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Short communication

Presence of Gumprecht shadows (smudge cells) in bovine leukemia virus-positive cattle

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A B S T R A C T

Enzootic Bovine Leukosis is a chronic disease caused by the bovine leukemia virus (BLV). Smudge cells, also known as Gumprecht shadows, are not simple artifacts of slide preparation, but ragged lymphoid cells found mainly in peripheral blood smears from human patients with chronic lymphocytic leukemia. In this study, we report the presence of Gumprecht shadows in peripheral blood from BLV-positive cattle.

Enzootic Bovine Leukosis (EBL) is a chronic disease caused by the bovine leukemia virus (BLV). BLV belongs to the Retroviridae family (genus Deltaretrovirus) and is distributed widely in cattle populations of many countries. The BLV genome consists of two identical RNA molecules that are transcribed to a double-stranded DNA by the reverse transcriptase enzyme and then integrated into the genome of B-lymphocytes as proviruses. Approximately 30% of infected cattle develop persistent lymphocytosis, 0.1–5% may develop tumors that invariably lead to death, and about 65–70% remain as asymptomatic seropositive carriers in an aleukemic state (Lagarias and Radke, 1989; Miller et al., 1969). Smudge cells, also known as Gumprecht shadows (Gumprecht, 1896), are ragged lymphoid cells found mainly in peripheral blood smears from human patients with chronic lymphocytic leukemia. Smudge cells are not simple artifacts of slide preparations (Gumprecht, 1896; Macdonald et al., 2003) and there is no correlation between the absolute lymphocyte count and the percentage of these cells (Heinivaara, 1959). This anomaly in lymphocytes caused by ruptured B cells is seen in routine blood smears from virtually all chronic lymphocytic leukemia patients. Recently, smudge formation has been found to be related to the content of the cytoskeletal protein vimentin present in leukemic cells (Nowakowski et al., 2009). Vimentin is an intermediate filamentous protein critical for lymphocyte rigidity and integrity (Brown et al., 2001). Because there are no reports regarding Gumprecht shadows in cattle, the goal of this work was to describe the presence of these anomalies in blood samples from BLV-positive cattle.

Blood samples with EDTA were collected from six bovines in an Argentine farm to evaluate the presence of Gumprecht shadows. Genomic DNA was extracted from 1 ml of blood by using a commercial kit (DNA Purification Kit, Promega, WI, USA). DNA concentrations were determined using a Nano Spectrophotometer Vue (GE Healthcare, Freiburg, Germany). To confirm the seropositive cattle and search for the presence of the provirus, a nested PCR of a segment of the BLV gp51 was performed by using the following primers: env 5032 5′-TCTGTGCCAAGTCTCCCAGATA-3′ -forward-, env 5608r: 5′-AACAACAACCTCTGGGAA-3′ -reverse-, resulting in the amplification of a 598-bp fragment and env 5099 5′-CCCACAAGGGCGGCGCCGGTTT-3′ -forward-, and env 5521r: 5′-GCGAGGCCGGGTCCAGAGCTGG-3′ -reverse- resulting in a 444-bp fragment. The primers used and the PCR conditions followed Licursi et al. (2003). Blood smears of the six samples were prepared from EDTA anticoagulated blood. A drop of blood was pulled along a slide to ensure smear uniformity. Each sample was stained using May-Grunwald-Giemsa (Merck, Darmstadt, Germany) and analyzed by optical microscopy to determine its morphological features. Slides were prepared by the same technician specialist in hematology of the Hematology Laboratory at the Faculty of Veterinary Sciences of the University of La Plata (Buenos Aires, Argentina).

To determine BLV-positive cattle, the cattle sampled were analyzed by nested PCR. Four of them were BLV-positive and the other

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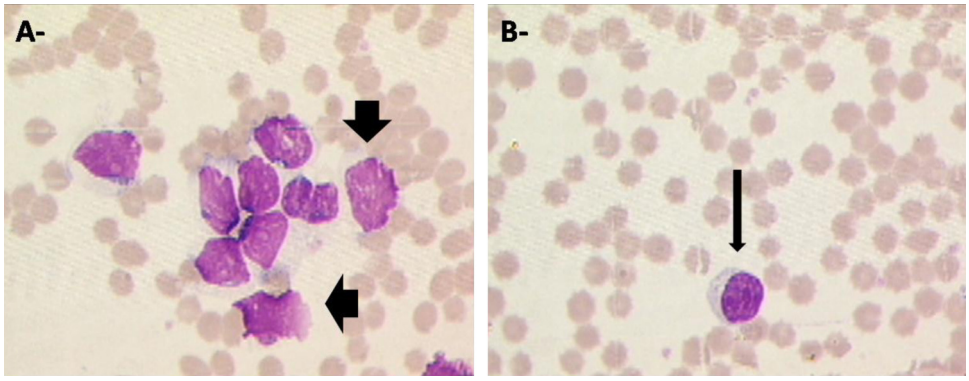

Fig. 1. (A) Smudge cells (broad arrows) on peripheral-blood smears from BLV-positive cattle stained with May-Grunwald-Giemsa. (B) Normal lymphocyte in BLV-negative cattle (narrow arrow).

Table 1
White blood cell count and percentage of smudge cells in the six bovines analyzed.

Bovine number	PCR-BLV	Leukocyte (μl)	Lymphocyte (μl)	Smudge cells (%)
1	Positive	38,000	25,460	11
2	Positive	6400	3456	5
3	Positive	8100	5022	8
4	Positive	11,900	5355	6
5	Negative	16,300	7982	0
6	Negative	4900	2548	0

two were BLV-negative. Total white blood cells, lymphocytes and percentage of smudge cells are indicated in Table 1.

Smudge cells or Gumprecht shadows were found only in the peripheral blood from BLV-positive cattle (Fig. 1). As expected, these morphological features were not found in BLV-negative cattle. The presence of smudge cells in peripheral-blood smears from chronic lymphocytic leukemia patients is a constant feature in B cells (Nowakowski et al., 2009). In the case of cattle, the cell type in which this anomaly occurs has not yet been determined, but it is probably that the cell type also corresponds to B cells as in humans (Heinivaara, 1959). Furthermore, we cannot yet affirm whether this anomaly is present in the peripheral blood of all BLV-positive cattle, because that would require greater sampling. Both the cell types involved and the number of BLV-positive cattle with this anomaly will be the focus of future research. We postulate that, as reported in humans by other authors, Grumprecht shadows or smudge cells also occur in bovines infected with BLV.

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