

# The prevalence of bovine viral diarrhoea virus in persistently infected cows in industrial dairy herds in suburb of Mashhad-Iran

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## Key words:

BVDV, dairy herds, Iran, Mashhad, persistent infection.

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Received: 1 June 2011

Accepted: 27 September 2011

## Abstract:

Bovine Viral Diarrhoea Virus (BVDV) is a *Pestivirus* of the *Flaviviridae* family that infects cattle, and its widespread distribution can cause substantial economic losses worldwide. The aims of this study were: (1) to estimate the prevalence of animals persistently infected (PI) with BVDV in industrial dairy cattle herds in suburb of Mashhad- Iran; and (2) to compare the occurrence of persistently infected (PI) animals in industrial dairy cattle herds where BVDV animals had been either removed from or retained in the herds. In total, 157 blood samples were taken from individual cows from 18 Holstein dairy cattle herds each containing 250-3000 cows in suburb of Mashhad. The blood samples were analyzed for the presence of BVDV using *Pestivirus* antigen-capture ELISA. Positive animals were retested after three weeks and animals those tested positive again were defined as PI. We found that only 3/18 herds (16.7%) contained antigen-positive cows, and that only 5/157 animals tested (3.2%) were antigen positive. All of the positive animals were confirmed as PI, and all of PI cows were less than 17 months old. The true prevalence of antigen-positive cows was calculated to be 3.31%. A significant proportion ( $p < 0.01$ ) of PI animals exhibited severe weight loss (4/5; 80%). and before this study, it was supposed to be sent to slaughterhouse by the farmers due to loss of weight. We conclude that the prevalence of PI cows in our study is consistent with previous reports. However, our study reveals that severe weight loss in young PI animals requires their removal to minimize the numbers of BVDV-infected animals in the herd.

## Introduction

Bovine Viral Diarrhoea Virus (BVDV) is a *Pestivirus* of the *Flaviviridae* family that infects cattle, causing reproductive, respiratory and enteric disorders (Grooms, 2006; Garoussi, 2007), and its widespread distribution can therefore cause

substantial economic losses among farmers worldwide. BVDV is classified into two biotypes, NCP (noncytopathic) and CP (cytopathic), based on *in vitro* cell assays (Dereget and Loewen, 1995). The NCP BVDV biotype is the one most commonly isolated in the field. However, CP biotypes have also been shown to cause mucosal disease (MD)

(Brownlie et al., 1984). Persistently infected (PI) animals, resulting from intrauterine infection, are of major epidemiological importance. PI calves may show clinical signs of infection, including poor weight gain or increased susceptibility to infection with other pathogens, although they may also remain healthy. PI animals form the main reservoir of BVDV within herds and play the most important role in spreading the disease (Bolin, 1990). In addition, such animals are at risk of developing MD, a fatal form of BVDV infection characterized by lesions in the gastrointestinal tract (Brownlie, 1991; Weiss et al., 1994; McGowan and Kirkland, 1995). Infection with BVDV has been reported to cause substantial economical losses worldwide (Houe, 1995; Pringle, 1999). The design of an effective control and eradication strategy depends on knowledge of the epidemiological parameters, including the prevalence of antibody-positive and PI animals, and information on the economic impact of BVDV infection in relation to the cost of different potential eradication schemes.

The objectives of this study were: (1) to estimate the prevalence of PI BVDV animals in industrial dairy cattle herds of Mashhad suburb-Iran; and (2) to investigate the significance of PI animals between culled and non-culled cows in industrial dairy cattle herds in the suburbs of Mashhad in Khorassan Razavi province, a major producer of livestock in Iran.

## Materials and Methods

### Sampling and dairy herd management:

Randomized blood samples were taken from dairy cattle herds according to a proportional geographical distribution in various parts of suburb of Mashhad, using a lottery method to select individual animals. It was previously reported that BVDV could be isolated from the lymphatic nodes of 13.1% of cows slaughtered in Iran (Sedighi Nejad, 1996). The required sample size for estimating the seroprevalence of BVDV in the cattle population with a 95% level of confidence, 5% absolute precision and an expected prevalence of 10%, was determined to be at least 138 using the following

formula (Thrusfield, 2005):

$$n = 1.96^2 P_{\text{exp}}(1 - P_{\text{exp}})/d^2,$$

where 'n' is the required sample size, 'P<sub>exp</sub>' is the expected prevalence, and 'd' is the desired absolute precision.

