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# Monitoring the bulk milk antibody response to bovine viral diarrhea in dairy herds vaccinated with inactivated vaccines

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## ABSTRACT

This study was designed to determine long-term responses in dairy herds after vaccination with 1 of 3 inactivated bovine viral diarrhea virus (BVDV) vaccines with regard to antibodies against p80 protein in bulk tank milk samples, as detected by ELISA. In the present study, 29 dairy herds were vaccinated with Bovilis BVD (MSD Animal Health, Milton Keynes, UK), 11 with Hiprabovis Balance (Laboratorios Hipra, Amer, Spain), and 9 with Pregsure BVD (Zoetis, Florham Park, NJ). In these herds, bulk tank milk samples were collected and examined at the time of the first vaccination and every 6 mo during a 3-yr period. Samples were analyzed with a commercial ELISA test for the p80 protein of BVDV. The results demonstrated that vaccination affected the level of antibodies against p80. Hence, vaccination status should be taken into consideration when interpreting bulk tank milk antibody tests.
**Key words:** bovine viral diarrhea virus (BVDV), vaccine, milk, diagnosis, ELISA

## INTRODUCTION

Bovine viral diarrhea virus (**BVDV**) is a member of the genus *Pestivirus* in the family Flaviviridae. It causes bovine viral diarrhea (**BVD**), one of the most important diseases of cattle worldwide (Gunn et al., 2005). The genus *Pestivirus* comprises other important pathogens of livestock, including classical swine fever virus and border disease virus of sheep. Pestiviruses are able to cross species barriers to infect different hosts within the order Artiodactyla (Nettleton, 1990).

Bovine viral diarrhea causes considerable economic losses in dairies because of reduced milk production; increased mortality of young animals; and the reproductive, respiratory, and intestinal problems that it produces. Infected animals are also more susceptible to other diseases (Greiser-Wilke et al., 2003; Diéguez et al., 2009). Gunn et al. (2004) noted that, without adequate control measures, BVDV infection might cost up to £37 per cow per year, which is equivalent to a loss of £37,000 for a 100-cow herd over a 10-yr period. In a few countries in which eradication campaigns have been implemented, the programs have shown to be cost effective (Houe, 2003). Vaccination programs still play an essential role in the prevention and control of the infection. Although protection is not 100% effective in every individual animal—due to some factors such as age, nutrition, genetics, and stage of pregnancy—it can be applied as an additional biosecurity measure (European Thematic Network on BVDV Control, 2001).

Two types of BVDV vaccines are currently available: modified live virus and killed virus. A possible concern with the use of BVDV vaccines is the interference of the induced antibody response with the interpretation of serological test results. Detection of antibodies is still the most rapid and cost-effective method to identify exposure to BVDV in herds. The most frequently used technique to estimate BVDV seroprevalence is ELISA. Control programs conducted in many countries are based on ELISA tests, which are run on bulk tank milk (**BTM**) samples periodically to assess the immune status of the sampled herds (Niskanen et al., 1991; Rikula et al., 2003; Hult and Lindberg, 2004; Eiras et al., 2012; Humphry et al., 2012)

The structural protein E2 of the virus is the major target for neutralizing antibodies, which confers protection after either infection or vaccination. Of the nonstructural proteins, the most studied is NS3 (**p80**). This protein is immunogenic and forms the basis of several commercially available immunoassays (Brownlie et al., 2000; Luo et al., 2012). Because nonstructural proteins are mainly produced during virus replication, cattle are more likely to develop antibodies to these antigens following natural infection. Thereby, it has been

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hypothesized that inactivated BVDV vaccines may not induce detectable antibodies against the p80 protein. As a result, they would not interfere with ELISA tests (Graham et al., 2003). However, several studies have been inconclusive when trying to clarify whether vaccination with inactivated BVDV stimulates development of serum antibodies to p80 (Makoschey et al., 2007; Raue et al., 2011; Álvarez et al., 2012; Luo et al., 2012).

