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Original Article
Özgün Araştırma

Serological evaluation of precolostral Bovine Parainfluenza 3 Virus infection in an organised dairy herd*

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Abstract: Calves are born agamaglobulinemic due to syndesmochorial placenta features that prevent immunoglobulin transfer to the fetus from the dam. The presence of precolostral antibody (Ab) directly induces in-utero infection. The purpose of this study was to investigate Bovine Parainfluenza 3 virus (BPI3V) infection in precolostral calves and their dams. Samples were obtained from large-scale dairy cattle farm, which was established nearly 10 years ago. Blood serum samples were collected immediately after birth from precolostral calves (n=123) and their dams for about three-month period. Blood serum samples tested with Serum Neutralisation test using reference strain SF-4. BPI3V specific Ab was found in 119 of 123 dams (96.7%), and Ab titers showed a regular bell curve distribution's and peak points were at 1/20 and 1/40 interval. Out of all precolostral calves, 31 (25.2%) were Ab positive between 1/5 and 1/80 titer values. Average Ab titer level was higher in the dams of Ab positive calves as expected a result of recent infection history. The obtained data revealed that almost all the adults were exposed to BPI3V and one of the four pregnant had transplacental infections. Current veterinary practices are based on the prevention of diseases, which is an important point for intensive dairy breeding enterprises. Precolostral Ab controls can be used as a convenient tool for estimating and eliminating the risks of upcoming postpartum infections.

Key words: Bovine Parainfluenza Virus 3, Cattle, Precolostral, Serocontrol.

Bir organize sütçü işletmede Bovine Parainfluenza 3 Virus enfeksiyonunun prekolostral olarak serolojik değerlendirmesi

Özet: Buzağılar anneden yavruya immunoglobulin geçişini önleyen syndesmochorial plasenta yapısı nedeniyle agamaglobulinemik olarak doğarlar. Prekolostral antikor varlığı direkt olarak in-utero enfeksiyonu gösterir. Bu çalışmada prekolostral buzağılar ve annelerinde Bovine Parainfluenza 3 virus (BPI3V) enfeksiyonunu araştırmak amaçlanmıştır. Örnekler yaklaşık on yıl once kurulmuş büyük ölçekli bir çiftlikten elde edildi. Kan serum örnekleri yaklaşık 3 ay süresince, doğumdan hemen sonra prokolostral buzağılardan (n=123) ve annelerinden alındı. Kan serum örnekleri referens suş SF-4 kullanılarak yapılan Serum Nötralizasyon ile test edildi. BPI3V spesifik antikorlar 123 annenin 119'unda (%96.7) tespit edildi ve antikor titrelerinin düzenli çan eğrisi gösterdiği ve 1/20 ile 1/40 aralığında pik yaptığı belirlendi. Prekolostral buzağıların ise 31'inde (%25.2) 1/5 ile 1/80 aralığında antikor titreleri belirlendi. Antikor pozitif buzağıların annelerinde ise beklendiği gibi yakın zamanda geçirilmiş enfeksiyona bağlı olarak daha yüksek olduğu görüldü. Elde edilen verilere göre neredeyse tüm ineklerin BPI3V'a maruz kaldığı ve her dört gebe ineğin birinin transplasental enfeksiyon geçirdiği anlaşıldı. Geçerli Veteriner uygulamalarının temeli, özellikle sütçü intensif yetiştiricilik açısından önemli olan koruyucu hekimliğe dayanır. Prekolostral antikor kontrolleri yakın gelecekte karşılaşılabilecek postpartum enfeksiyon risklerini tahmin ve elimine etmek için kullanılabilecek yararlı bir yöntemdir.

Anahtar kelimeler: Bovine Parainfluenza Virus 3, Prekolostral, Serokontrol, Sığır.

Introduction

The duration of viremia is mainly concerned with the formation of specific antibodies (Abs) in all species. The embryo or fetus is usually not affected by infections if the dam has a protective Abs level in the blood. However, even a short period of viremia can result in an intrauterine infection in some cases. Clinical findings of prenatal infections may vary depending on the type of infectious agent and virulence. Syndesmochorial placenta features in the ruminants form a syncytium between the endometrium and the fetal trophectoderm. These structures

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create a barrier between maternal and fetal blood resources. As a result, no immunoglobulin can pass from the uterus to the fetus (Arthur 1996).

