The Veterinary Journal 187 (2011) 330–334

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Antibody responses to inactivated vaccines and natural infection in cattle using bovine viral diarrhoea virus ELISA kits: Assessment of potential to differentiate infected and vaccinated animals

Rüdiger Raue *, Silke S. Harmeyer, Ian A. Nanjiani

Pfizer Animal Health, VMR&D, Ramsgate Road, Sandwich CT13 9NJ, UK

ARTICLE INFO

Article history:
Accepted 12 December 2009

Keywords:
Bovine viral diarrhoea virus
p80 blocking ELISA
Marker vaccination

ABSTRACT

Bovine viral diarrhoea virus (BVDV) is one of the most common and economically important viral infections of cattle. As vaccination is common in most European countries, differentiation between infected and vaccinated animals is one of the key challenges facing BVDV eradication campaigns. This study was designed to compare the ability of commercial ELISA kits to differentiate antibodies generated following vaccination with four different commercial inactivated BVDV vaccines from antibodies generated following challenge with virulent BVDV. Although none of the tested vaccine–ELISA combinations was able to differentiate an infected from a vaccinated animal (DIVA) at the individual animal level, p80 blocking ELISAs, in combination with inactivated BVDV vaccines, may have some value under certain circumstances at herd level. In most cases, antibody responses to BVDV vaccines cannot be clearly distinguished from responses seen in the early phase of natural infection. No commercial BVD vaccine showed true marker qualities for DIVA using p80 blocking ELISAs.

Introduction

Bovine viral diarrhoea virus (BVDV) is one of the most common and economically important viral infections in cattle (Houe, 1999). In adult animals, infection is usually mild or subclinical, although outbreaks of haemorrhagic disease associated with BVDV have been observed (Corapi et al., 1990; Pellerin et al., 1994). Vertical transmission during the first trimester of pregnancy may cause abortion or the birth of immunotolerant persistently infected (PI) calves. PI animals can die of mucosal disease caused by superinfection with antigenically similar cytopathic strains (Brownlie, 1991).

BVDV is a member of the genus *Pestivirus* in the family *Flaviviridae*. The single precursor polyprotein is post-translationally cleaved into structural and non-structural (NS) proteins. The structural proteins are the nucleocapsid protein C (p14), surface glycoproteins E0 or E_{rns} (gp48), E1 (gp32) and E2 (gp53). At least four NS proteins have been identified: N^{pro}, NS2/3, NS4 and NS5.

BVDV can be divided into cytopathic and non-cytopathic biotypes. NS2/3 remains intact in non-cytopathic BVDV strains, whereas in cytopathic BVDV strains NS2/3 is either cleaved to NS2 and NS3 after insertion of cellular mRNA (e.g. ubiquitin sequences) or there is duplication of viral RNA containing an additional NS3 region (Qi et al., 1998). BVDV isolates can be further classified into two main genotypes (BVDV-1 and BVDV-2), along with several subtypes (Pellerin et al., 1994; Becher et al., 1997; Vilcek et al., 2001).

Vaccination is still the most common way to control BVD in most European countries (Moennig et al., 2005) and is considered to be a complementary biosecurity tool in countries with high BVDV prevalence, to prevent accidental re-infections of herds in the early stages of control or eradication campaigns. Diagnostic assays play an important role in this context (Houe et al., 2006), particularly for identification of PI animals and, in the later stages, of field infection in vaccinated herds.

Real-time reverse transcriptase-PCR (RT-PCR) can be used to detect BVDV genomes, even in vaccinated herds (Sandvik, 2005) and is ideal for testing (pooled) blood samples or bulk milk (Radwan et al., 1995; Munoz-Zanzi et al., 2000). Furthermore, it is possible to discriminate between BVDV-1 and BVDV-2 in the same assay by employing separate sets of primers and probes in the same test (Letellier and Kerkhofs, 2003). However, at present, real-time PCR machines and automated RNA extraction are often only available in specialised laboratories due to the relatively high costs of equipment. In addition, the cost per test of a (pooled) sample is higher than for other techniques (such as ELISA), which limits the application of real-time PCR as a tool in eradication campaigns.

