Internal Medicine

Abnormal clonalities of B-lymphocytes in bovine leukemia virus-infected cattle with persistent lymphocytosis

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RUNNING HEAD: B-CELL CLONALITY IN PL CATTLE
Abstract. Peripheral B-lymphocyte clonality of 274 bovine leukemia virus - infected cattle with lymphocytosis was analyzed using clonality PCR based on sequences of the variable region of the bovine immunoglobulin H chain. None of the cattle showed monoclonal proliferation, while 10, 31, and 233 showed minor-clonal, oligoclonal, and polyclonal proliferation, respectively. A total of 163 cattle were analyzable the following year, and lymphocytosis was maintained in 157, indicating persistent lymphocytosis (PL). B-lymphocyte clonality of the 157 PL cattle was minor-clonal in 6 (3.8 %), oligoclonal in 8 (5.1 %), and polyclonal in 143 (91.1 %). A higher rate of enzootic bovine leukosis (EBL) onset within a year was observed in PL cattle with minor-clonal (50.0 % (3/6)) and oligoclonal (25.0 % (2/8)) proliferation compared to those with polyclonal (5.6% (8/143)) proliferation. Minor-clonal and oligoclonal proliferation in PL cattle may be a prognosis factor for developing EBL.

Keywords: bovine leukemia virus, minor-clonal proliferation, oligoclonal proliferation, persistent lymphocytosis, prognosis factor
Enzootic bovine leukosis (EBL) is a disease caused by infection with bovine leukemia virus (BLV), and its incidence has recently been increasing in Japan [9, 10]. Most BLV-infected cattle are asymptomatic, but 30% of them progress to persistent lymphocytosis (PL), and 10% of those with PL develop EBL [2, 3]. Lymphocytes in EBL cattle are tumorigenic and show monoclonal proliferation, whereas lymphocytes in PL cattle are non-tumorigenic and show polyclonal proliferation [4, 6]. In humans, the oligoclonal proliferation phase is observed in patients with adult T-cell leukemia, which results from infection by human T-cell leukemia virus, a virus closely related to BLV [5]. We recently reported an oligoclonal lymphocyte proliferation phase in PL cattle [11]. However, the clinical significance of abnormal clonal proliferation in PL cattle remains unclear, and the percentage of clonal abnormalities in PL cattle caused by BLV infection has never been examined. The present study aimed to examine the clonality status of B-lymphocytes in PL cattle and observe its temporal changes.

A total of 274 blood samples taken from BLV antibody-positive cattle with lymphocytosis from 18 farms in Ibaraki Prefecture in 2018 were used. Of the 274 cattle, 168 were Holstein-Friesian (HF) and 106 were Japanese Black (JB). Criteria for lymphocytosis used in this study were the European Community’s leukosis key [8] for HF and the criteria of Akagami et al. [1] for JB (Table 1). DNA was extracted from peripheral blood of these 274 cattle using an automated nucleic acid extractor (Automate Express Nucleic Acid Extraction System, Thermo Fisher Scientific, South San Francisco, CA, USA) and stored at -30°C until clonality analysis. DNA samples from lymph nodes or blood of cattle diagnosed with lymphoma were used as positive controls. EDTA peripheral blood samples from 4 BLV-negative and PL-negative cattle were used as negative controls.

For B-lymphocyte clonality analysis, PCR was performed using the primers BoVHF1: 5’-AGC CCT GAA ATC CCG GCT CA-3’ / BoVHR1: 5’-TCC AGG AGT CCT TGG
CCC CA-3', which target the region containing the variable region of the bovine immunoglobulin H (IgH) chain [7]. PCR products were analyzed for clonality using a capillary electrophoresis device (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA) and a DNA 1000 Lab Chip kit (Agilent Technologies). Samples showing a single peak exceeding the height of the marker were designated as monoclonal (Fig. 1A). We defined “minor-clonal” as a sample that does not show a high peak like monoclonal, but has a peak that is larger than the maximum height of the polyclonal waveform and does not exceed the height of the marker (Fig. 1B). Those with one or more small peaks were designated as oligoclonal (Fig. 1C). Samples showing a waveform without a peak were designated as polyclonal (Fig.1D).

