Screening of Persistently Infected (PI) heifers and cows in a brazilian farm for BVDV type 2 infection

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Bovine Viral Diarrhea (BVD) has global economic importance due to the productive and reproductive losses caused by the disease. Non-cytopathic BVDV infection in immunosuppressed cows, between 40 and 142 days of gestation, results in 100% of fetal infection with a high risk of persistent infection. That is the main source of virus in the dairy herd.

The purpose of this case report is to describe the screening of PI animals realized between September 2015 to March 2017 in the largest dairy farm in Brazil localized in São Paulo state, latitude 22°21'34.12"S and longitude 47°23'40.17"W. The herd was composed of approximately 3,700 Holstein animals, of which 1,750 cows are lactating with an average daily production of 38.5 liters of milk per cow.

The past history of this herd includes the purchase of cows from Paraná state. Moreover, some heifers and cows were sent to an embryo transfer center also localized in the south of Brazil. The embryos from these animals were implanted in different receptors from properties that did not control BVDV. After birth, half of heifers from this partnership were sent back to São Paulo.

The calves were housed in individual suspended cages from birth to weaning (at 75 days of age). Subsequently, they were transferred to collective pens containing sand bed with access to pasture. Heifers were kept in confinement pasture with approximately 100 animals, under the same conditions until the seventh month of gestation. In the eighth month of pregnancy, heifers and dry cows moved to the maternity pen with a cross-ventilation system with a capacity of 200 females. After calving, lactating cows were housed in an intensive cross-ventilation barn, subdivided into six pens consisting of approximately 290 animals.

The births were assisted and calves were separated immediately after calving and transferred to individual pens. Five Liters of colostrum were fed twice during the first 12 hours after birth. The immunological quality of colostrum was estimated using colostrometer (≥ 50 g/L of immunoglobulin). Vaccination of calves started at 60 days of life, with booster 30 days after the first dose of a commercial vaccine, followed by biannual revaccinations realized in April and October. In the past, commercial vaccines in Brazil were composed of inactivated strains of BVDV type 1. In 2012, the first commercial

vaccine containing both inactivated BVDV genotypes was introduced in Brazil.

Despite the vaccination protocol, the BVDV concern on this farm started in 2012 due to high occurrence of Bovine Respiratory Disease (BRD) in calves characterized by an interstitial bronchopneumonia. Between 2013 and 2014, the fetal infection for BVDV in this herd was confirmed by the detection of serum positive neonate calves before colostrum intake (7/52, 13.46%). Moreover we found 2/10 (20%) of positive RT-PCR for BVDV in transtracheal lavage from heifers calves manifesting bronchopneumonia. In 2015, a case of Mucosal Disease was detected in a young heifer. After these findings, the dairy cloused the herd and started PI screening.

For initial screening of calves, skin biopsy samples (ear notches) measuring 1 x 0.5 cm were obtained from the dorsal pinna margin of each calf using an stainless steel ear missing clamp type V pig, disinfected between calves (immerse in alcohol 70%). Ear notch biopsy samples were added in a sterile microtube, followed by freezing at -20ºC. Detection of BVDV antigen in ear notches was performed in a commercial laboratory that has used a commercially available Kit, according to the manufacturers instructions.

Screening of PI animals was realized in four steps. In the 1st step, 2,247 heifers and cows were tested by antigen-ELISA, including the last generation of animals, lactation cows that produced only male calves and cows without live descendants. In this step, it was possible to detect 34 infected animals distributed in the following age: nineteen animals from 1 up to 12 months of age, seven animals from 13 up to 24 months of age and eight animals from 25 up to 36 months of age. In the 2nd step, thirty animails were re-tested to differentiate between transient and persistent infection. Four animals were not re-tested because they died due to Bovine Respiratory Disease (BRD) or were culled. In this step, 19 animals were confirmed PI. In the 3rd step, all live dams of PIs (n=11) and dams of dead animals (n=4) also were tested. In this step, it was possible to detect four PIs dams with between 25 and 36 months of age. After the slaughter of the PI animals, the fourth step of PI screening was started. All newborn calves were tested monthly, detecting three PIs (3/103) births in the following two months after the last PI was detected in the previous step. So, the newborn screening was re-started looking at all calves born during the following nine months, no positive animals were detected. The genotype of BVDV found in this herd was type 2 using RT-PCR technique.

Probably, the vaccination protocol of this farm using a commercial vaccine composed only for BVDV type 1 was the gate for BVDV type-2 associated to the purchase and receipt of animals from south of Brazil. The PI screening was essential to decrease the rate of disease in calves and improve reprodutive rates. PI identification and vaccination are the key components of BVDV control.

Figure 1. Ear notches obtained from the dorsal pinna margin of calf using a stainless steel ear missing clamp type V pig.