Introduction
The prognostic significance of the immuno-histochemical assessment of Ki-67 proliferation marker has been largely demonstrated in many tumor types; however, its importance for canine lymphoma is still debated.

Evaluation of Ki-67 by flow cytometry (FCM) in fine needle aspirates has recently shown prognostic relevance in canine high-grade B-cell lymphoma.

The aims of this preliminary study was to investigate the agreement between IHC and FCM in the assessment of Ki-67 index in canine lymphoma, in order to determine whether these techniques can be used interchangeably for the evaluation of proliferative activity.

Materials & Methods
Dogs referred with a previously untreated lymphoma at the Veterinary Hospitals of vetmedUni Vienna and of the University of Bologna were considered for inclusion in this prospective study. In these patients, the proportion of proliferating tumor cells (Ki-67 index) was assessed by FCM on fine-needle aspirates, and the dogs undergoing subsequent diagnostic lymphadenectomy/biopsy with histopathological evaluation were ultimately included. For each case, Ki-67 index was also assessed immunohistochemically on formalin-fixed and paraffin-embedded tissue sections. By FCM, 10x10⁶ cells were categorized. By IHC, a manual count was performed in five 400x fields, without knowledge of FCM results. With both methods, Ki-67 index was expressed as percentage of positive tumor cells.

Results
Twenty-nine dogs were included in the study. There were 11 males and 18 females; the mean age was 8 years (range, 4-11). Tumors were 26 peripheral nodal, 1 mediastinal, 1 indolent splenic and 1 intestinal lymphoma. According to the final histopathological diagnosis, B-cell lymphomas (n = 21) included 18 diffuse large B-cell lymphoma, 1 lymphoblastic, 1 nodal marginal zone lymphoma and 1 splenic marginal zone lymphoma; B-cell lymphomas (n = 8) included 4 peripheral T-cell lymphomas, 1 lymphoblastic, 1 enteric and 2 T-zone lymphomas (Table 1).

The median time elapsed between FCM and histology was 3.5 days. No therapy was administered in between.

The mean Ki-67 index was 51 ± 27% (range, 2-80%) with FCM and 49 ± 23% (range, 8-86%) with IHC (Figure 1).

According to the Bland-Altman plot, the bias between the techniques was 2% and 95% limits of agreement were -47% to 52%. Spearman’s correlation coefficient was 0.45 (P = 0.014; Figure 2).

Table 1 Sample characteristics, histological diagnosis and Ki-67 index assessed both by FCM and IHC in 29 cases of canine lymphoma.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Histology</th>
<th>Ki-67 FCM (%)</th>
<th>Ki-67 IHC (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>M</td>
<td>Lymph node</td>
<td>58.0</td>
<td>57.0</td>
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<tr>
<td>2</td>
<td>11</td>
<td>M</td>
<td>Lymph node</td>
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When stratifying cases according to the growth pattern (nodular vs. diffuse), a significant correlation in Ki-67 index between FCM and IHC was not observed for nodular lymphomas (marginal zone and T-zone).

Discussion and conclusions
The assessment of Ki-67 index by FCM in canine lymphomas may represent a valuable alternative to the IHC evaluation since - it is faster and non-invasive as it does not require a surgical procedure; - being an automated count, the operator-dependent bias is minimal; - it allows to assess concurrently the immunophenotype of the proliferating cells; - the number of analysed cells is consistently higher than the one assessed immunohistochemically.

Due to the above reasons, FCM might prove more accurate than IHC in the assessment of proliferative activity. Although these preliminary results indicate an overall agreement between the two methods, in a subset of cases the discrepancies were prominent and the correlation was modest. This may be due to the above method-related differences in the number of evaluated cells or to difficulties in limiting the assessment of proliferative activity to neoplastic cells in IHC sections. Notably, the lowest agreement was observed in nodular lymphomas, in which neoplastic cells are admixed with a relevant number of resident lymphoid cells. Further prospective comparative studies are warranted to assess the prognostic relevance of Ki-67-index evaluated by FCM and its potential advantages over IHC.

Table 1

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References

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