Emergence of a new bovine herpesvirus 1 strain in Australian feedlots

Brief Report

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Summary. Restriction endonuclease analysis of 19 Australian bovine herpesvirus 1 (BHV1) isolates recovered between 1989 and 1993 has revealed the emergence of a new BHV1 genotype which appears associated with severe respiratory disease.

Bovine herpesvirus 1 (BHV1) is recognised as a serious disease threat to beef and dairy industries worldwide. In North America and more recently in Europe, BHV1 has been responsible for outbreaks of a severe form of infectious bovine rhinotracheitis (IBR) [3]. While serological surveys have suggested that the BHV1 seroprevalence rate within Australia may be as high as 30% [10], the virus has been regarded as a relatively minor cause of disease in Australian beef and dairy cattle.

The low incidence of clinical disease in Australia is similar to that reported in the United Kingdom prior to 1977, although the BHV1 seroprevalence rate in Australia is much higher than in the UK [3–5, 8]. In 1977 cattle and dairy producers in the U.K. experienced an alarming increase in the incidence and severity of IBR outbreaks. A number of studies were conducted to account for this sudden increase [4, 5, 8, 9]. Restriction endonuclease (RE) analysis of BHV1 viruses based on the scheme of Metzler [6, 7] was used in two retrospective studies to determine whether a 'new' BHV1 genotype might have been responsible [5, 9]. The unofficial classification scheme uses restriction endonuclease profiles of BHV1 isolates to group them into one of five subtypes; 1.1 (IBR-like), 1.2a and 1.2b (infectious pustular vulvovaginitis (IPV)-like) and 1.3a and 1.3b (encephalitic viruses now formally classified as bovine herpesvirus 5). The U.K. studies revealed that all BHV1 viruses isolated prior to the 1977 out-
break could be classified as belonging to the BHV-1.2b subgroup. Since 1977 there has been an increasing number of BHV1.1 isolates recovered in the U.K. [4, 5, 8, 9]. Investigators concluded that the most probable cause for this sudden increase in the incidence of IBR was the introduction of a BHV1.1 isolate with imported North American Holsteins [4, 8].

To date only two studies [1, 11] have used RE analysis to study the genetic diversity of different Australian BHV1 isolates. Based on the information contained in the first of these [1] it was concluded that the sixteen Australian and fourteen New Zealand BHV1 isolates examined were virtually identical to one another and that all isolates could be classified as IPV-like. All viruses in this initial study had been isolated between 1961 and 1982. These findings were supported by a second study which included a further eleven Australia BHV1 viruses which had been isolated between 1982 and 1988 [11]. The second study advocated the continued enforcement of strict quarantine measures to prevent the inadvertent introduction of more virulent BHV1 strains into Australia.

Within the last five years there has been growing anecdotal evidence of a dramatic increase in the incidence and severity of BHV1 associated respiratory disease within Australian feedlots. The present study was undertaken to determine whether this apparent increase in the incidence and severity of respiratory disease was due to the accidental importation of an exotic BHV1 strain into Australia.

Nineteen BHV1 isolates which had been recovered between 1988 and 1993 were examined in this study. Sixteen BHV1 viruses, isolated between July 1992 and June 1993, were provided under code by the Elizabeth Macarthur Institute of the New South Wales Department of Agriculture. The code was not revealed until all analysis had been completed. Of these sixteen, nine originated from a single New South Wales feedlot. A further two BHV1 viruses isolated from this same feedlot in 1989, and one additional virus isolated in 1989 from a small Queensland feedlot, were provided by the Animal Research Institute of the Queensland Department of Primary Industries. The Australian BHV1 isolate, V155, which was included in the two earlier restriction endonuclease studies of Australian BHV1 strains [1, 11] was included as a reference in the present study.

Approximately 0.1 pfu/cell of each virus isolate was used to infect bovine testes cells which had been grown to confluency in a 850 cm^2 roller bottle at 37°C. Cells were maintained in Dulbecco’s modified essential medium (Gibco-BRL) supplemented with 10% foetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin and 8 mM L-glutamine. Virus was harvested 48 h after inoculation. BHV1 DNA was extracted from virions essentially as described by Dorman [2]. Viral DNA was gently resuspended in 200 µl H_2O and quantified by measuring its absorbency at A_{260}nm.

Restriction endonuclease digestion of 2.0 µg of purified viral DNA in 20 µL digest volume was performed using HindIII, HpaI, EcoRI and BamHI according to the manufacturers recommendations (Boehringer Mannheim). Five micro-litres (0.5 µg of viral DNA) from each digest was analysed by electrophoretic