Investigation of the prevalence of bovine viral diarrhoea virus in dairy cows in South Korea

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Blood samples were collected from 1328 dairy cows of different parities in 46 herds in two regions of South Korea and tested for bovine viral diarrhoea virus (BVDV) by reverse transcriptase-PCR (RT-PCR) for the detection of viral sequences in whole blood and by a commercial ELISA for the detection of BVDV-specific antibodies. None of the animals was positive by RT-PCR but 770 (58 per cent) were seropositive. The proportion of seropositive cows increased with their parity, but there was no difference between the seroprevalence of BVDV among the cows in the two regions.

BOVINE pestiviruses are one of the most important viral pathogens of cattle and have been recorded in most countries where cattle are raised. Bovine viral diarrhoea virus (BVDV) is a single-stranded RNA virus in the genus *Pestivirus* within the Flaviviridae family (Nettleton and Entrican 1995). The viral RNA is approximately 12·3 kb in length and contains one large open reading frame flanked by 5′- and 3′- non-coding regions (Donis 1995). Strains of BVDV have been classified by biotype or genotype; there are two biotypes, designated as cytopathic and non-cytopathic, according to their ability to produce cytopathic effects in cell culture (Deregt and Loewen 1995), and two genotypes, categorised as genotypes I and II on the basis of genetic differences (Ridpath and others 1994).

Infection with BVDV can cause diseases of the alimentary and respiratory tracts and reproductive problems. Most primary infections in seronegative immunocompetent cattle are subclinical, but they can cause outbreaks of fever, loss of appetite, diarrhoea, salivation, leucopenia and changes in platelet function (Baker 1987, Perdrizet and others 1987, Bolin and Ridpath 1992, Walz and others 2001). The virus has been shown to have an immunosuppressive effect, it may enhance secondary bacterial and viral infections (Pritchard and others 1989), and it can cause reproductive problems such as embryonic death, mummification, abortion, stillbirth and a reduced rate of conception (Ross and others 1986, Rufenacht and others 2001, Valle and others 2001, Munoz-Zanzi and others 2004, Robert and others 2004). If a fetus becomes infected before day 125 of gestation it becomes persistently infected (PI) and unable to produce antibody to the virus. After birth these animals may appear stunted and poorly grown, be clinically normal, or develop mucosal disease as a result of superinfection by a cytopathic virus (Brownlie and others 1984, Houe 1995, Brock and others 1998). All PI animals are potent sources of virus to their cohorts, and their elimination is therefore an important strategy for the control and eradication of BVDV (Lindberg and Alenius 1999).

The prevalence of seropositive animals in herds ranges from 40 per cent to 90 per cent, and most studies have found the prevalence of PI animals in the general adult cattle population to be approximately 1 per cent (Baker 1987, Edwards and others 1987, Houe 1995, Mockeliuniene and others 2004, Loneragan and others 2005). Globally, the clinical effects of BVDV infection cause economic losses due to reductions in productivity and the need for treatment (Moerman and others 1994, Chi and others 2002), and as a result many countries try to control BVDV infection. One of the current methods is the elimination of PI cattle and vaccination of the herd (Bezek and Mechor 1992, Peters and others 2004).

The purpose of this study was to investigate the prevalence of BVDV in dairy cows in South Korea and to use the data to develop a programme to control the number of PI animals.

MATERIALS AND METHODS

Herd selection and blood samples

Blood samples collected for the purpose of metabolic profile testing from September 2004 to June 2005 were used (Payne and others 1970). The age, days in lactation, parity, milk production, body condition score, feed history and vaccination history of each animal were recorded. All the samples were collected from parous dairy cows, and 1328 samples were selected from 46 herds that had not been vaccinated against BVDV. The samples were taken from the tail vein into plain and EDTA Vacutainer (Becton Dickinson) collection tubes to obtain clotted and EDTA-treated blood samples. The plain samples were allowed to clot at ambient temperature for 40 minutes and then centrifuged at 1300 *g* for 15 minutes. The serum and EDTA-treated whole blood samples were kept on ice for transport to the laboratory. RNA was extracted immediately from the EDTA-treated whole blood samples, and the serum was kept at –80°C until analysed by ELISA.

RNA extraction from whole blood and identification

To improve the testing efficiency, an aliquot of blood from each of five samples was combined into a pooled sample. It was planned to analyse individual samples from positive pools to identify the infected animals. Viral RNA was extracted from 1·5 ml of sample using the QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions. The RNA samples were stored at –80°C until they were analysed by reverse transcriptase-PCR (RT-PCR).

RT-PCR assay

A one-tube method combining both reverse transcription and PCR was used for the RT-PCR (OneStep RT-PCR Kit; Qiagen). It was carried out in a total volume of 25 µl containing 1 µl template RNA, 1 µl Qiagen OneStep RT-PCR enzyme mix, 0·6 µl of each primer (0·6µM each), 1 µl dNTP mix, 5 µl 5× Qiagen OneStep RT-PCR buffer, and 15·8 µl RNase-free water. A previously reported primer pair (BVDV 1: 5′-GCCATGCCCTTAGTAGGACT-3′; BVDV CR1: 5′-GCAGCACCCTATCAGGCTGT-3′) was used. This BVDV-specific primer set detected the BVDV NY-1 strain at a concentration of 2×10^1 TCID50/ml, and did not amplify Akabane, parainfluenza-3 (PI-3) or infectious bovine rhinotracheitis (IBR) viruses (Park and others 2004). The reactions were carried out in an automated thermal cycler (Takara Bio) with the following steps: reverse transcription at 50°C for 30 minutes; initial PCR activation at 95°C for 15 minutes; 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds and 72°C for one minute; followed by a final extension at 72°C for 10 minutes. A positive control sample of BVDV-1 (NADL strain, accession number M31182) and a negative control lacking template were included in each round of amplification.

