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Surveillance of Infectious Bovine Rhinotracheitis in marker-vaccinated dairy herds: Application of a recombinant gE ELISA on bulk milk samples

Elvira Muratore[a], Luigi Bertolotti[a,*], Chiara Nogarol[a], Claudio Caruso[b], Laura Lucchese[c], Bryan Iotti[a], Dario Ariello[d], Angela Moresco[e], Loretta Masoero[b,1], Stefano Nardelli[c,1], Sergio Rosati[a,1]

[a] Department of Veterinary Science, University of Torino, Italy
[b] Istituto Zooprofilattico Sperimentale di Piemonte Liguria e Valle d'Aosta, Torino, Italy
[c] Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
[d] Azienda Sanitaria Locale TO3, Torino, Italy
[e] Azienda Provinciale Servizi sanitari, Trento, Italy

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ABSTRACT

Infectious Bovine Rhinotracheitis (IBR) occurs worldwide, requiring significant resources for eradication programs or surveillance purposes. The status of infection is usually detected by serological methods using the virus neutralization test (VNT) or enzyme-linked immunosorbent assay (ELISA) on individual sera. The gE DIVA (Differentiating Infected from Vaccinated Animals) vaccines approach, adopted in order to reduce the virus circulation and prevent clinical signs, have tightened the range of available methods for the serological diagnosis. Different gE blocking ELISA could be performed to detect specific antibodies in sera of infected or whole virus-vaccinated animals but with less sensitivity if applied to bulk milk samples, especially in marker-vaccinated herds. A new rec-gE ELISA was recently developed in Italy and applied with good performances on blood serum samples. The present paper focuses on the application of a rapid protocol for purification/concentration of immunoglobulin G (IgG) from bulk milk and on the use of the new rec-gE indirect ELISA. The study involved three different partners and 225 herds (12,800 lactating cows) with different official IBR diagnostic statuses. The diagnostic specificity of the method was demonstrated closed to 100% while the diagnostic sensitivity was strictly related to the herd-seroprevalence. Considering 2.5% as the limit of detection of within-herd seropositivity prevalence, the diagnostic sensitivity showed by the proposed method was equal to 100%. A single reactivation of a whole strain vaccine in an old cow was detected inside a group of 67 lactating cows, showing the field applicability of the method.

1. Introduction

Infectious Bovine Rhinotracheitis (IBR) is a widespread animal disease caused by Bovine Herpesvirus (BoHV-1) type 1, with a substantial impact on animal health and livestock productivity (Raaperi et al., 2014). Upon primary infection, BoHV-1 replicates in the mucous membranes of either the respiratory or the genital tract. From there, it will gain access to local sensory neurons for establishment of latency in the corresponding ganglia, reactivating itself in the presence of stress, immunosuppression or treatments with corticosteroids (Ackermann and Engels, 2006; Winkler et al., 2000).

Abbreviations: IBR, Infectious Bovine Rhinotracheitis; DIVA, differentiating infected from vaccinated animals; Ig, immunoglobulins; BM, bulk milk; PC, purified/concentrated; PC-BM, purified/concentrated bulk milk.

* Corresponding author at: Department of Veterinary Science, University of Torino, Italy Largo Paolo Braccini, 2 10095, Grugliasco, Torino, Italy.
E-mail addresses: elvira.muratore@unito.it (E. Muratore), luigi.bertolotti@unito.it (L. Bertolotti), chiara.nogarol@unito.it (C. Nogarol), claudio.caruso@izsto.it (C. Caruso), llucchese@izsvenezie.it (L. Lucchese), bryan.iotti@unito.it (B. Iotti), dariello@aslto3.piemonte.it (D. Ariello), angela.moresco@apss.tn.it (A. Moresco), loretta.masoero@izsto.it (L. Masoero), snardelli@izsvenezie.it (S. Nardelli), sergio.rosati@unito.it (S. Rosati).
[1] These authors contributed equally to this work.

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DIVA-vaccines, based on BoHV-1 glycoprotein E (gE) deleted strain, have been reported to be a safe and effective strategy against IBR that can be successfully used in countries with high prevalence of infection (Kaashoek et al., 1995).

Currently, different diagnostic tools are suitable for the surveillance of BoHV-1 infection. Evidences of viral circulations can be detected using the gold standard virus neutralization test (VNT), or indirect ELISAs based on whole-virus antigens. Diagnostically, specific ELISA protocols must be performed in herds where marker vaccines are used, to correctly differentiate between infected and vaccinated animals. Because gE deleted vaccines represent the most commonly used approach (Mars et al., 2001; Rijsewijk et al., 1999), serological investigations are based on the combination of a whole-virus-based indirect ELISA or a glycoprotein B (gB) based competitive ELISA with a gE –based competitive ELISA. An indirect ELISA test was recently developed based on the identification of anti BoHV1-gE antibodies (Bertolotti et al., 2015). In all previously mentioned cases the diagnostic approaches are based on blood serum and on the individual testing of the whole herds.

Bulk milk (BM) can represent a readily available sample matrix for screening tests. Indeed, the BM sampling procedure is non-invasive for the animals, avoiding the negative effects, such as stress, that an invasive blood sampling can cause (Reber et al., 2012); moreover, the lower expenses for farm visits, blood sampling procedures and necessary materials identify it as a valuable and inexpensive sampling method (Reber et al., 2012).