A total of 157 Holstein cow blood samples were taken from 18 industrial dairy cattle herds in suburb of Mashhad between February and May 2006. Forty-one (26.1%) samples were obtained from animals culled on the farms. Prior to this study, these animals were due to be referred to slaughter house as a consequence of weight loss. The body condition scoring of culled animals was poor.

Two production strata for dairy cattle exist in the suburbs of Mashhad. The first group consists of small farmers, who together own about 35% of the total cattle. In this group, herd size is approximately 2-10 animals per farm, with a low technology level and low milk production (mean = 10 L/day/cow). The second group is the commercial stratum, with an average herd size of 110 cows. The commercial industrial herds use more advanced technology and have a higher average milk production of about 6,800 L/cow/year. We included only large commercial farms in this study. The industrial dairy cattle herds are the major producers of milk in this area and farmers export milk to all areas of Iran. The industrial dairy cattle herds participating in this study each contained 250-3,000 animals. Therefore, this study covered about 15,500 cows in total. All of the cows were of the Holstein breed, and all were housed in intensive systems. Approximately 55% of the herds used the free-stall system, and cows were typically fed alfalfa, corn silage and concentrate in various proportions using the Totally Mixed Ration (TMR) system. Most cows (85%) were milked three times a day and the remainders were milked twice daily using milking machines. Most of the cows milked twice a day were over 5.5 months pregnant. Calves were kept in individual boxes until they were 2-3 months old. After this, male and female calves were reared separately in an intensive system. All herds used artificial insemination and heifers were artificially inseminated when they were 15-18 months old. Cows were not vaccinated against BVDV. However,

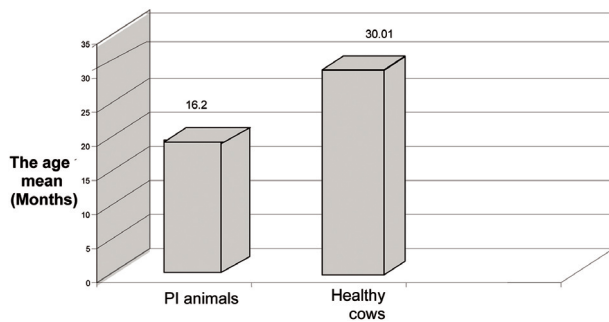


Figure 1: The comparison of the age between healthy animals and those persistently infected with bovine viral diarrhoea virus in dairy cattle herds in the suburbs of Mashhad, Iran.

animals were immunized against foot and mouth disease (FMD) and clostridial diseases, and all female cows were vaccinated against brucellosis. The nutrition and reproduction management of the herds were controlled using a computerized herd health management system.

Blood samples were taken from young calves (57/157, 36.3%) at three months of age in order to prevent false negative test results caused by maternally derived antibodies (Zimmer et al., 2004). The age of animals was recorded on identification cards within each herd.

**Antigen-capture ELISA:** All blood samples were treated with heparin to prevent coagulation, and whole blood samples were examined for the presence of BVDV antigen using a Pestivirus-Ag capture ELISA kit (Svanova Biothec AB, Uppsala, Sweden) according to manufacturer's instructions. The sensitivity (Se) and specificity (Sp) of the test was 98% and 97%, respectively, as indicated by the manufacturer.

**Statistical analysis:** Differences in the proportion of persistent infection between culled and non-culled animals were analyzed for statistical significance ( $P < 0.01$ ) using the Chi-square test in SPSS software version 9. The true prevalence of BVDV infection within the herds was estimated using Rogan and Gladen's (1978) correction of

apparent prevalence, using the equation:

$$\text{True prevalence} = (\text{Apparent prevalence} + \text{Sp} - 1) / (\text{Se} + \text{Sp} - 1).$$

## Results

Out of a total of 157 cows within 18 dairy cattle herds in suburb of Mashhad-Iran, 57 (36.3%) were calves, 36 (22.9%) were heifers and 64 (40.8%) were adult dairy cows. Three (16.7%) of the 18 industrial dairy cattle herds contained PI animals. A total of five (3.18%) animals from these herds were positive for BVDV and they were retested after three weeks to confirm their PI status (Table 1). Before the BVDV tests were done, four supposedly PI cows from two of the dairy herds had been culled by the farmers due to weight loss. The calculated true prevalence of PI cows in the sample herds was 3.3%. Significantly more PI BVDV cows were culled than not culled ( $p < 0.01$ ). The age of the PI animals averaged less than 17 months old (Fig 1), although one PI cow was 41 months old.