Proliferation in 274 samples from cattle with lymphocytosis in 2018 was minor-clonal in 10 (3.7%), oligoclonal in 31 (11.3%), and polyclonal in 233 (85.0%), with none showing monoclonal proliferation (Table 2). Of the 168 HF cattle, proliferation was minor-clonal in 7, oligoclonal in 18, and polyclonal in 143. Of the 106 JB cattle, proliferation was minor-clonal in 3, oligoclonal in 13, and polyclonal in 90.

Blood samples from the same cattle were continuously examined in 2019 to the extent possible. DNA were extracted from the peripheral blood of the PL cattle and analyzed for B-lymphocyte clonality with the same manner. When peripheral blood samples were not available due to death, slaughter, or trading in 2019, the presence or absence of EBL onset was confirmed for dead cattle that could be tracked by interviews with local veterinarians or meat inspection records. The prognosis (dead/alive and presence/absence of EBL) of cattle at the end of 2020 was also tracked by using the cow traceability system (https://www.id.nlbc.gp.jp) and/or interviews. Differences between HF and JB and the rate of EBL onset were analyzed using Pearson's chi-square test. Comparisons of cattle age and numbers of lymphocytes according to clonality were analyzed using the Kruskal-Wallis test. The
significance level was set to 5%.

In 2019, blood count and clonality analyses were possible for 163 cattle. Among these, 157 (109 HF and 48 JB) still had lymphocytosis and were considered PL cattle. Proliferation in the 157 PL cattle was minor-clonal in 6 (3.8 %), oligoclonal in 8 (5.1 %), and polyclonal in 143 (91.1 %), with none showing monoclonal proliferation (Table 2, 3 and supplemental Table 1). In the 109 HF cattle, proliferation was minor-clonal in 4, oligoclonal in 7, and polyclonal in 98. In the 48 JB cattle, proliferation was minor-clonal in 2, oligoclonal in 4, and polyclonal in 40. There was no significant difference in the ratio of clonal abnormalities between HF and JB, suggesting that breed-specific differences in clonality might not exist in PL cattle. Median ages of PL cattle with minor-clonal, oligoclonal, and polyclonal proliferation in 2019 were 8, 7, and 5 years, respectively (Table 3). Median ages of cattle with minor-clonal and oligoclonal proliferation were significantly older than that of cattle with polyclonal proliferation (p=0.0004). Median lymphocyte counts of PL cattle with minor-clonal, oligoclonal, and polyclonal proliferation in 2019 were 7,100, 7,800, and 8,000, respectively (Table 3 and supplemental Table 1), with no significant difference between the 3 groups. The remaining 6 cattle among the 163 showed normal lymphocyte counts in 2019.

A total of 59 cattle with lymphocytosis in 2018 were slaughtered in 2019; 2 cattle (6-year-old HF and 12-year-old JB) had EBL onset as determined by inspection at the slaughterhouse, and the remaining 57 were confirmed to be EBL-negative. Other 52 cattle were untraceable due to various reasons, such as death or trading with other farms.

We found that 8.9% of PL cattle showed clonal abnormalities – minor-clonal or oligoclonal proliferation. As the sensitivity of the PCR assay for IgH rearrangement used in the present study was reported to be 68.6% [7], approximately 30% of clonal abnormalities may have been overlooked. Thus, the actual number of PL cattle with minor-clonal or oligoclonal proliferation might be higher than the present results suggest.
Oligoclonal proliferation of lymphocytes is recognized as one type of onset in human ATL [5], and is also observed in human patients with chronic lymphocytic leukemia [12]. Thus, it is possible that cattle having oligoclonal proliferation may already have tumors, although direct evidence is lacking. In this study, minor-clonal proliferation was included as a category in addition to monoclonal and oligoclonal proliferation. Both minor-clonal and oligoclonal proliferation suggest the emergence of weak neoplastic clones. The only difference between minor-clonal and oligoclonal proliferation is the number of bands detected. The actual significance of minor-clonal proliferation of lymphocyte is not well understood [3]. Further studies will be needed with more cases of minor-clonal proliferation to better understand the clinical significance of this clonality category.

