Identification of a pestivirus isolated from a free-ranging pudu (*Pudu puda*) in Chile

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PESTIVIRUSES are small, enveloped RNA viruses that belong to the family Flaviviridae, which includes bovine viral diarrhoea (BVD) virus, classical swine fever virus and Border disease virus (Meyers and Thiel 1996, Nettleton and others 1998).

In cervids, pestiviruses have been isolated from free-ranging roe deer (*Capreolus capreolus*) (Romvary 1965, Frölich and Hofmann 1995), fallow deer (*Dama dama*) (Edwards and others 1988), red deer (*Cervus elaphus*) (Nettleton and others 1980) and mule deer (*Odocoileus hemionus*) (Van Campen and others 2001). These studies have established the susceptibility of cervids to BVD virus infection, but the clinical signs reported are mild or non-existent (Van Campen and others 1997, Tessaro and others 1999). The development of adequate control programmes for BVD virus in cattle depends on knowing whether wild ruminants have their own pestivirus strains or whether they are reservoirs of cattle strains. The aim of this short communication was to identify a pestivirus isolated from a free-ranging pudu (*Pudu puda*), an indigenous deer species living wild in Chile.

In May 2001, a free-ranging pudu was found dead near Chillán, Chile (36°36′S 72°06′W) and was submitted to the Pathology Department at the Facultad de Medicina Veterinaria, Universidad de Concepción, Chillán. Postmortem examination revealed small ulcerative lesions of approximately 3 to 5 mm diameter on the skin near the nose, the buccal and gingival mucosa, interdigital surfaces and oesophagus. Generalised congestion was observed in the mucosa, submucosa and musculature layers of the gastrointestinal system. No lesions were observed in other organs.

Serum obtained from a blood sample collected from the heart was negative for antibodies to foot-and-mouth disease virus and positive for virus-neutralising antibodies to BVD virus (reciprocal titre of 80). This implied that the pudu had suffered an infection with a virus antigenically very similar to BVD virus and was probably not persistently infected with BVD virus. Twenty per cent oesophagus homogenate in minimum essential medium was inoculated on to BVD virus-free primary fetal bovine testicle cells (FBTs), and after five culture passages, pestivirus was detected by indirect immunoperoxidase using pestivirus-specific monoclonal antibodies (IPEX-BVD; CVL). The FBTs were found to be infected with a non-cytopathic pestivirus.

Total RNA was extracted from infected cells using Trizol LS (Life Technologies) according to the manufacturer's instructions. Reverse-transcriptase PCR was used to amplify a region from the 5′ untranslated region (5′ UTR) of the pestivirus genome using the panpestivirus-specific primers 324/326 (Vilcek and others 1994). The product of in vitro amplification was directly sequenced by forward and reverse PCR primers using the fmol DNA Cycle Sequencing System (Promega) according to the manufacturer's protocol, and the sequence obtained was designated Pudu-CH and deposited in the GenBank data library (accession number AY679726).

The sequences were aligned using the ClustalW software (De Moerlooze and others 1993, Baule and others 1997).