Ruminant embryos are completely defenceless to infections at the first trimester of pregnancy. Lytic type viral infections can causes death of the embryo. The outcome of infections that occur during the 90-110 days of gestation at the time of development of the immune system may vary (Kendrick 1971). Virulence of the agent is the main determinant in this stage. Possible consequences include; abortion, intrauterine growth retardation, premature birth, stillbirth, congenital anomalies and symptomatic or asymptomatic infection. So far, few viral, bacterial and parasitic infections have been studied in the precolostral phase (Bosch et al. 2000; Staubli et al. 2006; Schefers et al. 2008; Ozel and Gur 2015). The only bovine viral diarrhoea virus (BVDV) infection has been well researched in this respect due to its complicated pathogenesis (Kendrick 1971; Scherfers et al. 2008).

Bovine Parainfluenza-3 Virus (BPI3V) infection in cattle is associated with respiratory system disorders. The virus was first isolated in the USA (Reisinger 1959). Later, it was proved that the disease spread worldwide (Figueroa-Chavez et al. 2012). The agent is classified as *Paramyxovirus* of *Respirovirus* genus in the family *Paramyxoviridae* (Rima et al. 2019). Primer entry site is the upper respiratory mucosa. Proliferation of the virus preferably occurs in ciliated cells (Zhang et al. 2005). Fever, depression, cough, serous nasal and ocular discharge, anorexia, dyspnea, increased rate of respiration and breathing sounds are the main clinical signs (Bryson et al. 1989). Another name of the infection is shipping fever, since the immunosuppression caused by animal transport often results in activation of the virus. Fulton et al. (2000) detected 68% seroconversion after transportation of the calves, and detected antigen in 7 of 9 lung tissues. Experimental inoculations result in atelectasis, consolidation in the cranio-ventral aspects of the lungs, interlobular edema, bronchial/bronchiolar epithelial cell necrosis, syncytium formation, bronchial-alveolar epithelial cell hyperplasia and bronchiolitis obliterans (Tsai and Thomson 1975; Bryson et al. 1999).

BPI3 mostly occurred during the months from October to March (Stott et al. 1980). The mortality rate is very low, but can reach up to 10% in severe cases, and persistent pulmonary damage may remain in survivors (Thomas et al. 1978). BPI3 infection has been shown to lead to dysfunction of alveolar macrophages, which play an important role in bacterial opsonization (Brown and Ananaba, 1988). The most important aspect of BPI3V is to serve as an initiator to the development of secondary bacterial pneumonitis. The agent is one of the main factors of the bovine respiratory disease complex (BRD). BPI3 infection usually lasts with subclinically or mild disorders (Hodgins et al. 2002), if not complicated by other infectious agents. Severe cases have been reported in the presence of internal or external immunosuppressive factors (Snowder et al. 2006). Due to difficulties in the differential diagnosis of BRD, laboratory controls are a necessity in defining and challenging this economically important problem. Infection prevention studies are an important part of herd management. When an acute infection begins, viral antigen diagnosis cannot always be done quickly and accurately in field conditions. Antibody screening in adults will not be supply efficient data in order to make definitive assessments. We hypothesize that, sera-controls of precolostal calves may be a useful method to identify the recently circulating agents and to understand the actual contagious infection profile of the herd. These studies have been done for only a few infections.

The aim of this study is to assess the BPI3V infection in a closed system dairy breeding herd through serological controls of precolostral calves and their dams at the same time.

Materials and Methods

Materials

Sampled animals

In this study, samples were taken from a dairy cattle enterprise which was established nearly ten years ago in the Afyonkarahisar province, Central Anatolia. More than 1.500 animals in different age groups are breeding in a closed system intensive breeding management. The number of fertile cows was roughly 1.100. Immediately after birth, blood samples were taken simultaneously from the offspring (n=123) in the precolostral period, and simultaneously from their dams, over a period of nearly three months.

All the sampled animals were clinically normal during sampling. According to the farm's health records; Foot and Mouth Disease, *E. coli*, Rotavirus and Coronavirus vaccines were administered in the last two years before sampling. Except these infections, no any vaccination has been carried out for BPI3V or other prominent infections like BHV1 and BVDV.

It has been reported that no new animals have been taken to the farm since the day it was founded.

As learned from the farm records and veterinarians, the major health problems in the herd are diarrhea and pneumonia in calves, various reproductive problems and mastitis problem in cows have been increased especially in the last year. Blood samples were taken via vein-puncture from the jugular vein into Vacutainer tubes and transferred to the laboratory in cool chain, centrifuged at 3000 rpm for 10 minutes. Serum fractions were separated into stock tubes and stored at -20°C until the tests.

Cell culture

Madine Darby bovine kidney (MDBK) cell culture (ATCC, CCL-22) was used for virus propagation, titration and virus neutralization test. Cells were cultured in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 2-10% fetal calf serum (FCS).

