Pleural effusion in a 1 year old heifer

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Signalment

1 year-old Limousine Heifer

History

The heifer was presented for anorexia, dyspnea, tachycardia and marked edema extending from the sternum to the umbilicus

Diagnostic procedures and Laboratory data

- Thoracic ultrasound revealed a severe left thoracic effusion and a thickened pericardium
- Hematology and biochemistry analyses gave the following results :

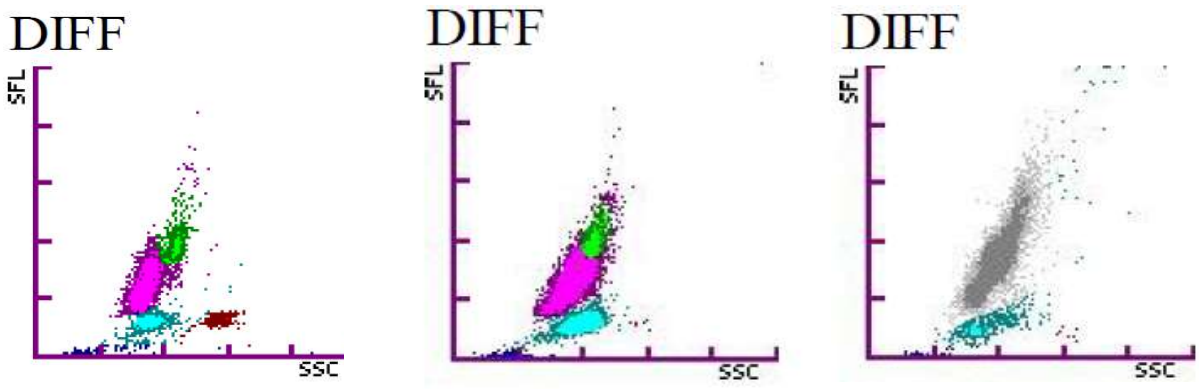
Hematology (Sysmex XT-2000iV, Sysmex, Kobe, Japan)		
	Value	**Reference interval**
RBC (10^6/µL)	5.95	4.8 - 7.8
HGB (g/dL)	10.0	8.2 - 13
HCT (%)	27.8	24 - 39
MCV (fL)	46.7	41.2 - 58.7
MCH (pg)	16.8	14.3 - 19.6
MCHC (g/dL)	36.0	32.4 - 35.8
PLT (10^3/µL)	191	100 - 800
WBC (10^3/µL)	23.74	4.4 - 10.8
NEUT (10^3/µL)	7.97	0.8 - 5.0
LYMPH (10^3/µL)	13.42	1.8 - 4.9
MONO (10^3/µL)	1.95	0.3 - 1.2
EO (10^3/µL)	0.01	0.0 - 0.1

Biochemistry (Vitros 350, Ortho Clinical Diagnostics, Raritan, NJ, USA)		
	Value	**Reference interval**
Urea (mmol/L)	13.9	2.1 - 7.9
Creatinine (µmol/L)	164.9	44 - 97
AST (U/L)	212	58 - 100
GGT (U/L)	72	22 - 64
Total Proteins (g/L)	90.1	58 - 75
Albumin (g/L)	36.6	24 - 35

Thoracic ultrasound revealed severe left thoracic effusion and thickened pericardium.

Pleural effusion had moderate to high cellularity (13.93 x 10^3/µL) and a low total protein concentration (10g/L).

