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BRIEF REPORT

Bovine leukemia virus becomes established in dairy herds before the first lactation

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Abstract In this work, we studied seven groups of pregnant heifers from a consortium of dairy farms heavily infected with bovine leukemia virus (BLV). ELISA testing showed that the seroprevalence ranges of BLV in heifers between 36.1 and 66.5 %. No significant differences in proviral load were found when comparing heifers with adult cattle. Before their first delivery, more than 9.8 % of heifers show a high proviral load. Because BLV infection can occur during the first two years of life, the rationale of any strategy should be to take action as early as possible after birth.

Keywords BLV · Heifers · Seroprevalence · Proviral load

Bovine leukemia virus infection is underdiagnosed on most dairy farms in the Americas and numerous other locations where large amounts of milk are produced. With no official national plans to stop transmission in most of these countries, endemicity has reached high levels during the last decades. Lactating cows in highly productive areas of Argentina show a mean prevalence of around 90 % [1], and high rates have also been reported in the United States and Canada [2, 3]. Even when most BLV-infected animals show no evident symptoms, dairy farms are adversely impacted by unregistered lost profits because of deaths due to lymphosarcoma in 5-10 % of lactating cows. A recent economic study done in Argentina with the aim of analysing the feasibility of developing an intervention strategy against BLV showed that fatal lymphosarcoma results in an economic cost of 5000 USD per dead cow [4]. This amount results from (i) a lack of milk production, (ii) a reduction in the number of calves born and, therefore, fewer heifers, and (iii) the inability to sell infected animals after the end of lactation when cows are sent to the slaughterhouse. Moreover, some authors have reported that subclinical BLV infection is a risk factor for lower milk production [3, 5]. Nevertheless, this impact is extremely difficult to demonstrate under field conditions with the prevalence as high as it currently is.

Our epidemiological studies show that BLV initiates its spread within the dairy herd by targeting young animals. About 10 % of calves from heavily infected farms are already infected at birth, and their high proviral loads represent a risk of infection for other calves [6]. The infection rates gradually increase up to more than 80 % in adult cows during the second lactation, resulting in efficient virus transmission [7].

With the aim of determining the incidence of infection in dairy herds in Argentina, we studied the prevalence of infection in seven groups of pregnant heifers from a consortium of heavily infected dairy farms. We analysed the antibody levels in all groups, as well as the blood BLV proviral load of two subgroups of seropositive heifers before they entered the milking herd, and compared the

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proviral loads of the latter group with those of adult cows from one milking herd.

We investigated BLV infection in pregnant heifers between the ages of 20 to 27 months from this group of heavily infected dairy farms. These farms handle newborn calves from different dairy farms in a facility to which they are moved after intake of colostrum from their dams. The calves are kept outdoors, individually tethered, for a period of sixty days, receiving fresh bulk tank milk from the nearest dairy facility of the consortium (with high prevalence of infection, as previously shown [1]) and dry feed. After 60 days, calves are moved to the field with other growing heifers. Insemination at 15-18 months of age takes places at this same location, and animals are only moved to the final dairy facility after their first delivery. Hence, animals do not share farmlands with adult milking cows until after their first delivery.

ELISA testing [8] of seven groups (718 samples) showed that the seroprevalence of BLV in heifers ranged between 36.1 and 66.5 % (Fig. 1A) before they entered the milking herd. These data are similar to those previously shown for this age category [9]. BLV blood proviral loads of two subgroups of seropositive heifers (group 6, n = 22, and group 7, n = 46) were measured by real-time PCR [7]. No significant differences in proviral load were found when comparing heifers with adult cattle, not only from the same dairy consortium (n = 146, Fig. 1B) but also from others that were analysed previously (data not shown). Before the first delivery, more than 9.8 % of heifers showed a high proviral load, with more than 1 % of peripheral white blood cells infected, suggesting a very early occurrence of primary infection and clonal expansion [10]. A cutoff point for high and low proviral load was established previously using published data, our own criteria, and samples from reference infected and uninfected animals [7].