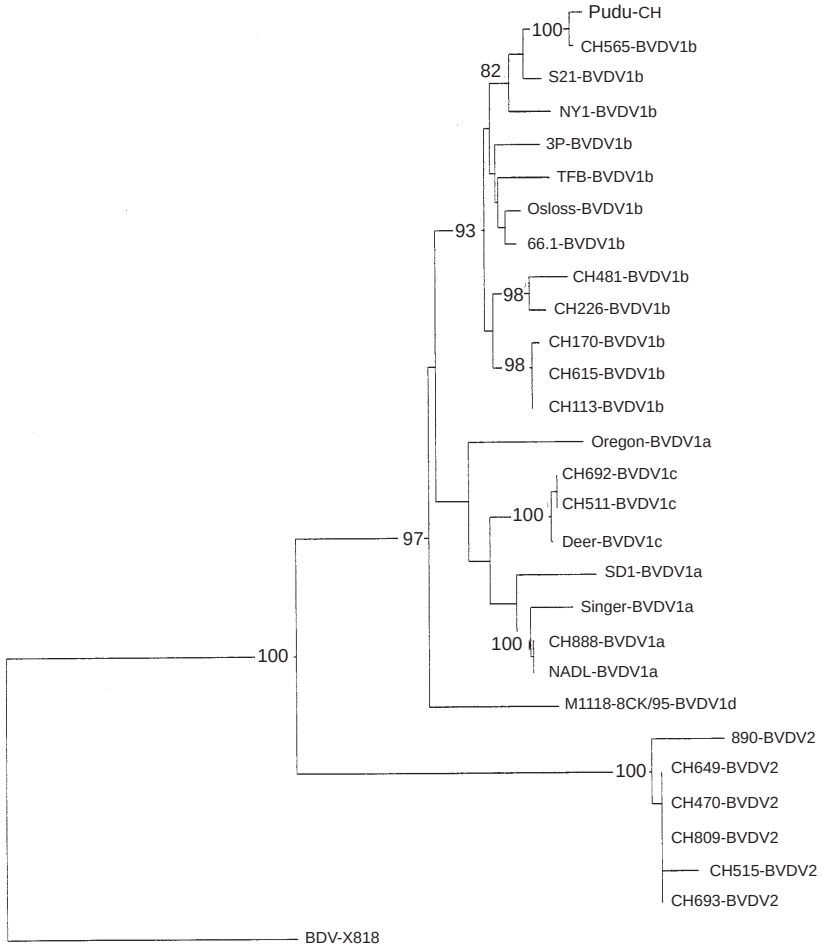

FIG 1: Phylogenetic tree of 5′ untranslated region sequences from the Pudu-CH isolate, Chilean bovine viral disease virus (BVDV) isolates and other BVDV isolates representing the major BVDV genotypes and subgroups. The aligned sequences correspond to nucleotides 142 to 371 in the NADL sequence

Phylogenetic analysis was performed by the neighbour-joining method using TREECON 1.3b (Van de Peer and De Wachter 1994). The robustness of the phylogenetic analysis and the significance of the branching order was determined by bootstrap analysis carried out on 100 replicates. Evolutionary distances between sequences were estimated by using the Kimura two-parameter method (Kimura 1980).

As shown in the phylogenetic tree (Fig 1), the 5′ UTR sequence of Pudu-CH confirmed that it was a pestivirus closely related to BVD virus type 1b (BVDV-1b) circulating among cattle. The similarity between the Pudu-CH isolate and other cattle BVDV-1b isolates ranged from 91·2 to 99·1 per cent. The highest similarity (99·1 per cent) to the Pudu-CH isolate was seen with the CH565 Chilean cattle isolate (S isolate), and the lowest similarities were with the Chilean cattle isolates CH113, CH170, CH226, CH481 and CH615 (C isolates), with a range of 91·2 to 92·5 per cent. The C isolates were obtained from cattle from central Chile and the S isolate was obtained from a cow from southern Chile near the region where the pudu was found. Therefore, it is likely that there are different populations of BVD virus isolates circulating among the cattle in both of these regions of Chile (J. Pizarro-Lucero, M. O. Celedón, unpublished observations).

Nucleotide analysis of the variable region II (VR-II) of cattle BVDV-1b isolates (Fig 2), a region of the 5′ UTR particularly rich in informative bases (Deng and Brock 1993), showed that

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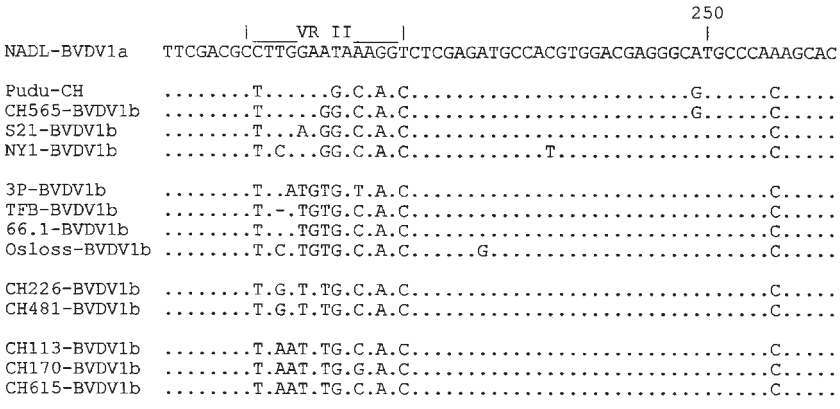

FIG 2: Alignment of 5′ untranslated region nucleotide sequences from the Pudu-CH isolate, Chilean bovine viral disease type 1b (BVDV-1b) isolates and other BVDV-1b isolates. The aligned sequences correspond to nucleotides 202 to 261 in the NADL sequence. Dots represents nucleotides that are identical to the NADL sequence, and the dash indicates a putative nucleotide deletion. The variable region II (VR-II) (nucleotides 209 to 223 of the NADL sequence) is indicated

the Pudu-CH isolate had a pattern of nucleotide substitutions very similar to the CH565, S21 and NY1 BVD virus isolates, thereby supporting the hypothesis that the Pudu-CH isolate was of bovine origin.

The origin of the infection in the pudu was unknown, but it was probably the result of viral transmission between cattle and pudu. To the authors' knowledge, this is the first detection of BVD virus in a pudu. Although the role of the Pudu-CH isolate in the disease seen in the animal was not established, it is possible that the isolate played an important role in the clinical presentation and lesions observed.

The present findings highlight the need to establish how much impact BVD virus has on the health of free-ranging pudu and other cervid populations in Chile.

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