The aim of the present paper was to determine the long-term response in dairy herds to vaccination with 3 different inactivated BVDV vaccines with regard to antibodies against p80 protein, as detected by ELISA, in BTM samples.

## MATERIALS AND METHODS

### Study Area and Herds Surveyed

The study was carried out in northwestern Spain, which is the major cattle-farming region of the country, accounting for 38% of milk production in Spain and 1.5% in the European Union. In 2004, this was the first region in Spain to establish a voluntary BVDV control program. Farms willing to join the program are required to undertake additional BVDV serological testing on all cattle older than 1 yr, in addition to the compulsory annual testing for tuberculosis, brucellosis, and enzootic bovine leucosis. For those farms that participated in the voluntary BVDV eradication scheme, serum samples were analyzed with a commercial BVDV ELISA, based on detection of antibodies against p80. Subsequently, herd monitoring was also carried out by performing anti-p80 antibody ELISA on BTM samples every 6 mo in combination with the testing of serum samples (by the same technique) from a selection of unvaccinated heifers.

### Study Design

The same testing strategy as used in the control program was used for the study. Participating herds had to (1) be commercial dairies enrolled in the voluntary control program, (2) be free of active BVDV infection (since at least 2004), (3) have fewer than 25% seropositive animals, and (4) be getting vaccinated for the first time. The number of herds that matched these criteria was 49. Twenty-nine out of the 49 herds were vaccinated with Bovilis BVD (MSD Animal Health, Milton Keynes, UK) by applying 2 doses 4 wk apart and then additional doses at 6-mo intervals. Eleven herds were vaccinated with Hiprabovis Balance (Laboratorios Hipra, Amer, Spain) and 9 with Pregsure BVD (Zoetis, Florham Park, NJ), in both cases applying 2 doses 4 wk apart, followed by booster vaccinations once a year. All vaccinations were performed following each manufacturer's instructions.

In the studied herds, BTM samples were collected and examined at the time of the first vaccination (**t1**) and every 6 mo, as previously mentioned, during a 3-yr period (**t2** to **t7**). Another 65 farms, also enrolled in the control program and free of active BVDV infection, were used as nonvaccinated controls.

### Serological Analysis

The BTM samples were analyzed with a commercial ELISA test (BVDV p80 Ab; Pourquier Laboratories, Institut Pourquier, Montpellier, France). Analyses were performed following the recommendations of the manufacturer: samples were considered positive at a percentage inhibition (**%inh**) of ≤80%; %inh was calculated from optical densities (OD) of samples and controls, as follows: %inh = (OD of the analyzed sample/mean OD of the negative control) × 100.

According to Eiras et al. (2012), low prevalence herds (<5% of seropositive animals) would have a %inh >84.3%; herds with seroprevalence of 5 to 25% would have a %inh from 84.3 to 56.7%; those with seroprevalence of 25 to 65% would have a %inh from 56.7 to 27.3%; and high prevalence herds (>65%), where BVDV is more likely to be present, would have a %inh <27.3%.

### Statistical Analysis

Data were analyzed with SPSS 11.0 (SPSS Inc., Chicago, IL). A one-way repeated-measures ANOVA was conducted to examine changes in %inh obtained in the serial BTM samples using Wilks' Lambda statistic and polynomial contrast.

## RESULTS

The mean %inh of the 29 herds vaccinated with Bovilis BVD was 79.7 at t1. Six months after collecting the first BTM sample (t2), %inh increased to 88.7 and tended to decrease toward the end of the study (85.6 at t3, 74.1 at t4, 76.6 at t5, 77.7 at t6, and 72.9 at t7; Table 1). The largest decrease was observed between t3 and t4 (by 11.5%; i.e., t4 – t3). These differences in mean %inh were statistically significant (Wilks' Lambda = 0.416; $P = 0.003$). The polynomial contrasts also indicated a significant linear component ($F = 12.997$; $P = 0.001$). Accordingly, %inh decreased linearly with time after vaccination (which implies a linear increase in the level of antibodies with successive vaccine doses). According to the manufacturer cut-off points, 44.8% (13/29) of the herds were negative (%inh >80) at t1.