BVDV antigen ELISAs are commercially available, rapid and cost effective and are useful in the detection of PI animals. However, some ELISAs have been found to be unsuitable for use in control

* Corresponding author. Tel.: +44 1304 645542; fax: +44 1304 651008.
E-mail address: rudiger.raue@pfizer.com (R. Raue).

1090-0233/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2009.12.013

programmes (Graham et al., 1998), since maternally derived or vaccine antibodies can mask the antigen and lead to false negative ELISA results (Palfi et al., 1993; Zimmer et al., 2004).

Detection of antibodies is still the most rapid and cost effective method for detection of exposure to BVDV in unvaccinated herds. Since NS proteins are mainly produced during virus replication, cattle are most likely to develop antibodies to these proteins following infection with infectious field or vaccinal virus, whereas the use of inactivated vaccines is less likely to induce antibodies to NS proteins.

This study was designed to determine whether commercial blocking ELISA kits that detect antibodies against BVDV p80 (NS3) could be used to differentiate infected from vaccinated animals (DIVA) following the use of inactivated BVDV vaccines. We compared two commercially available ELISA kits coated with whole virus particles and seven p80 blocking ELISAs for their reactivity with antibodies in serum after vaccination of cattle with PregSure BVD, Bovilis BVD, Bovidec and Mucobovin/Vacoviron or following challenge with virulent BVDV.

Materials and methods

Vaccination trial

The vaccination trial (Harmeyer et al., 2005) used groups of 9–10 seronegative heifers aged 3–8 months that were vaccinated either with PregSure BVD (Pfizer Animal Health), Bovilis BVD (Intervet), Bovidec (Novartis Animal Health) or Mucobovin/Vacoviron (Merial) according to the manufacturers' recommendations (Table 1). A group with nine calves received saline as a control. All animals were seronegative for BVDV at the start of the study (day 0). The animals were co-mingled and randomly allocated to treatment groups. Clinical observations were made by a veterinarian on days 0, 7 and 28 within 2 h of vaccination. General health was observed at least once daily throughout the study period by an experienced stock person.

On study day 49 (21 days after completion of immunisation), all animals were challenged with 50% tissue culture infectious doses ($TCID_{50}$) per calf of the European non-cytopathic BVDV-1 strain 11249 (provided by the Institute for Animal Health, Compton, UK) and were examined within 4 h after challenge to check for potential adverse effects. Experimental procedures were conducted in compliance with the Act on Experimental Animals in The Netherlands (Dutch government licence No. 40100) and regulated by an Ethical Review Committee from the Centre of Veterinary Investigation, Lelystad, The Netherlands.

Collection of serum samples

Blood samples were collected for testing in the serum neutralisation test (SNT) and by ELISA on days 0 or 7 prior to first vaccination and on days 28, 35, 49 (21 days after completion of the vaccination course, prior to challenge), 56 and 63 (14 days after challenge). Blood samples were allowed to clot and then centrifuged at approximately 2000 *g* for at least 10 min. Aliquots of serum were stored frozen at −20 ± 5 °C until required for further testing.

Serum neutralisation test

Serum samples from group T01 (saline; Table 1) taken up to and including the day of challenge were tested for the presence or absence of BVDV neutralising antibodies by SNT using serum dilutions of 1/4 and 1/8. Only samples with SNT titres $\geqslant 4$ were titrated further for neutralising antibodies. Samples from all remaining groups (T02, T03, T04, T05) and all samples taken after challenge virus administration were titrated for BVDV neutralising antibodies using serial two-fold dilutions in cell culture medium starting at 1/4.

