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Short Communication

Characterization of colostrum from dams of BLV endemic dairy herds

Gerónimo Gutiérrez [1,*], Marina Lomonaco [1], Irene Alvarez, Fernando Fernandez, Karina Trono

Laboratorio de Virus Adventicios, Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, INTA, Nicolás Repetto y De los Reseros s/n, 1686 Hurlingham, Buenos Aires, Argentina

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ABSTRACT

Bovine Leukemia Virus (BLV) is endemic in Argentina, where the individual prevalence is higher than 80% in dairy farms. The aim of this work was to find preliminary evidence to know if the high level of infection of the dam would implicate a higher challenge to her own offspring. We collected 65 sets of samples consisting of dam's blood and colostrum from two heavily infected dairy farms, and investigated the correlation between the dam's blood proviral load and the presence of provirus in colostrum. We also described the dual antibody/provirus profile in the colostrum. Provirus was detected in 69.23% of the colostrum samples, mostly from dams with a high proviral load, 36/45 (80%). Colostrum proviral load was significantly higher in dams with high blood proviral load ($p < 0.0001$). Provirus was detected in colostrum samples all along the antibody distribution, even in those with a low amount of antibodies. These results show that even when high blood proviral load dams offer higher levels of infected cells to their offspring through colostrum they also offer higher levels of protection of antibodies. On the contrary, low blood proviral load dams also offer infected cells but a poor content of antibodies, suggesting that these animals could play an important role in the epidemiological cycle of transmission.

1. Introduction

Bovine Leukemia Virus (BLV) is endemic in Argentina, where the individual prevalence is higher than 80% in dairy farms of the main productive areas of the country (Gutiérrez et al., 2012). In the herds of these farms, about 10% of the calves are born infected, and blood proviral load reaches high levels (more than 1% of white blood cells infected) in more than 40% of infected animals (Lomonaco et al., 2014). In this work we investigated the correlation between the dam's blood proviral load and the presence of provirus in colostrum, in two heavily infected dairy farms. Concurrently, we described the dual antibody/provirus profile in the colostrum of naturally infected dams. Even when the effect of consuming colostrum from infected dams is still a matter of controversy, the aim of this work was to find preliminary evidence to know if the high level of infection of the dam would implicate a higher challenge to their own offspring.

2. Materials and methods

2.1. Farms and samples

The study was carried out using samples from 2 commercial dairy farms naturally infected with BLV, coded

* Corresponding author. Tel.: +54 11 4621 9050; fax: +54 11 4621 9050.
E-mail addresses: gutierrez.geronimo@inta.gob.ar (G. Gutiérrez), lomonaco.marina@inta.gob.ar (M. Lomonaco), alvarez.irene@inta.gob.ar (I. Alvarez), fernandez.fernando@inta.gob.ar (F. Fernandez), trono.karina@inta.gob.ar (K. Trono).

[1] These authors contributed equally to this work.

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as farm A and B (86.5% and 89.3% of individual prevalence, respectively). Both farms had typical Holstein dairy herds, with about 350 milking cows each. We collected 65 sets of samples (39 from Farm A and 26 from Farm B) including whole blood and colostrum from dams at the peripartum period. Farm owners' consent was obtained before animal sampling. The procedures followed for extraction and handling of samples were approved by the Institutional Committee for Care and Use of Experimental Animals of the National Institute of Agricultural Technology (CICUAE-INTA) under protocol number 35/2010, and followed the guidelines described in the institutional Manual.

2.2. Antibody testing

Plasma antibodies against the whole BLV viral particle were detected by ELISA as previously reported (Trono et al., 2001). The antibody titers were assayed by the end-point dilution method using two-fold dilutions of sera. Titers were expressed as the reciprocal of the dilution.

2.3. BLV detection and proviral load quantification

Total DNA was extracted from whole blood using a DNA extraction kit (High Pure PCR Template Preparation kit, Roche, Penzberg, Germany) according to the manufacturer's instructions. BLV proviral DNA was detected by nested PCR (Wu et al., 2003), and the relative quantification of the proviral load (PVL) was assessed as described by Lew et al. (2004) using the SYBR Green Detection technology. All samples were tested in duplicate by using 50 ng of DNA as template. A fragment of the BLV pol gene was amplified together with a fragment of the constitutive 18 S gene used as reference. As calibrator for both reactions, we used 50 ng of DNA from fetal lamb kidney (FLK) cells, containing four copies of BLV proviral DNA per cell (Van den Broeke et al., 2001), in a final concentration of 1% in peripheral blood mononuclear cells (PBMCs) purified from a non-infected cow, according to Hopkins and DiGiacomo (1997) for aleukemic animals. The relative PVL was expressed as the ratio obtained by the sample for the BLV gene in comparison to the 18 S reference gene, based on the efficiency and the cycle threshold deviation from the internal control sample (Pfaffl, 2001). With this method, the relative PVL (ratio) of the calibrator sample was set to 1 and all samples were referred to it. Samples showing a ratio ≥ 1 were considered with high PVL while samples showing a ratio <1 were considered with low PVL according to our own criteria. The reaction showed a limit of detection of 1 BLV-infected cell in 10,000 non-infected cells.