Figure 1



Healthy cattle blood
DIFF Scattergram

Heifer blood
DIFF Scattergram

Heifer pleural effusion
DIFF Scattergram

Figure 2a : blood smear, MGG, x1000 magnification

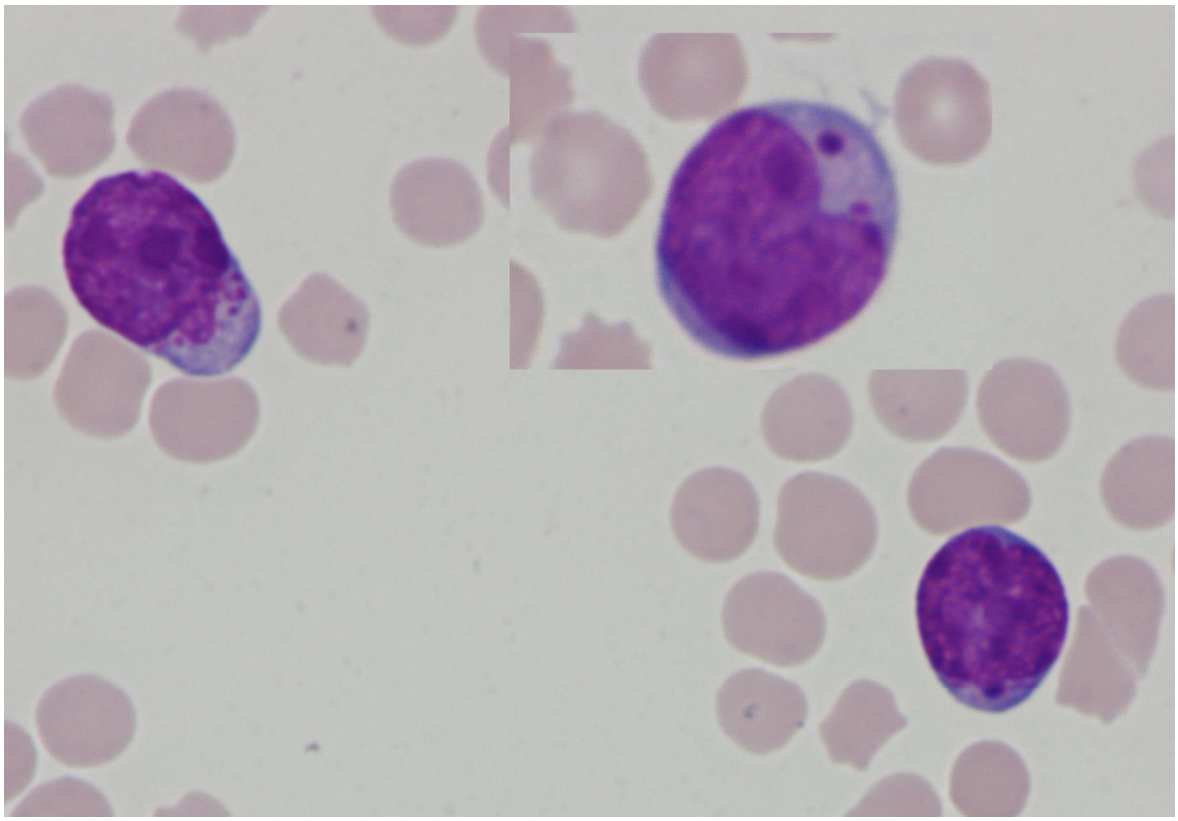
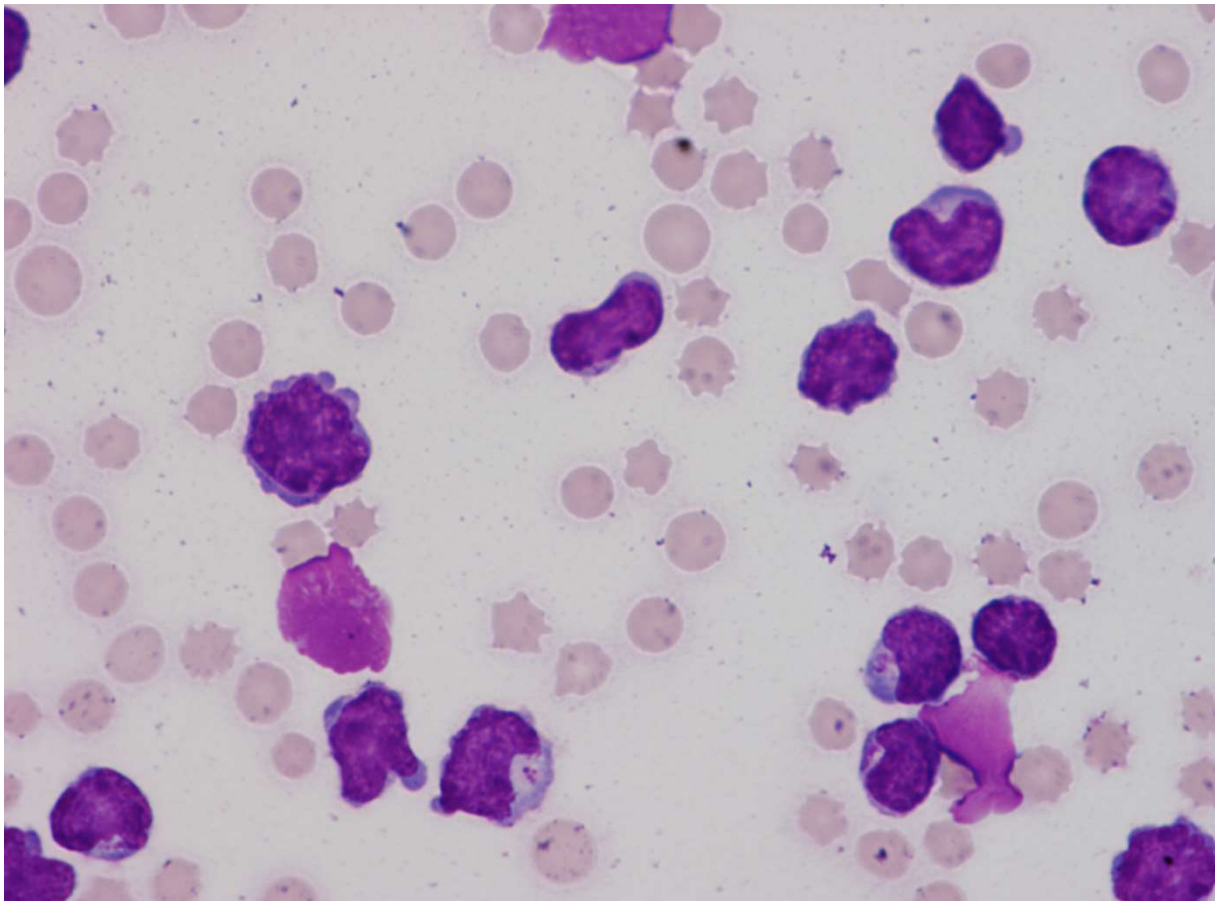