Among the 6 PL cattle with minor-clonal proliferation in 2019, 4, 1, and 1 cattle had minor-clonal, oligoclonal, and polyclonal proliferation in 2018, respectively (Table 3). A total of 3 cattle (2 HF and 1 JB) among 6 (50.0 %) with minor-clonal proliferation in 2019 were confirmed to have EBL in 2020 by inspection at the slaughterhouse, and the remaining 3 were alive at the end of 2020 (Table 3). Among the 8 PL cattle with oligoclonal proliferation in 2019, 1, 3, and 4 had minor-clonal, oligoclonal, and polyclonal proliferation in 2018, respectively (Table 3). Two cattle among 8 (25.0 %) with oligoclonal proliferation in 2019 were confirmed to have EBL in 2020 by inspection at the slaughterhouse (Table 3). Other 4 cattle were alive at the end of 2020 (Table 3). A total of 143 PL cattle showed polyclonal proliferation in 2019. Of these, 6 had shifted from oligoclonal proliferation, and the remaining 137 have remained polyclonal since 2018 (Supplemental Table). A total of 8 cattle (3 HF and 5 JB) among 137 (5.8 %) with polyclonal proliferation in 2019 were confirmed to have EBL in 2020 by inspection at the slaughterhouse (Supplemental Table).

Given the lack of data on lymphocyte counts of BLV-infected cattle before 2018, we could not determine whether lymphocytosis detected in 2018 was PL or not. However, our
findings suggest that peripheral B-lymphocyte clonality in PL cattle does not always progress in one direction. Rather, it can change reversibly. Clonality changed over time, and not all cattle showing oligoclonal or minor-clonal proliferation developed EBL. This change in clonality may be related to the immune function of cattle and tumorigenesis of B lymphocytes. It will be important to investigate in further detail the relationship between changes in clonality status of B-lymphocytes in PL cattle and EBL cattle in future studies.

With regard to EBL onset, 50.0% (3/6) of PL cattle with minor-clonal proliferation and 25.0% (2/8) with oligoclonal proliferation developed EBL in the following year. These percentages were significantly higher than that of PL cattle with polyclonal proliferation (5.6% (8/143)), suggesting that minor-clonal and oligoclonal status in PL cattle may be a prognosis factor for developing EBL. We also found that cattle with minor-clonal or oligoclonal proliferation were significantly older than those with polyclonal proliferation. This may be related to the high incidence of EBL in older cattle. A larger-scale study will be needed to clarify the significance of clonality abnormalities in PL cattle.

Conflicts of interest
The authors declare no conflict of interest.

Acknowledgements
This work was supported by JSPS KAKENHI Grant Numbers 20H03142 and 20J10567.

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| Breed | Age (years old) | Numbers of lymphocyte (/μl) |  |
|-------|----------------|-----------------------------|---|
|       |                | Normal | Suspected | Lymphocytosis |
|       | <1             | < 10,000 | 10,000 - 12,000 | > 12,000 |
|       | <2             | < 9,000 | 9,000 - 11,000 | > 11,000 |
| HF \textsuperscript{6)  | <3             | < 7,500 | 7,500 - 9,500 | > 9,500 |
|       | <4             | < 6,500 | 6,500 - 8,500 | > 8,500 |
|       | \geq 4         | < 5,000 | 5,000 - 7,000 | > 7,000 |
| JB \textsuperscript{7) | <1             | < 6,300 | | \geq 6,300 |
|       | \geq 1, < 2    | < 5,900 | | \geq 5,900 |
|       | \geq 2, < 3    | < 5,500 | | \geq 5,500 |
|       | \geq 3, < 6    | < 4,500 | | \geq 4,500 |
|       | \geq 6, < 10   | < 4,300 | | \geq 4,300 |
|       | \geq 10        | < 3,700 | | \geq 3,700 |

Table 1. Criteria of lymphocytosis used in this study.
Table 2. Time lapse analysis of clonality status in peripheral B-lymphocyte or prognosis of cattle with lymphocytosis in 2018 and with or without persistent lymphocytosis (PL) in 2019