The results indicate that BLV infection can progress during the first two years of life, when young animals are still not in contact with adult cows. At this stage, the only potential sources of virus are their own mothers, bulk tank milk, and calves that were born infected, which reached 8.3 % of the population in a recent study [9]. This finding is in line with our previous studies that suggest that colostrum and milk from cows with high proviral load could be the primary source of infection [7, 11]. Detection can overlap with the presence of antibodies during the first year of life, also suggesting an arrest of infected cells from the dam in a latency-like state, without viral expression. Later on, without any obstacles to progress, BLV infection can spread rapidly to susceptible animals in the herd.

Once infection is established in dairy herds, BLV is extremely difficult to eradicate using standard control strategies. Segregation and/or elimination of infected animals is economically and even socially unsustainable, despite data showing the adverse effect on public health of consumption of milk or meat from infected animals [12]. There is still no therapy against persistent BLV infection, although leukemia can be cured with lysine deacetylase inhibitors [13].

The central question is how to inhibit the spread of BLV within a productive farm system that seems to foster (rather than slow down) virus transmission. In this context, we recently proposed a vaccine strategy based on the use of an attenuated BLV strain. The vaccine induces an efficient immune response but shows only limited ability to replicate and be transmitted *in vivo*. A trial is currently ongoing to evaluate the efficacy of this strategy to stop natural virus

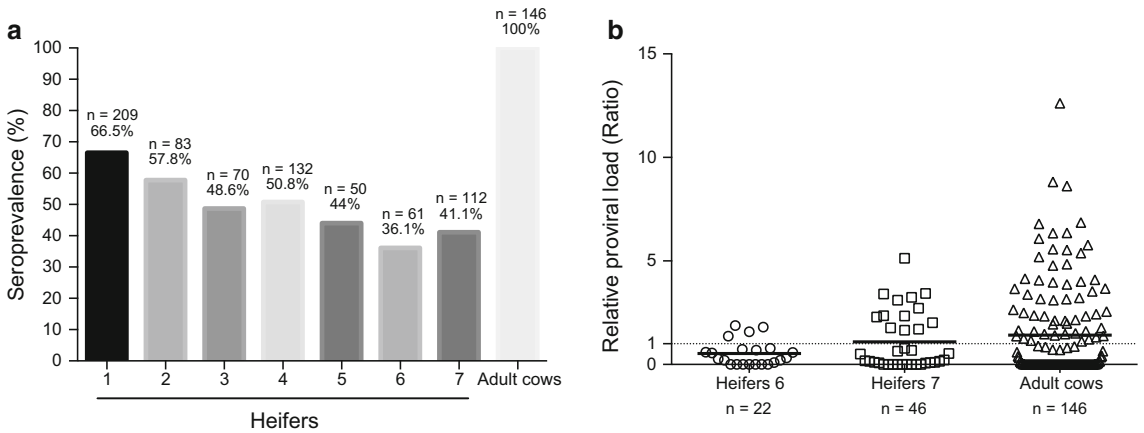

Fig. 1 BLV seroprevalence (a) and proviral load distribution (b) in heifers and adult cows. a) Seven groups of 20 to 27-month-old heifers and one group of adult cows from a consortium of dairy herds were analysed for the presence of anti-BLV antibodies using an ELISA test. b) The BLV proviral load in seropositive animals from two out of seven groups of heifers (groups 6 and 7) was measured and compared with the proviral load of seropositive adult cows. No significant differences were found according to the Mann-Whitney test. A ratio of 1 is the cutoff point that arbitrarily discriminates between high and low proviral loads. n, number of animals in each category

challenges and to eradicate BLV infection [14]. Considering the rapid progression of infection during the first years of life, our report shows that this strategy should be undertaken as early as possible after birth. This could become an effective alternative to deal with a problem that not only affects cattle health but also is relevant for public health and food safety.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The procedures 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.

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