In Countries or regions where IBR eradication has already been achieved, surveillance can be easily performed by indirect ELISA test on BM samples. Unfortunately, if BM samples are tested by competitive gE ELISA when vaccination is still ongoing, IBR diagnosis can feature limited sensitivity (Kramps et al., 2004). Even if individual milk was shown to be a reliable biological matrix for diagnosis of BoHV-1 (Schroeder et al., 2012), blocking ELISAs can prove unable to detect the seroconversion caused by an IBR wild strain infecting a vaccinated herd because of the required dilution of the samples and the lower concentration of antibodies in milk. Some methods for improving the sensitivity of ELISA tests were recently proposed (Schroeder et al., 2012) based on the concentration of immunoglobulins from the milk.

In this paper we describe a cost-effective approach for IBR surveillance in vaccinated herds. Immunoglobulins (Ig) in the BM samples are purified, concentrated and subsequently tested with a new indirect ELISA test based on the reactivity against the BoHV-1 gE (Bertolotti et al., 2015). In particular, the recombinant indirect gE ELISA was used in association with a rapid protocol for the concentration and purification of IgG from BM samples. The aim of this study was the evaluation of the performances of this new serological method on samples collected from three Italian Provinces (Turin, Cuneo and Trento).

2. Materials and methods

2.1. Purification and concentration of bulk milk IgG

A protein G (pG) based affinity matrix was prepared following a standard recombinant approach. Briefly, the Streptococcal pG gene subunit, lacking the albumin-binding region, was cloned into pGEX-6His, expressed as GST/pG fusion protein and purified under native condition by immobilized metal ion affinity chromatography (Smith and Johnson, 1988). The purified protein was covalently bound to agarose beads using low density ABT glyoxal resin (Agarose Bead Technologies MADRID, Spain) following manufacturer's recommendation and at 6 mg of GST/pG per ml of settled gel ratio. Following bond stabilization, the affinity matrix was washed with PBS and stored as 50% slurry in 25 mM phosphate buffer (pH 7.0) 20% ethanol at 4 °C.

The purification and concentration protocol was applied to each BM adapting an immunoprecipitation protocol, with minor modification (Harlow and Lane, 1988). Briefly, 10 ml of BM were subjected to rennet-based casein precipitation. After curd breaking and 5 min of incubation on ice, the sample was centrifuged at 3600*g* for 10 min at 4 °C. Following the separation from lipids and curd, about 6 ml of milk whey were decanted into a new tube and incubated with 100 μl of 50% affinity matrix for 10 min at room temperature under gentle agitation. The adsorbed matrix was centrifuged and the pellet was loaded into a mini spin column. The matrix was washed twice (Tween 0.05%, EDTA 0.5 mM, NaCl 160 mM) and the IgG were eluted in 200 μl of 0.1 M glycine buffer pH 2.8 onto a collection tube preloaded with 1/10 vol of Tris 1 M, pH 8.2. A Bradford quantification assay was performed to confirm presence of at least 1.5 mg/ml of IgG in the purified/concentrated (PC) eluted sample, using Bovine Gamma Globulin (BIORAD Quick Start) standard curve. Only samples with a sufficient IgG concentration have been considered suitable for testing in the indirect ELISA. Moreover, the effectiveness of purification was initially confirmed in a consistent subset of samples by SDS-PAGE.

2.2. Indirect gE ELISA

In a previous work, we developed and validated an indirect ELISA assay based on the reactivity of bovine sera against BoHV-1 glycoprotein E (gE) expressed in recombinant form. Briefly the ectodomain of gE was cloned into a mammalian expression vector and expressed as secreted protein in protein free medium as described (Bertolotti et al., 2013). The test showed very good performances, especially in terms of specificity. Compared to the original protocol, the ELISA assay was slightly modified and adapted to milk samples. Briefly, each sample, represented by purified/concentrated bulk milk (PC-BM) was diluted 1:2 directly in plate in PBS 1.25% casein and placed into two adjacent wells, the first coated with the recombinant BoHV-1 gE while the second one with a negative antigen. The plates were incubated at room temperature for 60 min. After four washing cycles, a peroxidase labeled secondary antibody, diluted at 10 ng/ml in PBS 1.25% casein, was added to each well. After 45 min of incubation at room temperature, four washing cycles were performed before the addition of the substrate solution (3,3′,5,5′-tetramethylbenzidine, TMB). The reaction was stopped with 0.2 M H_2SO_4 after 15 min at room temperature, and the absorbance value determined at 450 nm. The net reactivity (net optical density, or OD) was calculated for each sample as the difference between the absorbance of the gE and negative antigen wells. The results were expressed as the percentage of reactivity against the net OD of the positive control included in each plate.

Considering that samples represent a pool of individual milks, we modified the cut-off of the test compared to the previous work as suggested (Böttcher et al., 2012). The samples with a reactivity percentage greater than 40% were classified as positive, those with reactivity percentage between 30% and 40% as doubtful, and those with a reactivity percentage lower than 30% were considered negative.

2.3. Evaluation of analytical and diagnostic performances

A selection of 7 individual milk samples belonging to IBR-positive farms was used to evaluate the efficacy of IgG purification/concentration method in rec-gE ELISA protocol. The purified/concentrated milk samples were obtained as described above. Twofold dilution of each sample was tested by rec-gE ELISA and compared to the reactivity of the corresponding untreated