Veterinary Microbiology 180 (2015) 180–185

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Serological detection of infection dynamics for respiratory viruses among dairy calves

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ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 17 August 2015
Accepted 27 August 2015

Keywords:
Respiratory viruses
Calves
BRSV
PI-3
BHV-1
BVDV

ABSTRACT

The aim of this study is to reveal infection dynamics of bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (PI-3), bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine adenovirus type 3 (BAV-3) and bovine coronavirus (BCoV), which are important viral pathogens of respiratory disease complex in ruminants. Through such an analysis, the regression period of maternally derived antibodies and optimum vaccination time in calves can be recommended. A total of 10 farms were grouped as large (4)-, medium (2)- and small (4)- sized enterprises according to their animal population. Newborn calves (*n*: 94) delivered during a calendar month on the farms were studied. Blood samples were collected from these calves during their 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th months of age. Blood samples were also taken from their dams during the first sampling. Neutralizing antibody titers were detected using the serum neutralization test (SN_{50}). New PI-3 and BVDV infections at the early stages of life were determined in the calves. Maternal antibodies began to decrease in the 2nd month for BRSV, BHV-1 and BAV-3 (97.8%, 25.5% and 91.4%) and in the 3rd month for PI-3, BVDV and BCoV (85.1%, 67% and 93.6%). It was concluded that maternal antibodies begin to decrease after the 1st month and that the possible first exposure of calves to respiratory viruses is after the 2nd month. Therefore, it is recommended that the first vaccination program including prime and booster doses can be applied between 2 and 4 months of age. Furthermore, re-vaccination of animals at 6 months after the booster dose is also suggested.

1. Introduction

Bovine respiratory disease (BRD) is a common health problem in cattle herds (Gulliksen et al., 2009b), and BRD can be classified as the most important welfare health problem in calves. Financial losses related to BRD are due to animal mortality, body weight loss, yield loss, veterinary fees and medical cost. Although respiratory disease can be found in cattle of all ages, during the decline of passive immunity, weaning time and entrance into the feedlot, calves are most susceptible due to the intensive stress and exposure to pathogens (Muggli-Cockett et al., 1992). On a Dutch dairy farm where 60% of the heifers younger than 3 months old were infected, €31.2 in economic losses were estimated per heifer due to pneumonia. In the same study, it was shown that the calculated losses for one seasonal outbreak were €27.0 per heifer up to 15 months old (Van Der Fels-Klerx et al., 2001). Furthermore, the estimated annual economic loss due to respiratory tract infections is over $600 million in the USA (Smith, 2000). The contribution of BRD to the total morbidity cases in the USA was estimated to be approximately 75% and was similarly reported to be 50–70% of all feedlot deaths (Edwards, 1996; Galyean et al., 1999; Loneragan et al., 2001). The spread of BRD infections among young populations is extremely rapid: 91% of calves can be infected with BRD within the first 27 days after arrival to a feedlot (Buhman et al., 2000). An insufficient level of maternally derived antibodies (Kimman et al., 1988) and adrenocortical-immunosuppressive factors, i.e., stress, crowded barns or bad shelter conditions, can lead to an increased incidence of infection in calves (Gulliksen et al., 2009b).

One or more viral agents as well as some bacteria have been reported in the etiology of BRD (Härtel et al., 2004). The most important viral agents in BRD cases are bovine respiratory syncytial virus (BRSV), bovine herpesvirus type 1 (BHV-1), bovine parainfluenza virus type 3 (PI-3), bovine viral diarrhea virus (BVDV) and bovine adenovirus serotype 1-2-3 and 7 (BAV-1, BAV-2, BAV-3, BAV-7) (Härtel et al., 2004; Hägglund et al., 2006; Autio et al., 2007; Gulliksen et al., 2009a). Bovine rhinovirus, bovine coronavirus and bovine reovirus serotype 3 have occasionally been

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http://dx.doi.org/10.1016/j.vetmic.2015.08.025
0378-1135/

isolated (Kurogi et al., 1976; Richer et al., 1988; Decaro et al., 2008). In addition to *Mycoplasma bovis, Mannheimia haemolytica, Histophilus somni* and *Pasteurella multocida* can accompany these viral agents, with the prognosis of clinical illness becoming dramatically worse (Härtel et al., 2004; Dabo et al., 2008; Rice et al., 2008).

As it is commonly accepted that the primary pathogens of BRD are viral agents, the immunity gap (i.e., the time lapse between the regression period of maternally antibodies and the formation of antibodies by vaccination or between vaccination and clinical protection) becomes even more important in BRD. BVD viruses can persist in the cattle population through persistently infected individuals (IPI) (Mcclurkin et al., 1984), and BHV-1 may create lifelong latency after primary infection (Ackermann et al., 1982); it was also reported that BRSV and BCoV may persist within herds (Heckert et al., 1991; Valarcher et al., 2001). Virus clearance between outbreaks (Alenius et al., 1991; Elvander, 1996) and the re-introduction of new viral strains (Larsen et al., 2000) has been reported, and the seasonal incidence of BRD cases is generally higher during autumn and winter (Stott et al., 1980).