| Clonality status | Number of cattle | Clonality status or prognosis | Number of cattle |
|------------------|------------------|------------------------------|-----------------|
| Monoclonal       | 0                | PL cattle                    | -               |
| PL cattle        |                  | Monoclonal                  | 0               |
| Minor-clonal     | 10               | PL cattle                    | 0               |
| Monoclonal       | 0                | Minor-clonal                | 4               |
| Minor-clonal     | 10               | Normal lymphocyte counts    | 0               |
| Oligoclinal      | 31               | EBL (Slaughtered)           | 0               |
| Oligoclinal      | 31               | Non-EBL (Slaughtered)       | 2               |
| Oligoclinal      | 31               | Untraceable                 | 3               |
| Total            | 10               | Total                        | 31              |
| PL cattle        |                  | Oligoclinal                 | 12              |
| Monoclonal       | 0                | EBL (Slaughtered)           | 2               |
| Minor-clonal     | 1                | Non-EBL (Slaughtered)       | 5               |
| Oligoclinal      | 3                | Untraceable                 | 6               |
| Total            | 31               | Total                        | 31              |
| Polyclonal       | 233              | PL cattle                    | 0               |
| Polyclonal       | 233              | Monoclonal                  | 0               |
| Polyclonal       | 233              | Minor-clonal                | 1               |
| Polyclonal       | 233              | Oligoclinal                 | 4               |
| Polyclonal       | 233              | Normal lymphocyte counts    | 4               |
| Polyclonal       | 233              | EBL (Slaughtered)           | 0               |
| Polyclonal       | 233              | Non-EBL (Slaughtered)       | 50              |
| Polyclonal       | 233              | Untraceable                 | 43              |
| Total            | 274              | Total                        | 274             |

EBL: enzootic bovine leukemia
Table 3. Time lapse analysis of lymphocyte numbers, B-lymphocytes clonality and prognosis of persistent lymphocytosis cattle with minor- and oligo-clonal proliferation in 2019.

| Cattle No. | Breed | Date in 2019 | Numbers of lymphocytes in 2018 (µL) | Clonality Status in 2018 | Numbers of lymphocytes in 2019 (µL) | Prognosis in 2020 |
|------------|-------|--------------|------------------------------------|-------------------------|------------------------------------|------------------|
| 1          | HF    | 7            | 9,700                              | Minor                   | 10,300                             | EBL (Slaughtered) |
| 2          | HF    | 8            | 5,500                              | Minor                   | 5,000                              | EBL (Slaughtered) |
| 3          | JB    | 12           | 9,300                              | Oligo                   | 9,300                              | EBL (Slaughtered) |
| 4          | HF    | 4            | 6,500                              | Minor                   | 7,100                              | Alive            |
| 5          | JB    | 14           | 5,000                              | Minor                   | 5,000                              | Alive            |
| 6          | HF    | 9            | 9,700                              | Poly                    | 5,600                              | Alive            |

Median: 8, 6,500 to 7,100

| Cattle No. | Breed | Date in 2019 | Numbers of lymphocytes in 2018 (µL) | Clonality Status in 2018 | Numbers of lymphocytes in 2019 (µL) | Prognosis in 2020 |
|------------|-------|--------------|------------------------------------|-------------------------|------------------------------------|------------------|
| 7          | JB    | 6            | 5,600                              | Oligo                   | 5,600                              | EBL (Slaughtered) |
| 8          | JB    | 15           | 5,000                              | Poly                    | 5,000                              | EBL (Slaughtered) |
| 9          | HF    | 9            | 11,500                             | Minor                   | 10,000                             | Alive            |
| 10         | HF    | 2            | 13,400                             | Oligo                   | 15,000                             | Alive            |
| 11         | HF    | 6            | 10,700                             | Poly                    | 14,900                             | Alive            |
| 12         | JB    | 7            | 6,000                              | Poly                    | 5,100                              | Alive            |
| 13         | HF    | 7            | 5,700                              | Oligo                   | 5,200                              | No EBL (Slaughtered) |
| 14         | JB    | 12           | 6,100                              | Poly                    | 4,500                              | No EBL (Slaughtered) |

Median: 7, 8,350 to 7,800

EBL: enzootic bovine leukosis, *: No lymphocytosis
Fig. 1. Representative patterns of (A) monoclonal, (B) minor-clonal, (C) oligoclonal, and (D) polyclonal B-lymphocyte proliferation by clonality PCR using a capillary electrophoresis device (Agilent 1000).
Supplemental Table 1. Time lapse analysis of lymphocyte numbers, B-lymphocytes clonality and prognosis of BLV infected cattle with polyclonal B-lymphocyte proliferation in 2019.