## Discussion

This study showed that only three out of 18 herds (16.7%) contained PI animals. In our data, the prevalence of PI animals remaining within the herds was significantly lower than that observed in culled cows. The PI and positive-antigen animals were young, with a mean age of less than 17 months (Fig 1). We found if these PI animals were culled from infected herds at a young age then BVDV could be eliminated from the herd. Since positive samples were mainly obtained from the animals with poor body condition scoring, it is likely that poor body condition scoring could be used as an indicator of PI BVDV animals. It is essential to identify and remove PI BVDV animals from the herd as these PI animals are likely to shed the BVD virus constantly within their herd and infect other animals. Furthermore, animals in the herd might show high levels of BVDV antibody in blood samples as well as in bulk tank milk (BTM) of the related dairy herds (Talebkhani Garoussi et al., 2009, 2008, 2007). Interestingly, one

Table 1: Distribution of bovine viral diarrhoea virus in persistently infected cows among the dairy cattle herds of Mashhad suburb-Iran.  
\*Significant differences ( $p < 0.01$ )

Persistently infected	Infected herds (%)	Persistently infected animals		Total (%)
		Non-Removed cows (%)	Retained Cows (%)	
+	3 (16.66)	1 (0.63%)*	4 (2.57%)*	5 (3.18%)
-	15 (83.33)	115 (73.24%)*	37 (23.56%)*	152 (96.81%)
Total	18	116(73.88%)	41(26.11%)	157

PI cow was found to be 41 months old; therefore, this animal was already old enough to give birth to further PI animal(s). Two studies have shown that the virus can circulate in a herd for 2-3 years despite no PI animals being present and no direct contact with PI cows occurring (Barber and Nettleton, 1993; Moerman et al., 1993). Therefore, the actual prevalence of PI animals in our study may be higher than the results we obtained.

In the present study, we confirmed that 16.7% of randomly selected dairy cattle herds in suburb of Mashhad-Iran contained PI animals. Several other studies have also estimated the proportion of herds with PI animals. For example, a BVDV prevalence was estimated at 10% in Portugal, based on a finding that the probability of PI cattle being present was 20% for herds with antibody-positive BTM samples and 4% for those with doubtful or negative BTM-antibody test results (Niza-Ribeiro and Pereira, 2004). It has also been reported that 53% of cattle herds in Denmark, 15% in the United States, 45% in Germany and 100% in Greece contain PI animals (Houe and Meyling, 1991; Houe et al., 1995; Frey et al., 1996; Billinis et al., 2005). The prevalence of PI BVDV beef cattle in the United States is estimated to be 4% (Van Campen, 2010). However, in a previous study we found that BVDV was detected in 11% of BTMs from industrial dairy cattle herds in the suburb of Mashhad (Talebkhani Garoussi et al., 2007). Moreover, our previous studies have shown that the true prevalence of BVDV antibody in serum samples and in BTMs from industrial dairy cattle herds of Mashhad suburbs were 72.2% and 94.0%, respectively (Talebkhani Garoussi et al., 2009, 2008).

Many studies show a similar prevalence of PI positive animals, i.e. approximately 0.5-2.0% (Houe,

1999). The estimated prevalence of PI animals among a subsample of 5129 calves in Canada was reported to be less than 0.1% (Talyor et al., 1995). In this study, the true prevalence (3.3%) of PI BVDV animals in industrial dairy cattle herds was lower than could be expected from the prevalence of the antigen-positive animals (Schonbauer et al., 2000).

In conclusion, we found that the prevalence of PI animals in dairy cattle herds around Mashhad in Iran was not high. However, there were high young PI BVDV cows among the culled animals in the farms, which revealed that PI BVDV cows had been identified and culled early from the herds. Several fundamental issues associated with the prevalence of BVDV such as: pregnancy stages and animals with transient infection of BVD virus, will require further investigation in dairy cattle herds in this area. However, on the basis of both our current results and our previous publications, we concluded that the high levels of seropositivity among dairy herds in this region could be attributed to the presence of PI animals that constantly shed the BVD virus into the herds. Therefore, thorough preventative measures are recommended in order to minimize the economic losses caused by this disease.

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