The aim of this study is to reveal the infection dynamics of the most important viral agents involved in BRD and to determine the regression period of maternally derived antibodies and the optimum age for the first vaccination.

2. Materials and methods

2.1. Study area and farm visits

This study was carried out in three locations (Karacabey, Mustafakemalpaşa and Yenişehir) in the Bursa province of Turkey. Blood samples were collected from a total of 10 cattle herds. Depending on the animal density (total number of animals including calves, cows and heifers), the herds were classified as small-scale enterprises (total animal number <20), medium-scale enterprises (total animal number is between 20 and 100) and large-scale enterprises (total animal number >100) (Table 1). The farms were visited monthly accompanied by the farm's veterinarians. Blood samples were collected during the visits. The records for clinical cases in the herd between visits were supplied by the veterinarian, and these clinical cases were also sampled for laboratory diagnosis (data not shown).

2.2. Sampled animals, blood samples and records

All the animals studied at the 10 dairy cattle farms were the Holstein-Fresian breed and were either semi-intensively or closely managed (Table 1). Calves that were born in a one-month period (July 2010) on these farms were selected for this study and then followed for 12 months. Blood samples were collected for serological testing from 94 calves at their 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th months of age between June 2010 and July 2011. None of the calves were vaccinated against the examined viruses during the study.

Dams (*n*: 94) were also sampled at the first sampling period (June 2010) for the demonstration of immunologic status. The dams at the large- and medium-scale enterprises had been vaccinated with an inactivated complete virion vaccine against some of the examined viruses (Table 2). Vaccination programs applied for dams in the farms included first vaccination at 4th month of age and the booster dose at 6th month of age followed by rappel vaccination one year apart. Inactivated BCoV vaccine is applied to the dams 2 months before they give birth.

Blood samples were collected into vacutainer tubes by venipuncture. Serum was separated by centrifugation at 3000 rpm, +4 °C, for 10 min, inactivated in a water bath at 56 °C for 30 min and stored at −20 °C until testing.

During the monthly farm visits, data about the clinical status of the studied calves as well as other calves on the farms were also obtained from the farm veterinarians (Table 3) to follow the circulation of viruses in the herds.

2.3. Viruses and cell cultures

The sampled animals were tested for immunological status against BVDV, BHV-1, PI-3, BRSV, BAV-3 and BCoV. BHV-1 strain Cooper, PI-3 strain SF-4 and BAV serotype 3 were formerly obtained from Department of Virology at Ankara University Faculty of Veterinary Medicine, Turkey. BCoV strain Mebus was obtained from Pendik Veterinary Control and Research Institute, Istanbul, Turkey. BVDV strain NADL and Atue strain of BRSV were obtained from Institute for Virology at Justus-Liebig University Faculty of Veterinary Medicine, Giessen-Germany. The Madin-Derby bovine kidney (MDBK) cell line was used for virus propagation and serum neutralization tests. Dulbecco's MEM supplemented with 10% fetal calf serum (FCS) was used for the cell cultures. Furthermore, the cell line and FCS were tested for the absence of pestivirus contamination throughout the study.

2.4. Detection of viral antibodies

In this study, a serum neutralization test (SN_{50}) was performed for the detection of viral antibodies, as described (Frey and Liess, 1971). A pre-dilution of serum samples, which is also accepted as the minimum positive titer values, at 1:2 for BHV-1 and BRSV, 1:5 for BVDV, PI-3 and BCoV, and 1:16 for BAV-3 were used.

For each serum sample, 2 parallel columns and 6 rows in 96-well microplates were used. To the first rows, 50 μl of pre-diluted sample was added, and twofold dilutions were prepared (1:2–1:64 for BRSV and BHV-1; 1:5–1:160 for PI-3, BVDV and BCoV; 1:16–1:512 for BAV-3). Then, an equal volume of 100$TCID_{50}$ diluted test virus was added. Two wells were used as virus controls (100 μl of the 100$TCID_{50}$ diluted virus) and other two as blanks (100 μl of DMEM). For BHV-1, two hours of incubation period

Table 1
Enterprises used for sampling and their management properties.

Enterprise no.	Region	Enterprise size	Number of animal in the farm	Enterprise type
Enterprise 1	Karacabey	Large	500–1000	Semi-intensive
Enterprise 2	Karacabey	Large	>1000	Semi-intensive
Enterprise 3	Karacabey	Large	>1000	Semi-intensive
Enterprise 4	Yenişehir	Large	500–1000	Semi-intensive
Enterprise 5	Mustafakemalpaşa	Medium	20–100	Semi-intensive
Enterprise 6	Mustafakemalpaşa	Medium	20–100	Semi-intensive
Enterprise 7	Mustafakemalpaşa	Small	<20	Semi-intensive
Enterprise 8	Mustafakemalpaşa	Small	<20	Close barn
Enterprise 9	Mustafakemalpaşa	Small	<20	Close barn
Enterprise 10	Mustafakemalpaşa	Small	<20	Close barn