| Cattle No. | Breed | Age in 2019 | Numbers of lymphocytes in 2018 (μl) | Clonality Status in 2018 | Numbers of lymphocytes in 2019 (μl) | Prognosis in 2020 |
|-----------|-------|-------------|--------------------------------------|------------------------|--------------------------------------|-------------------|
| 15        | HF    | 6           | 9,600                                | Oligo                  | 8,600                                | EBL(Slaughtered)  |
| 16        | JB    | 5           | 6,700                                | Oligo                  | 6,300                                | EBL(Slaughtered)  |
| 17        | JB    | 5           | 5,100                                | Oligo                  | 5,100                                | EBL(Slaughtered)  |
| 18        | HF    | 8           | 6,000                                | Poly                   | 5,600                                | EBL(Slaughtered)  |
| 19        | HF    | 4           | 11,800                               | Poly                   | 9,400                                | EBL(Slaughtered)  |
| 20        | JB    | 4           | 5,700                                | Poly                   | 5,000                                | EBL(Slaughtered)  |
| 21        | JB    | 6           | 8,600                                | Poly                   | 4,300                                | EBL(Slaughtered)  |
| 22        | JB    | 2           | 6,400                                | Poly                   | 6,400                                | EBL(Slaughtered)  |
| 23        | HF    | 6           | 16,700                               | Oligo                  | 13,600                               | No EBL (Slaughtered) |
| 24        | HF    | 4           | 21,500                               | Oligo                  | 20,600                               | No EBL (Slaughtered) |
| 25        | JB    | 6           | 11,200                               | Oligo                  | 7,700                                | No EBL (Slaughtered) |
| 26        | HF    | 5           | 6,400                                | Poly                   | 6,500                                | No EBL (Slaughtered) |
| 27        | HF    | 6           | 9,300                                | Poly                   | 9,900                                | No EBL (Slaughtered) |
| 28        | HF    | 6           | 25,200                               | Poly                   | 19,600                               | No EBL (Slaughtered) |
| 29        | HF    | 5           | 7,300                                | Poly                   | 5,600                                | No EBL (Slaughtered) |
| 30        | HF    | 5           | 7,800                                | Poly                   | 8,300                                | No EBL (Slaughtered) |
| 31        | HF    | 5           | 9,400                                | Poly                   | 7,900                                | No EBL (Slaughtered) |
| 32        | HF    | 4           | 6,500                                | Poly                   | 8,500                                | No EBL (Slaughtered) |
| 33        | HF    | 5           | 19,100                               | Poly                   | 16,200                               | No EBL (Slaughtered) |
| 34        | HF    | 8           | 10,200                               | Poly                   | 10,100                               | No EBL (Slaughtered) |
| 35        | HF    | 7           | 8,300                                | Poly                   | 7,400                                | No EBL (Slaughtered) |
| 36        | HF    | 5           | 7,300                                | Poly                   | 5,000                                | No EBL (Slaughtered) |
| 37        | HF    | 5           | 11,100                               | Poly                   | 9,200                                | No EBL (Slaughtered) |
| 38        | HF    | 4           | 8,100                                | Poly                   | 8,000                                | No EBL (Slaughtered) |
| 39        | JB    | 12          | 7,000                                | Poly                   | 3,900                                | No EBL (Slaughtered) |
