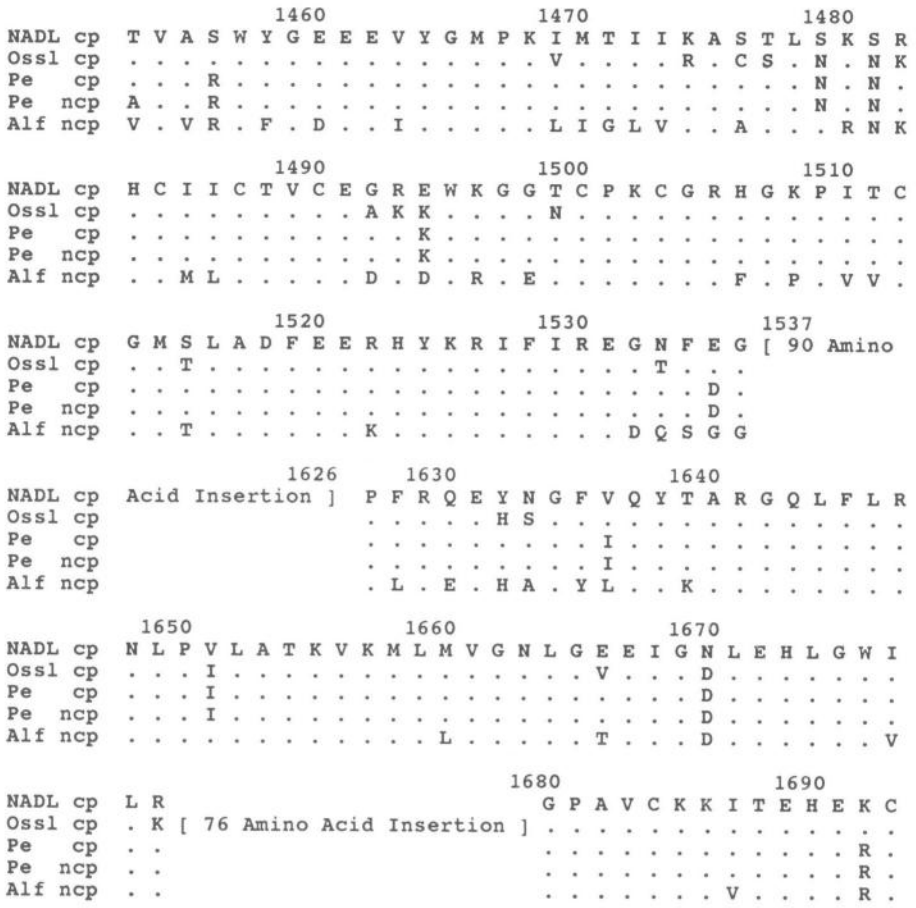

Fig. 3

host insertion. No insertion was observed when comparing the Pe515 cp and ncp sequences. The area immediately preceding the p54 coding region has recently been amplified in both biotypes. Fragments obtained by PCR and analyzed on agarose gels appear equal in size and preliminary sequence data indicates the same high degree of homology as that observed for the p125.

This report confirms that the techniques of reverse transcription, amplification and direct sequencing of PCR products can allow rapid analysis of specific areas of the BVDV genome. Any host sequence insertions in Pe515cp, similar to the NADL or Osloss insertions, should have been detected using this methodology since they only span relatively small areas. De Moerlooze et al. [5] have amplified and sequenced 10 pestiviral strains across this region of the p54 and found that NADL and Osloss cp strains were the only viruses with insertions. The significance of these insertions in cp isolates is still unconfirmed. However, one of the disadvantages of PCR when compared to cDNA cloning, is that duplications of sequences may not be detected, particularly if they span large areas. Meyers et al. [11] reported

finding a duplication of the entire p80 sequence in one of their cytopathogenic viruses and this would have been indistinguishable from the original sequence by the methods employed here. With more homologous pairs of viruses being examined, the raison d'être for the cytopathogenic biotypes will soon be resolved.

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