3. Results

We quantified BLV-specific antibodies and proviral load in 65 sets of samples, each set of samples including blood and colostrum from BLV-infected dams. Provirus was detected in 69.23% of the colostrum samples (Fig. 1). Provirus-positive colostrum samples were mostly from dams with a high blood proviral load, 36/45 (80%) (Fig. 1). Of these cows, the vast majority 92.3% (36/39) presented provirus-positive colostrum (Fig. 1). In addition, colostrum proviral load was significantly higher in dams with high blood proviral load ($p < 0.0001$). The amount of provirus in colostrum was always below a ratio of 0.22 (Fig. 2), more than 100-fold lower in mean than proviral load in blood. Colostrum samples were categorized as Low (≤ 16), Medium (32–256) or High (≥ 512), depending on the level of antibodies; each category representing 21.54%, 61.54% and 16.92% of the samples, respectively (Fig. 3). Provirus was detected in colostrum samples all along the antibody distribution, even in those with a low amount of antibodies. The prevalence of this detection was 21.43%, 77.5% and 100% for categories low, medium and high, respectively (Fig. 3). Colostrum proviral load was significantly lower in samples from the low category. Medium and high antibody categories showed the lowest difference in proviral load ($p = 0.048$) (Fig. 3).

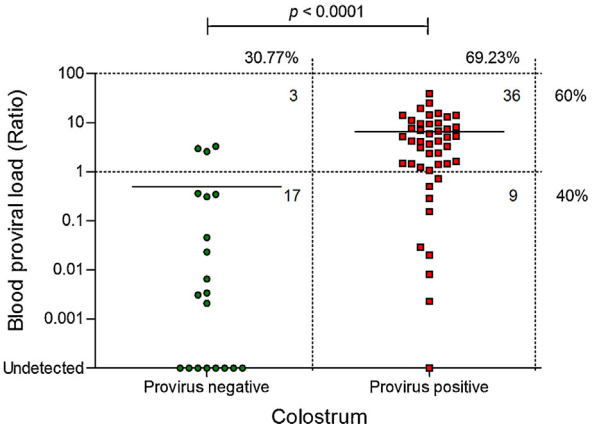

Fig. 1. Provirus-positive colostrum samples were mostly from dams with a high blood proviral load. Dams' blood proviral loads were plotted according to the detection (or not) of provirus in their respective colostrum sample. Means were significantly different ($p < 0.0001$, Mann–Whitney test).

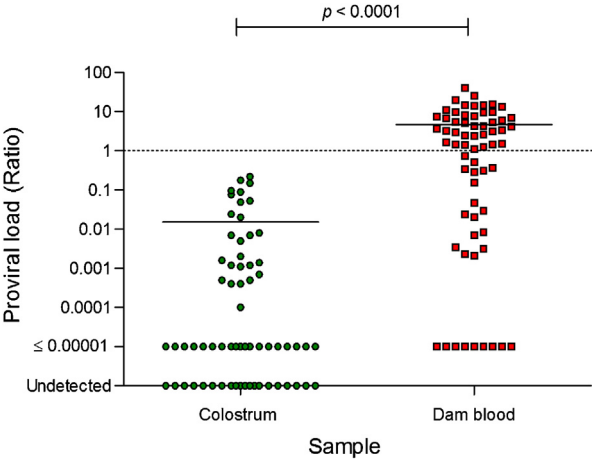

Fig. 2. Blood proviral load was significantly higher than colostrum proviral load. Proviral loads in dam's blood and in their respective colostrum samples were plotted. Means were significantly different ($p < 0.0001$, Wilcoxon matched pairs test).

