Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis

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Summary. A polymerase chain reaction-based assay capable of detecting a broad range of pestiviruses from pigs, cattle, or sheep was developed. Of six sets of primers selected from different parts of the pestivirus genome, the best results were provided by a pair from the highly conserved 5' non-coding region which gave amplification with all 129 isolates tested. This panel consisted of 33 isolates from pigs, 79 from cattle, and 17 from sheep. Differentiation between the viruses was achieved by cutting the PCR-amplified products with the restriction endonucleases AvaI and BglII. Using this procedure it was possible to distinguish at least 3 genogroups; group I (HCV) contained 32 of the pig isolates, group II (BVDV) contained all the cattle isolates tested plus 6 sheep isolates and group III (BDV) contained 11 sheep isolates and 1 pig isolate.

Introduction

Pestiviruses cause economically important diseases of pigs, cattle, and sheep [33]. The genus Pestivirus has been classified recently in the family Flaviridae [48] and consists of hog cholera virus (HCV), bovine viral diarrhoea virus (BVDV) and border disease virus (BDV) of sheep. These pathogens are responsible for substantial losses and their effective control relies on accurate laboratory diagnosis.

The current detection of pestiviruses in clinical specimens is based on direct virus isolation in cell culture or the detection of viral antigen by immunological methods such as immunohistochemistry and immunoassay. These techniques can be time-consuming and, although HCV can be discriminated from the

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ruminant pestiviruses using a panel of monoclonal antibodies (Mabs), the distinction between BVDV and BDV isolates is less clear [16].

The introduction of in vitro amplification of nucleic acids by the polymerase chain reaction (PCR) [44] has led to the development of faster, more sensitive and specific laboratory tests for the detection of microorganisms, especially viruses [5]. Several investigators have reported the utilisation of the reverse transcriptase-PCR (RT-PCR for the amplification of pestivirus RNA [1, 4, 6, 7, 21, 23, 27, 28, 40, 42, 45, 47, 49]. The primers for PCR have been selected from different regions of the pestivirus genome to amplify specific viruses or to attempt to detect all pestiviruses. The differentiation of the pestiviruses is desirable in order to understand further the epidemiology of these agents which will aid control and eradication programmes of the diseases they cause.

The publication of several complete sequences for pestivirus genomes (BVDV strains NADL [9], Osloss [38] and SD-1 [13]; and HCV strains Alfort [30] and Brescia [34]) has given more detailed information on the pestivirus genomic organisation. The comparison of these five sequences has allowed the identification of conserved regions in the 12.5 Kb RNA genomes. In this paper we report detection of a broad range of pig, cattle and sheep pestiviruses by RT-PCR using six sets of PCR primers. In addition, it has been possible to differentiate at least three genogroups of pestiviruses by restriction endonuclease digestion of the amplified product from the single most successful primer pair.

**Materials and methods**

**Virus isolates**

The 33 porcine pestiviruses were selected from the panel of reference strains held at CVL, Weybridge to represent antigenically divergent isolates from around the world: Germany (7), Britain (5), Japan (4), Malaya (4), Belgium (3), Brazil (3), France (2), Netherlands (2), USA (2) and Italy (1). All viruses were stored at −70 °C as infected PK-15 cell culture supernatants.

Of the 79 pestiviruses isolated from cattle three (NADL, Osloss and Oregon C24V) were cytopathic (CP) reference strains and two were non-cytopathic (NCP) viruses used as reference strains at Moredun Research Institute namely G982 [3] and KY1203 [24]. A sixth cattle pestivirus was a Czechoslovakian vaccine strain, while the remaining 73 pestiviruses were field isolates from cattle in Scotland. They had been isolated in secondary bovine embryonic kidney cells as previously described [2] from specimens submitted for virus diagnosis from apparently healthy cattle or those suffering congenital, enteric or respiratory infections. The isolates were selected randomly from viruses collected between 1979 and 1993 which had been stored at −70 °C as infected cell cultures at low passage level. Twenty three of the field isolates showed evidence of cytopathic effect in cell cultures. Nineteen of these isolates were from cattle with clinical signs of enteric disease strongly suggestive of mucosal disease, one was from a bull with respiratory disease and no history was given with the other three.

Sheep were the source of 17 pestiviruses tested. These included the Weybridge NCP reference strain [19], Moredun CP and NCP reference strains [46] and the NCP AV-2 virus isolated from a case of Aveyron disease in France [8]. The other 13 pestiviruses were NCP isolates from field cases of border disease in the U.K. They had been isolated in