Test virüs

BPI3V reference strain SF-4 ($10^{-3.7}$ $TCID_{50}$/0,1ml) kindly provided by Ankara University, Faculty of Veterinary Medicine, Department of Virology was used as a test virus. Tissue culture infective dose 50% ($TCID_{50}$) of the virus suspension was calculated by Spearman and Karber method (Osterkorn 1982).

Methods

Serum Neutralisation test

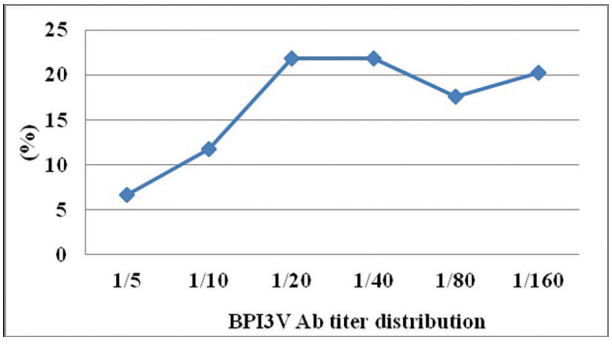
For the detection of BPI3V specific antibody presence, micro serum neutralisation test was carried out (Frey and Liess 1971). For this purpose, the blood samples were centrifuged at 3000 rpm for 10 min. Sera were separated, inactivated at 56 °C for 30 min and kept at -20 °C until analysis. Initially, all serum samples were diluted 1/5 with cell culture medium. Later on, 50 µl of the dilution was placed into two wells of the 96 well tissue culture plates with the same volume of virus suspension containing approximately 100 $TCID_{50}$ per 50 µl. After 1h of incubation, 50 µl cell suspensions (300.000 cells/ml) were added and incubated at 37 °C with 5% CO_2 conditions for one day. Test results were determined based on micromorphology of cells using an inverted microscope in subsequent two days.

All the Ab positive serum samples were diluted into1/5, 1/10...1/160 rates and re-tested to determine the Ab titer values.

Results

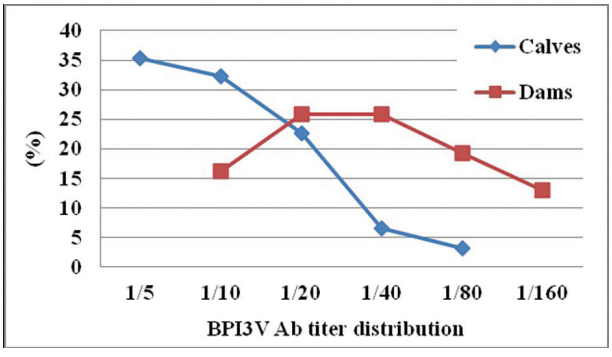
According to the test results, 1/5 dilution and above was accepted as seropositive. BPI3V specific Abs was found to be 119 of 123 dams (96.7%). As can be seen in figure 1, regular bell curve distribution's peak point was 1/20 and 1/40 interval. A slight increase was observed from 1/80 dilution point.

Figure 1. BPI3V Ab titer distribution of all dams



Out of their precolostral calves, 31 (25.2%) was Ab positive between 1/5 and 1/80 titer values. Positive calves and their dam's Ab titer values are shown in Figure 2.

Figure 2. BPI3V Ab titer distribution of precolostral calves and their dams



Discussion

BPI3V has worldwide spread (Frank 1992; Fulton 2009a).The infection was reported for the first time at 1971 in Turkey, 96% and 100% seropositivity detected in two cattle herds (Erhan et al. 1971). Subsequently, many studies were conducted from different regions across the country. Total negativity has not been reported in any study so far. In serological studies conducted in different parts of the country, these rates were reported; %50.63 in Central Anatolia (Ozturk and Yavru 1988), 88.2% in East and Southern East Anatolia (Cabalar and Can-Sahna 2000), 43% in Marmara (Yesilbag and Gungor 2008),

38.2% in West Anatolia (Erol et al. 2007), 52.7% in nine provinces all over the Turkey (Alkan et al. 1997).

BPI3V was serologically investigated in small scale private cattle herds throughout the Afyonkarahisar province recently. The seropositivity rate ranges from 58.8% to 97.5%, with a total of 1.279, 1.058 (82.7%) cattle exposed to the virus under field conditions (Gur 2018).

