Reviewer's report

Title: BOVINE LEUKEMIA VIRUS DISCOVERED IN HUMAN BLOOD

Version: 0 Date: 09 Nov 2018

Reviewer: Maria Carolina Ceriani

Reviewer's report:

To the authors, I highly appreciate your contribution to this study. The manuscript aimed to determine the presence of BLV in human blood. BLV as a δ-retrovirus is closely related to the human T-lymphotropic virus type 1 (HTLV-1) and both known as direct oncoviruses. Detection of BLV in human blood would be of great importance and may help improve preventive strategies, and to induce governments to implement measures in an effort to eradicate the virus mostly from those herds that are heavily infected.

However, there are some major and minor comments I want to address.

Lane 33: you seem to be convinced that BLV might lead to leukemia in humans, but all the reported studies in the literature are only referred to the association of BLV and the development of breast cancer. Do you have any certainty of this fact? Have you ever made any test? I strongly recommend for publishing to include some testing of human lymphomas to see if you can detect BLV.

Lane 52: reference [4] does not mention that mammary epithelial cells exfoliate into milk.

Lane 55: you mention three different research groups, but with the exception of the paper from Mesa and col, the other groups have one author in common, which could mean that they are not really different groups.

Lane 84: I don’t believe PBMC are viable after 14 days in the refrigerator. Not more than 2-3 days is recommended. You don’t show in the results the percentage of hemolysis, but after 14 days it is expected that the blood should be completely hemolysed.

In Results section, you mention the frequency of amplification for one, two or three genes, but you amplified four genes. Did you have any sample that amplified all the four tested genes simultaneously?

Lane 139: "...it the most highly conserved region and has the highest degree of sequence variation." Doesn’t it sound like a discrepancy? If it is highly conserved, it is expected to have very little variations.

Lane 162: Did you check all the positive blood samples for the mutation? Because later, you mention that env gene is usually deleted in clonal cells.
Lane 183-188: the primers sequences to amplify env gene, are included in Table 1. That means that env was amplified to check the sequence in several samples. How many of the tested samples were positive for env? Or it was deleted? The sequence of only two LTR is shown in the manuscript.

Lane 218: what do you mean by "well-matched for age".

Lane 223: if you isolate PBMC, and perform genomic DNA extraction, there is not doubt that the viral genome is integrated. You need to demonstrate that they can produce infective particles released to the supernatant, as PBMC from cows.

Lane 235: On what assumption can you speculate that human to human transmission is feasible?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

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