All serum samples were heat inactivated at 56 °C for approximately 30 min. Titres of neutralising antibodies against BVDV type 1 were determined by incubation with the cytopathogenic reference BVDV type 1a strain Oregon (30–300 $TCID_{50}$ per well). Both a positive and a negative reference serum were included in each assay. The virus–serum mixtures (two replicates per serum) were incubated for approximately 60 min, then EBTr cells were added and the plates incubated for 5 ± 1 days at 37 °C with 5% CO_2 in a humidified environment. BVDV was detected using peroxidase staining (Smith et al., 1988). Serum neutralising antibody titres were calculated using the Spearman–Karber method and expressed on a $\log_2$ scale.

BVDV antibody ELISA kits

The following commercial blocking ELISA kits were used for detection of antibodies against NS3 (p80): Ceditest BVDV (Cedi Diagnostics B.V.; Ceditest), Pourquier ELISA BVD/MD/BD P80 Antibodies Screening (Institute Pourquier; Pourquier), SerELISA BVD p80 Ab Mono Blocking (Synbiotics Europe; SerELISA), Bio-X BVDV ELISA Kit (Bio-X Diagnostics; Bio-X), BVDV-Ab (Cypress Diagnostics, Cypress), Ingezim BVD Compac (Ingenesa; Ingezim) and BVD/BD p80 Blocking One Step LSI Kit (Laboratoire Service International; LSI).

The following commercial indirect ELISA kits were used to detect BVDV antibodies by indirect ELISA: HerdCheck BVDV-Ab (IDEXX Laboratories; HerdCheck) and Svanovir BVDV-Ab (Svanova Veterinary Diagnostics; Svanovir). The plates of both ELISA kits were coated with whole BVDV-1 particles.

Statistical analysis

The individual animal was regarded as the experimental unit for each treatment group. All statistical testing was two-sided, using the 5% significance level ($P \leqslant 0.05$). Serological response (BVDV neutralising antibody titres on a $\log_2$ scale) to vaccination and challenge with BVDV was summarised by treatment group and analysed with a linear mixed model for repeated measures (with fixed effects for treatment, day and their interaction and random effects for block and animal). If the overall treatment effect or the interaction between treatment and day was significant, a priori contrasts were used to compare treatments at each post-treatment day (pre- and post-challenge data were analysed separately).

Each treatment was compared to the saline control (T01) and PregSure BVD (T02) was compared to each positive control. Least square (LS) means were back-transformed to give geometric means, which were plotted over time for each treatment. Serological response to vaccination and challenge with BVDV in the different ELISA kits tested was summarised by treatment group.

Results

General health observations

Forty-seven calves were reported as abnormal in the period before challenge (before day 49). Reported abnormalities included signs of upper respiratory tract disease (cough, nasal and ocular discharge), signs of lower respiratory tract disease (increased respiratory rate, harsh lung sounds), locomotion disorders (lameness, interdigital dermatitis, panaritium, joint swelling), diarrhoea, depression and fever. Twenty-two calves received medication during this period. Eleven calves were treated for respiratory tract disease with florfenicol (Nuflor, Schering-Plough Animal Health):

Table 1
Design of the vaccination trial.

Treatment	Group	*n*	Test article administration			Challenge[a]		Blood collection[b] (day)
			Dosage (mL)	Regime	Route	Volume (mL)	Study day	
Saline	T01	9	4	Day 7, 28	SC	2	49	Day 7, 28, 35, 49, 63
PregSure BVD	T02	9	2	Day 7, 28	SC	2	49	Day 7, 28, 35, 49, 63
Bovilis BVD	T03	10	2	Day 0, 28	IM	2	49	Day 0, 28, 35, 49, 63
Bovidec	T04	9	4	Day 7, 28	SC	2	49	Day 7, 28, 35, 49, 63
Mucobovin/	T05[c]	10	2	Day 0	SC	2	49	Day 0, 28, 35, 49, 63
Vacoviron			2	Day 28	IM			

n, Number of animals; SC, subcutaneous; IM, intramuscular.
[a] Every animal received a challenge dose of $10^{5.3}$ $TCID_{50}$ European non-cytopathic BVDV type 1a strain.
[b] Prior to vaccination or challenge, where applicable.
[c] Mucobovin was administered to Group T05 at day 0 followed by Vacoviron administered at day 28.