Figure 2b : pleural effusion, MGG, x400 magnification



Blood smear examination revealed mild toxic changes in neutrophils, and an important highly monomorphous atypical lymphoid population. Lymphoid cells were of small to medium size, with a high NCR, irregular nuclear borders and clumped chromatin. The cytoplasm was profoundly basophilic and contained some azurophilic granulations, gathered in a perinuclear area. Thoracic fluid examination revealed similar cells, in moderate to high number. Anisokaryosis, anisocytosis, mitoses and binucleations were frequent. Rare macrophages and neutrophils were also present.

Additional testing

The heifer died 3 days after admission, during a week-end night. Necropsic examination was realized 2 days later and revealed a very thick subcutaneous edema in the dewlap and thoraco-abdominal wall, fibrinous adherences between the pericardium, the left pulmonary lobe and thoracic wall, associated to 10 L of sero-hemorragic effusion, and a voluminous intra thoracic mass of 40x30 cm. The mass was irregular, lobulated, white and moderately firm. It was compressing the cranial vena cava. Prescapular, axillar, iliac, tracheobronchial and mediastinal lymph nodes were enlarged (10x6, 4, 8x6, 4 and 15x5 cm respectively) and the cortex was infiltrated by a white homogeneous material.
Histologically, the mass was highly cellular, with numerous sheets of round cells of lymphoid origin, and a supportive stroma composed of thick spans of connective tissue with occasional osseous metaplasia. Neoplastic cells were highly monomorphous, of small size, high NCR, with a hyperchromatic and round nucleus. Moderate anisocytosis and few mitoses were seen. Tracheobronchial and axillar lymph nodes examination revealed an infiltration by a similar lymphoid population. Final histologic diagnosis was mediastinal lymphoma with multicentric nodal infiltration.
Immunochemical staining was attempted on the mass and one lymph node : cells were weakly positive for CD79a, and negative for CD3.

Discussion

Sysmex CBC DIFF-scattergram shows an overlap between the lymphocytes and monocytes clusters, with an arbitrary cut between the two populations. Furthermore, the monocytes cluster seems to prolong the lymphocytes one, instead of being aside on the right. This graph then reveals a continuum of cells going from the normal lymphocytes location (and then of lymphoid origin), and extending up to overlap monocytes. Cells that appear upper on the graph are the cells with higher fluorescence, e.g. containing more nucleic acid, and in this case are suspected to be the largest blastic lymphoid cells.
Sysmex DIFF-scattergram for the pleural effusion showed a very similar pattern. This may allow the observer to assume a similar atypical neoplastic lymphoid component to the effusion, before looking at the cytological slides.

Bovine leukemias are separated into Enzootic Bovine Leukosis (EBL), related to Bovine Leukemia Virus (BLV) and Sporadic Bovine Leukosis (SBL), independent of BLV [1,2]. EBL is due to a clonal proliferation of B cells, and it has long been described as a disease affecting adult cattle over 3 to 5 years old. However, recent studies report onsets on juvenile individuals [1]. SBL has been associated with B cells or T cells proliferation, and is typically found in young cattle. It presents as 3 forms,

depending on the age and location of the tumor: the juvenile form occurs as a systemic disease before 2 years old, generally around 6 months; the thymic form occurs between 6 months and 2 years old; and the cutaneous form occurs between 1 and 3 years old, as a multifocal skin lesion [1,2,3].
Correct diagnosis is then based on epidemiology, clinical presentation, B or T cell identification, and BLV status.

Based on the age of the heifer at presentation and necropsy findings, the main hypothesis for this case was a thymic form (or less likely a juvenile form) of SBL. The fact that metropolitan France is officially free of EBL, and that latest EBL serological screening tests in the farm came back negative would support a viral-independent disease.
Immunophenotyping would have been helpful in discriminating between EBL and SBL if T-cells had been identified, but results were consistent with B-cell proliferation, which has been described in both EBL and SBL [3]. The B-cell immunophenotype was a surprise given the morphology of the cells, and particularly the cytoplasmic granulations. Such granulations are indeed typically associated with cytotoxic T-cells or Natural Killer cells [4]. We were consequently expecting a CD3+ CD79a- profile.
However, the death of the heifer during a week-end night prevented quick necropsy and tissue sampling. With this delay, absence of staining with the CD79a marker could be attributed to absence of expression ("true negative") or to a false negative due to cell autolysis. The weak positivity of CD3 marking similarly couldn't be interpreted with confidence. Then, the possibility of an atypical early onset of EBL, such as what has been described in Japan remains, eventhough unlikely.
Immunological characterization of neoplastic lymphoid cells has previously been performed on cattle leukosis, but less frequently in SBL cases than in EBL [5]. Cytological features are rarely properly described, nor related to the immunological profile [1,5]. To the author's knowledge, this is the first case report of bovine leukosis with large granular lymphocyte morphology.

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