| 40        | JB    | 5           | 5,700                                | Poly                   | 5,100                                | No EBL (Slaughtered) |
| 41        | HF    | 8           | 9,000                                | Poly                   | 6,600                                | Unknown (Dead)    |
| 42        | HF    | 6           | 10,500                               | Poly                   | 11,200                               | Unknown (Dead)    |
| 43        | HF    | 6           | 9,000                                | Poly                   | 11,800                               | Unknown (Dead)    |
| 44        | HF    | 5           | 7,600                                | Poly                   | 8,000                                | Unknown (Dead)    |
| 45        | HF    | 5           | 10,800                               | Poly                   | 8,100                                | Unknown (Dead)    |
| 46        | HF    | 4           | 7,000                                | Poly                   | 7,200                                | Unknown (Dead)    |
| 47        | HF    | 7           | 10,400                               | Poly                   | 6,200                                | Unknown (Dead)    |
| 48        | HF    | 5           | 18,800                               | Poly                   | 13,900                               | Unknown (Dead)    |
| 49        | HF    | 4           | 12,500                               | Poly                   | 12,700                               | Unknown (Dead)    |
| 50        | HF    | 2           | 9,100                                | Poly                   | 14,300                               | Unknown (Dead)    |
| 51        | JB    | 5           | 5,600                                | Poly                   | 5,600                                | Unknown (Dead)    |
| 52        | HF    | 5           | 8,400                                | Oligo                  | 7,000                                | Alive             |
| 53        | HF    | 9           | 5,900                                | Oligo                  | 9,000                                | Alive             |
| 54        | HF    | 5           | 6,900                                | Oligo                  | 8,000                                | Alive             |
| 55        | JB    | 8           | 5,100                                | Oligo                  | 4,400                                | Alive             |
| 56        | JB    | 5           | 10,000                               | Oligo                  | 10,700                               | Alive             |
| 57        | JB    | 4           | 5,300                                | Oligo                  | 5,300                                | Alive             |
| 58        | HF    | 3           | 10,800                               | Poly                   | 12,700                               | Alive             |
| 59        | HF    | 6           | 14,500                               | Poly                   | 9,100                                | Alive             |
| 60        | HF    | 6           | 6,000                                | Poly                   | 10,800                               | Alive             |
| 61        | HF    | 5           | 8,300                                | Poly                   | 10,100                               | Alive             |
| 62        | HF    | 4           | 7,900                                | Poly                   | 7,400                                | Alive             |
| Id | Type | Width | Material | Length | Status |
|----|------|-------|----------|--------|--------|
| 63 | HF   | 4     | Poly     | 16,800 | Alive  |
| 64 | HF   | 4     | Poly     | 9,800  | Alive  |
| 65 | HF   | 3     | Poly     | 12,900 | Alive  |
| 66 | HF   | 5     | Poly     | 11,200 | Alive  |
| 67 | HF   | 7     | Poly     | 6,800  | Alive  |
| 68 | HF   | 6     | Poly     | 5,400  | Alive  |
| 69 | HF   | 6     | Poly     | 8,700  | Alive  |
| 70 | HF   | 6     | Poly     | 11,300 | Alive  |
| 71 | HF   | 4     | Poly     | 13,600 | Alive  |
