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Relationship of bovine viral diarrhea virus persistent infection to incidence of diseases on dairy farms based on bulk tank milk test by RT-PCR

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Abstract

The prevalence of bovine viral diarrhea virus (BVDV) in dairy herds in Hokkaido, Japan, was estimated by reverse transcription polymerase chain reaction (RT-PCR) using bulk tank milk samples. Sixteen out of 265 dairy herds were identified as BVDV positive, and at least one persistently infected (PI) cattle was recognized in each of the positive herds except for two herds of which, owners did not agree to examine individual cows. The proportion of positive herds with a history of BVDV PI was significantly higher than that with no history of BVDV PI (odds ratio (OR) 4.25, 95% confidence interval (CI) 1.471–12.278, $p = 0.004$). The herds examined for BVDV were divided into two groups, high and low disease incidence groups based on the occurrence of diseases such as diarrhea, pneumonia or abortion in the past 1 year. The BVDV positive herds in the high disease incidence group were significantly more than that in the low disease incidence group (OR 2.92, CI 1.110–7.683, $p = 0.024$). It was observed that there were significantly ($p = 0.008$) more PI calves or heifers in farms of high disease incidence group than in farms of low disease incidence group. These results suggested that bulk tank milk test was available method for the detection of PI animals in dairy herds, and the existence of PI non-lactating cows in herd correlated with the incidence of diseases of the diarrhea or respiratory disorders.
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Keywords: BVDV; BVDV persistent infection; Bulk tank milk; Disease incidence; Herd diagnosis; RT-PCR

1. Introduction

Bovine viral diarrhea virus (BVDV) is a ubiquitous pathogen of cattle (Baker, 1995; Radostits and Littlejohns, 1988). Together with classical swine

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fever virus (CSFV) and border disease virus (BDV) of sheep, they belong to the genus *Pestivirus* within the family Flaviviridae (Heinz et al., 2000). The genome of *pestiviruses* consists of positive-stranded RNA of approximately 12.5 kb in length. It consists of a 5′-untranslated region (UTR) of almost 400 nucleotides, a single long open reading frame encoding approximately 4000 amino acids, and a 3′-UTR of more than 200 nucleotides (Meyers and Thiel, 1996). The 5′-UTR is highly conserved within the pestiviruses. It is, therefore, useful for the general detection of the *pestiviruses* or for the specific recognition of CSFV, BDV, and BVDV by the reverse transcription polymerase chain reaction (RT-PCR) (Vilcek et al., 1994; Sakoda et al., 1999).

Infection with BVDV is generally subclinical, however, when a dam is infected with BVDV during pregnancy, transplacental infection may occur, and as a result, fetal abortion, mummification or congenital defects may occur depending on the gestation stage (Baker, 1995). More importantly, infection in the first trimester of pregnancy can result into the birth of immunotolerant calves that are persistently infected (PI) with BVDV. The PI animals are a major source of virus spread and thus, it is very important to identify and remove them from the cattle herd (Lindberg, 2003). In general, PI cattle show varied clinical manifestations such as diarrhea, pneumonia (as a result of immunosuppression), poor growth, some succumb to mucosal disease, and some PI cattle indicate no clinical manifestations. The PI cattle on dairy farms are suspected as the cause of milk production loss and/or increase in occurrence of secondary or opportunistic infections (Baker, 1995; Chi et al., 2002; Kelling et al., 2002; Lindberg, 2003). To detect PI cattle, it is, therefore, necessary to examine all cattle in the herd and not to depend only on clinical manifestations.

Bulk tank milk has been utilized for screening of BVDV infection in dairy herd. The RT-PCR technique has previously been described for detection of BVDV in somatic cells from bulk milk samples (Radwan et al., 1995; Drew et al., 1999; Renshaw et al., 2000). In the present study, the prevalence of herds with PI animals was determined by RT-PCR using bulk tank milk. And the relationship between prevalence of PI animals and incidence of diseases such as diarrhea, pneumonia, and abortion on farms was estimated.

2. Materials and methods

2.1. Sampling and grouping of herds

Bulk tank milk samples were collected randomly from 265 herds in Hokkaido, Japan. Of the 265 herds, 35 herds had a recent history (within approximately 5 years) of the identification of PI cattle. The other 230 herds had no history of BVDV PI animal detection. In the herds where BVDV was detected by the bulk milk test, peripheral blood samples were collected from all cattle on the farm including calves, heifers, and dry and lactating cows, and the sera tested to identify BVDV infected cattle. To assess whether BVDV infection was transient or persistent, all positive animals were examined again after 3–4 weeks using both RT-PCR and virus isolation (VI) test.

At the same time of bulk tank milk sampling, epidemiological information of these farms was obtained from the farmers and veterinary practitioners in-charge. The information included history of detection of BVDV PI animals, record of vaccination against BVDV, and the outbreak of diseases (diarrhea, respiratory problems and abortion) in the farm per year. The outbreak of diseases was defined as clinical treated cases. The disease cases were examined from the health care record in individual farm and clinical record in the clinic in-charge. Based on the occurrence of diseases in the past 1 year, 265 herds were divided into two groups; high and low disease incidence groups. The criteria of the division was as follows; high incidence was more than five cases in 30 or less cows per farm, more than 10 cases in 30–50 cows per farm, and more than 15 cases in 51 or more cows per farm, respectively. Low incidence was less than above numbers in the respective farm size.

2.2. RNA extraction

The preparation of bulk milk sample was done as previously described (Radwan et al., 1995). Briefly, 50 ml of milk was centrifuged at 1000 $\times$ g for 15 min at 4 °C to pellet somatic cells and the supernatant was removed. The cell pellet was resuspended in 20 ml of PBS and centrifuged at 200 $\times$ g for 15 min at 4 °C and the supernatant was discarded. Total RNA extraction from somatic cells was done using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's

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