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TABLE 1: Regional prevalence of bovine viral diarrhoea virus-seropositive cows

Region	Number of herds	Number of samples	Number (%) seropositive
North*	22	604	343 (56·8)
South†	24	724	427 (59·0)
Total	46	1328	770 (58·0)

* Chungcheong, Gyeonggi
† Gyeongsang, Jeolla

TABLE 2: Numbers (%) of cows of different parities that were seropositive to bovine viral diarrhoea virus

Parity	Number (%) of samples	Number (%) seropositive
1	487 (36·7)	238 (48·9)
2	336 (25·3)	192 (57·1)
3	235 (17·7)	146 (62·1)
≥4	270 (20·3)	194 (71·9)
Total	1328 (100)	770 (58·0)

Identification of the PCR products

After the amplification, 10 µl of the final product was analysed by 1·5 per cent agarose gel electrophoresis in Tris acetate-EDTA buffer, using a 100 base pair (bp) DNA ladder (Bioneer Bio) as a molecular weight standard. The RT-PCR product bands were visualised under UV light (340 nm) and photographed. Amplification products of 235 and 546 bp were predicted for BVDV types I and II, respectively.

ELISA

Undiluted serum samples were tested for antibodies to BVDV using a commercially available indirect ELISA (SVANOVIR BVDV-Ab ELISA; Svanova Biotech) according to the manufacturer's instructions. Relative to the serum neutralisation test, as in the manual, this assay has a sensitivity of 100 per cent and a specificity of 98·2 per cent. The plates were read in an automatic plate reader at 450 nm, and the results expressed as optical density. Samples with a corrected optical density value below 0·20 were considered negative.

Statistical analysis

The antibody prevalences, grouped according to the predefined regions or parity of the cows, were compared using the chi-squared test.

RESULTS

No positive results were found by the RT-PCR analysis of the whole blood samples, but antibodies to BVDV were detected in 770 of the 1328 serum samples (58 per cent), and 45 of the 46 herds were seropositive for BVDV.

There was no difference between the prevalence of BVDV in the two regions (Table 1), but the seropositive rate rose as the parity of the cows increased ($P<0·0001$) (Table 2).

DISCUSSION

The prevalence of BVDV determined by an antibody ELISA was similar to findings from other countries, but that determined by RT-PCR was not (Vilcek and others 2003, Mockeliuniene and others 2004, Loneragan and others 2005). Antibody was detected in all but one of the herds, but no PI animals were found.

To diagnose animals with BVD-mucosal disease, both virus isolation and serological data are needed for each animal (Duffell and Harkness 1985). Previous studies have demonstrated that the viraemic stage of BVDV infection is rarely detected (Duffell and Harkness 1985). In the viraemic stage, the animals lack antibody but have virus in their blood. A high percentage of seropositive cattle is directly related to the presence of one or several PI animals in a herd (Houe 1992). It appears that the inability to detect PI animals in the present study was due to the selection of samples, specifically, that calves and heifers were not included. According to data from the Korea National Agricultural Cooperative Federation, in 2005 the mean age of dairy cows at first calving was 27·4 months. There is also some information on the distribution of PI animals by age. Rufenacht and others (2000) found that all PI animals were less than 30 months old, and Houe and others (1995) found that in Denmark 82·9 per cent, and in Michigan 71·4 per cent, of PI animals were less than 24 months old. The other characteristic of the sampling was healthy animals were tested for the purpose of metabolic profile testing (Payne and others 1970). A previous study in Korea found that 20 of 254 aborted fetuses derived from bovine reproductive studies were positive by RT-PCR for BVDV (Park and others 2004). Collectively, these findings indicate that BVD infection is widespread in South Korea.

In this survey, there was no difference between the prevalence of seropositivity to BVDV in the north and south regions. In Korea in 2005, there were 478,865 dairy cattle and 8923 herds (Korean Statistical Information System 2007). The rate of seropositivity increased with increasing age ($P<0·0001$), a result consistent with other studies (Rufenacht and others 2000), indicating that there was active and acute infection with BVDV, and suggesting that the RT-PCR assay was not sensitive enough to detect this form of infection in the cattle tested.

The failure to detect PI animals was probably due to the age range of the animals sampled and the fact that they were generally healthy lactating dairy cows. A study including younger animals would be required to obtain a more accurate measure of the proportion of PI animals in the general population. The lack of clinical disease in the population studied suggests that BVDV was probably endemic, and that acute, transient infections may have been occurring, as indicated by the increase in seropositivity with age. Evidence from other countries, especially in Scandinavia, has shown that this insidious disease can be controlled, even without the use of vaccines (Bitsch and Ronsholt 1995).

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