| 72 | HF   | 4     | Poly     | 12,700 | Alive  |
| 73 | HF   | 4     | Poly     | 13,400 | Alive  |
| 74 | HF   | 4     | Poly     | 9,400  | Alive  |
| 75 | HF   | 3     | Poly     | 10,600 | Alive  |
| 76 | HF   | 4     | Poly     | 12,700 | Alive  |
| 77 | HF   | 4     | Poly     | 8,000  | Alive  |
| 78 | HF   | 5     | Poly     | 7,100  | Alive  |
| 79 | HF   | 5     | Poly     | 8,900  | Alive  |
| 80 | HF   | 5     | Poly     | 14,500 | Alive  |
| 81 | HF   | 5     | Poly     | 5,300  | Alive  |
| 82 | HF   | 3     | Poly     | 11,900 | Alive  |
| 83 | HF   | 3     | Poly     | 9,200  | Alive  |
| 84 | HF   | 4     | Poly     | 7,900  | Alive  |
| 85 | HF   | 4     | Poly     | 12,200 | Alive  |
| 86 | HF   | 4     | Poly     | 11,200 | Alive  |
| 87 | HF   | 2     | Poly     | 12,400 | Alive  |
| 88 | HF   | 2     | Poly     | 10,100 | Alive  |
| 89 | HF   | 6     | Poly     | 5,600  | Alive  |
| 90 | HF   | 12    | Poly     | 19,400 | Alive  |
| 91 | HF   | 6     | Poly     | 5,700  | Alive  |
| 92 | HF   | 6     | Poly     | 7,200  | Alive  |
| 93 | HF   | 5     | Poly     | 7,600  | Alive  |
| 94 | HF   | 4     | Poly     | 6,600  | Alive  |
| 95 | HF   | 4     | Poly     | 12,500 | Alive  |
| 96 | HF   | 3     | Poly     | 9,300  | Alive  |
| 97 | HF   | 8     | Poly     | 14,600 | Alive  |
| 98 | HF   | 6     | Poly     | 12,600 | Alive  |
| 99 | HF   | 6     | Poly     | 14,200 | Alive  |
| 100| HF   | 6     | Poly     | 12,500 | Alive  |
| 101| HF   | 4     | Poly     | 7,100  | Alive  |
| 102| HF   | 4     | Poly     | 11,100 | Alive  |
| 103| HF   | 4     | Poly     | 8,100  | Alive  |
| 104| HF   | 6     | Poly     | 9,600  | Alive  |
| 105| HF   | 7     | Poly     | 17,300 | Alive  |
| 106| HF   | 7     | Poly     | 7,000  | Alive  |
| 107| HF   | 5     | Poly     | 10,800 | Alive  |
| 108| HF   | 5     | Poly     | 18,400 | Alive  |
| 109| HF   | 5     | Poly     | 7,900  | Alive  |
| 110| HF   | 4     | Poly     | 8,800  | Alive  |
| 111| HF   | 4     | Poly     | 7,300  | Alive  |
| 112| HF   | 4     | Poly     | 7,200  | Alive  |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
|113| HF | 4 | 7,900 | Poly | 7,200 | Alive |
|114| HF | 3 | 7,700 | Poly | 8,000 | Alive |
|115| HF | 3 | 9,200 | Poly | 9,200 | Alive |
|116| HF | 3 | 9,100 | Poly | 8,600 | Alive |
|117| JB | 5 | 8,400 | Poly | 9,200 | Alive |
|118| JB | 11 | 5,000 | Poly | 4,300 | Alive |
|119| JB | 8 | 5,600 | Poly | 4,800 | Alive |
|120| JB | 6 | 8,600 | Poly | 8,400 | Alive |
|121| JB | 7 | 4,500 | Poly | 5,000 | Alive |
|122| JB | 5 | 6,200 | Poly | 5,500 | Alive |
|123| JB | 3 | 5,900 | Poly | 5,700 | Alive |
|124| JB | 5 | 6,200 | Poly | 4,600 | Alive |
|125| JB | 10 | 14,000 | Poly | 8,600 | Alive |
|126| JB | 11 | 5,600 | Poly | 5,500 | Alive |
|127| JB | 6 | 8,000 | Poly | 8,500 | Alive |
|128| JB | 5 | 9,800 | Poly | 8,000 | Alive |
|129| JB | 6 | 6,200 | Poly | 5,500 | Alive |
|130| JB | 5 | 9,700 | Poly | 5,600 | Alive |
|131| JB | 5 | 11,100 | Poly | 12,200 | Alive |
|132| JB | 13 | 6,900 | Poly | 5,300 | Alive |
|133| JB | 12 | 11,700 | Poly | 9,500 | Alive |
|134| JB | 12 | 5,400 | Poly | 4,700 | Alive |
|135| JB | 11 | 5,600 | Poly | 4,700 | Alive |
|136| JB | 10 | 5,000 | Poly | 4,500 | Alive |
|137| JB | 6 | 5,000 | Poly | 5,500 | Alive |
|138| JB | 6 | 7,000 | Poly | 5,700 | Alive |
|139| JB | 6 | 5,100 | Poly | 4,600 | Alive |
|140| JB | 5 | 6,300 | Poly | 7,200 | Alive |
|141| JB | 5 | 8,500 | Poly | 12,200 | Alive |
|142| JB | 5 | 14,200 | Poly | 8,900 | Alive |
|143| JB | 4 | 6,300 | Poly | 5,400 | Alive |
|144| JB | 4 | 14,300 | Poly | 11,200 | Alive |
|145| JB | 4 | 5,900 | Poly | 5,000 | Alive |
|146| JB | 8 | 6,660 | Poly | 5,200 | Alive |
|147| JB | 5 | 4,680 | Poly | 6,500 | Alive |
|148| JB | 3 | 8,200 | Poly | 8,200 | Alive |
|149| JB | 5 | 4,700 | Poly | 5,800 | Alive |
|150| JB | 5 | 9,700 | Poly | 11,200 | Alive |
|151| JB | 12 | 5,300 | Poly | 4,500 | Alive |
|152| JB | 3 | 6,900 | Poly | 7,800 | Alive |
|153| JB | 5 | 5,000 | Poly | 4,600 | Alive |
|154| JB | 3 | 6,100 | Poly | 5,500 | Alive |
|155| JB | 15 | 3,900 | Poly | 4,600 | Alive |
|156| JB | 4 | 4,700 | Poly | 4,800 | Alive |
|157| JB | 14 | 6,900 | Poly | 14,000 | Alive |

| Median | 5 | 8,200 | 8,000 |

**EBL:** enzootic bovine leukosis