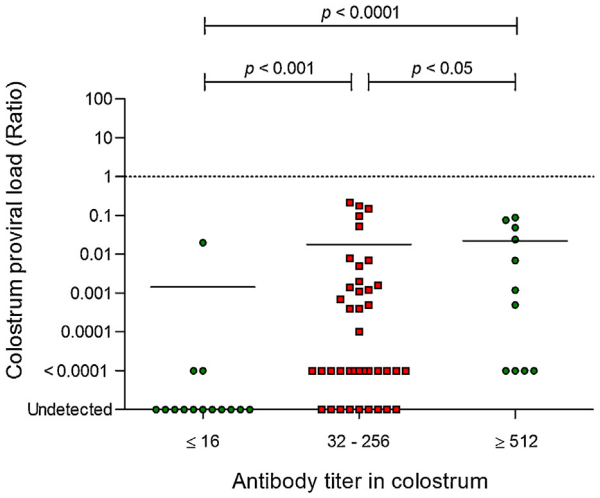

Fig. 3. Provirus was detected in colostrum samples all along the antibody distribution. Colostrum samples were categorized as Low (≤16), Medium (32–256) or High (≥512), depending on the level of antibodies. Colostrum proviral loads were plotted according to these antibody titer categories. Means were significantly different (Mann–Whitney test).

4. Discussion

The effect of consumption of colostrum within a BLV control strategy is still a matter of discussion between farmers of dairy herds. During the 1980s some works were conducted that demonstrated the infective and also the protective potential of colostrum from infected cows under experimental conditions (Ferrer and Piper, 1981; Van Der Maaten et al., 1981; Lassauzet et al., 1989). Later, some groups published that the consumption of colostrum was a risk factor for BLV within-herd transmission (Sprecher et al., 1991; Meas et al., 2002). Nonetheless, other epidemiological studies have shown the reduction of BLV incidence in concordance with the consumption of colostrum from BLV positive dams, interpreting the feeding of colostrum to newborn calves as a protective factor (Nagy et al., 2007; Kobayashi et al., 2010). Thus, the role of colostrum on natural transmission is still unclear. In this work, we describe the dual profile of antibodies and provirus in colostrum from naturally infected dams, and the relationship of this profile with the level of infection in dams' blood.

The most interesting finding was that even when the dams with high blood proviral load showed a higher prevalence of provirus-positive samples (Fig. 1) and a higher proviral load in colostrum, the provirus was detectable in all categories of colostrum samples, even in those with a low amount of antibodies (Fig. 3). If the colostrum carries viable cells with provirus and shows poor antibody content, it could be a vehicle for transmission, as with those shown in Fig. 3 categorized as Low or Medium, that count for more than 80% of the studied samples. Moreover, when bloody colostrum is fed to newborn calves, as often occurs in the dairy system, the consequences could be even worse, since the offer of infected blood cells would be even higher. In this context, the susceptibility to infection could be modified not only by the presence, but also by the amount of antibodies and provirus. The final result of the colostrum consumption is unpredictable, and would depend on the dual antibody/provirus profile, together with the duration of antibodies in the calf, and also of the immediate future challenge posed by the raw milk and the commingled infected newborn calves. A well-designed dose-response trial could be modeled using these data as the base line to build experimental groups that receive different antibody/provirus amounts in colostrum, and that should be followed up to analyze the future incidence of infection. The positive correlation found between the dams' amount of antibodies in blood and colostrum (data not shown) suggest that newborn calves could be supplemented with colostrum from a bank made with secretions of highly infected dams to provoke a higher level of passive immunology. The critical point should be the use of cell-deprived, frozen or heat inactivated secretions to render them non-infectious as previously reported (Baumgartener et al., 1976; Rubino and Donham, 1984; Kanno et al., 2014).

These results suggest that colostrum can constitute a vehicle for transmission, as reported for HTLV, a similar complex Retrovirus that infects humans (Kinoshita et al., 1984). The use of fresh raw colostrum from low proviral load dams is thus not as good as we previously supposed, at least until the protective/infective dose of colostrum is estimated. We can speculate that the consumption of colostrum with infected-cells and a poor content of antibodies could play a critical role in BLV propagation during young age. Van Der Maaten et al. (1981) showed that 10^6 mononuclear cells from an infected cow can infect a susceptible newborn calf by the oral route, but cannot do so in the presence of antibodies.

We consider that colostrum and milk could play an important role in the epidemiological cycle of transmission and should not be unattended, especially during the first two months of age when, after natural intake of colostrum from their dams, the calves are fed with raw bulk tank milk from the dairy. The use of colostrum supplementation from a pasteurized or frozen bank made with secretions from highly infected dams to finally provoke a high antibody titer in the calf could be a good alternative to resist a future challenge. Finally, conversely to blood borne BLV transmission, we can presume that dams with low proviral load could be those that pose the highest risk of transmission by feeding colostrum or milk to their own offspring.

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