Considering the absence of vaccination for the BPI3V and no new animal introduction since the establishment, it was assessed that obtained data reflect natural infection (96.7%). This rate is extremely high even for an organized enterprise. However, the detection of high infection prevalence in the small private herds (82.7%) in the same localisation shortly before, makes reasonable of the obtained value (Gur 2018). The presence of low-level Abs in the majority of adults indicates previous exposure. However, an increase after 1/80 dilution reveals recent acute infection in a limited number of dams (Fig. 1). Precolostral serocontrols showed that 31 of 123 (25.2%) calves were exposed to the virus in-utero in the last five months of gestation. Precolostal Ab levels started at 1/5 and decreased rapidly to 1/40. Surprisingly, it was found to be in a high Ab titer (1/80) in a calf. Dams of positive calves generally have higher levels of Abs compared to other cows, as a result of recent infection, as expected (Fig. 2). According to test results, it would not be wrong to say that the virus is in circulation in the herd. In addition, the presence of Abs in almost all animals even at different levels, explains the absence of clinical disorders in both dams and in other offsprings fed from milk tank.

Passively induced immunity is assumed to be protected until 3 to 6 months of age (Kirkpatrick et al. 2001). In fact, the duration of maternal deprived protection is directly related to the level of antibodies taken from the colostrum and milk. It cannot be said that the maternal antibodies completely prevent the natural infection, but it is known to reduce the severity of the disease. Initial exposure to the virus may cause clinical disease, but subsequent exposures are subclinical because of the presence of secretory IgG in the blood serum for approximately one year. In addition, the presence of IgA in respiratory mucosal secretions provides protection from re-infections for about 2 months (Graham et al. 1999).

Bovine respiratory disease (BRD) has a global spread. Impaired phagocytic activity of alveolar macrophages during acute BPI3 infection (Liggitt et al. 1985, Slauson et al. 1987), and decrease in surfactant production due to viral replication in the alveolar type II cells (Tsai and Thomson 1975) are facilitates the development of seconder bacterial infection. BPI3V plays a significant role in this complex problem among many other viral and bacterial microorganisms. Besides BPI3V, well-known etiologic agents in cattle: *Bovine Herpesvirus type 1* (BHV1), *Bovine Respiratory Coronavirus* (BRCV), *Bovine Viral Diarrhea Virus* (BVDV), *Bovine Respiratory Syncytial Virus* (BRSV), *Bovine Adenovirus* (BAV), Mycoplasmas, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somnus*, *Mycoplasma bovis* and *Bibersteinia trehalosi* (Fulton et al. 2009b; Jericho et al. 1982; Griffin et al. 2010; Panciera and Confer 2010). Ghram et al. (1989) reported that prognosis is heavier in the dual BHV1 and PI3 infections.

Severity of BRD prognosis was determined by environmental factors, virus, bacteria and host interaction (Taylor et al. 2010). There are many different ways to fight infections. The strategy will be chosen depending on the agent characteristics and its pathogenetic pathway. In the meantime, the identification of infections in the current circulation has priority. Like BRD, it is difficult to distinguish many viruses that affect the alimentary and the genital system due to lack of any pathognomonic or specific clinical signs (Hodgins et al. 2002; Snowder et al. 2006; Murray et al. 2018). Precolostral controls may be preferred for the discriminate the etiological agents which carry true risk potential for the herd. It should be considered that, the presence of stress factors such as transport, seasonal change, malnutrition, heat imbalance, weaning, parasitism, hormonal fluctuations, etc. determines the clinical picture and mortality of BPI3V infection (Maillard et al. 2006; Snowder et al. 2006).

Morbidity tends to be quite high during outbreaks. Under normal conditions, it can be said that BPI3V is part of the respiratory tract flora considering viral shedding from asymptomatic animals and the emergence soon after transports (Hodgins et al. 2002; Murray et al. 2018). In an experimental study, Tsai and Thomson (1975) were demonstrated that the virus budding through the basal alveolar membrane and the presence of virus particles in the interstitial tissue, besides they detected the virions in the subepithelial area, suggested that persistent infection may develop in some hosts. It is understood that viral shedding may continue after acute infection. In addition, it was stated in the same study that the infection was seen without observable clinical signs in some calves.

There are many therapeutic options for bacterial infections but not for viral ones. As virulence and pathogenetic pathways of virus families are different from each other, distinct methods need to be used while applying control and eradication strategies. At this point, an exact definition of the causative agent is crucial. Viral diagnosis under field conditions can be cumbersome and requires great attention. Incorrect results are always possible during long processing. During pregnancy, especially in the last trimester, many viral agents including BPIV3 can cross the placenta and cause the formation of immunoglobin. The data that can be obtained from Ab presence and level controls in random samples will only provide rough estimates in adults. On the other hand, precolostral Ab controls are easy and reliable methode and directly reveals the infections that occured at the last five months of the gestation.

Holistic view of sustainable herd management is the backbone of intensive dairy enterprises, especially in countries that have a high presence of the infections with major veterinary importance. The absence of therapeutic options in viral infections makes it necessary to know currently circulated viruses. At this point, procolostral sero-controls could be recommended due to its high potential on the determining successful vaccination schedule.

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