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Case Report

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Congenital lymphoma of B-cell lineage in a newborn calf

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Abstract: In this report, congenital lymphoma of B-cell lineage in a Brown Swiss calf is described. A large mass was seen on the head of the calf at birth. At necropsy, multiple masses were found on the skin and internal organs. A histopathological examination showed atypical lymphoid cells separated by connective tissue. None of the tumor cells showed a positive reaction to CD3, but the cells were immunopositive for CD79αcy. A CD45$^+$ reaction confirmed a hematopoietic origin of the neoplasm. Tumor cells were also positive for lambda light chain IgG (λ IgG). Proliferating cell nuclear antigen (PCNA) immunostaining showed diffuse nuclear positivity. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) staining found numerous apoptotic bodies. The neoplasm was diagnosed as a congenital lymphoma of B-cell lineage.

Key words: B-cell, calf, congenital, lymphoma

Yeni doğmuş bir buzağıda B-hücreli doğmasal lenfoma

Özet: Bu olguda, İsviçre esmeri bir buzağıda doğmasal lenfoma tanımlandı. Doğumda buzağının baş bölgesinde iri bir kitle gözlendi. Nekropside deri altı ve iç organlarda çok sayıda tümör kitleleri görüldü. Histopatolojik olarak, ince bir bağdoku ile çevrelenmiş atipik lenfoid hücreler tespit edildi. İmmunhistokimyasal boyamalarda, tümör hücrelerinin CD3 negatif ve CD79αcy pozitif reaksiyonları belirlendi. Tümör hücrelerinin CD45 pozitif reaksiyonu, tümörün hemopoietik orjinli olduğunu gösterdi. Yine tümör hücrelerinin λ IgG kuvvetli pozitif reaksiyonu görüldü. Ayrıca tümör hücreleri PCNA yaygın nüklear pozitif reaksiyon gösterdi. TUNEL boyama ile tümör dokusunda çok sayıda apopitotik cisimcik saptandı. Tümör B hücre orjinli doğmasal lenfoma olarak teşhis edildi.

Anahtar sözcükler: B hücre, buzağı, doğmasal, lenfoma

Introduction

Bovine lymphoma is one of several common neoplasms identified in cattle, with considerable confusion with regard to their nomenclature and classification (1-3). Neoplasm in cattle is classified into enzootic bovine leukosis (EBL) and sporadic bovine leukosis (SBL), according to the pathogenesis and clinicopathology. EBL is almost always associated with the enzootic leukemia virus, and cattle affected with this form of lymphoma are usually 4-8 years of age, and tumors are commonly detected in uterine, abomasum, heart, and peripheral lymph nodes. SBL

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is not associated with the bovine leukemia virus and is classified into 3 additional types: calf type, thymic type, and skin type, on the basis of preferential sites of the neoplasia and age of the affected animal (2,4-6). Although lymphomas are relatively uncommon in calves, the neoplasms are interesting because of their occurrence early in life. These tumors have also been subdivided into 3 additional groups in calves: a) Spontaneous tumors of the congenital type, occurring in fetuses, and newborn and very young calves, b) Spontaneous tumors of the juvenile type, occurring in older calves (2-12 months of age), and c) Iatrogenic tumors, such as skin papillomas caused by the papilloma virus after tattooing or dehorning (7). To the best of our knowledge, congenital occurrence of lymphoid tumors has not been reported in bovines in Turkey. Therefore, in this case report, congenital lymphoma in a newborn calf is described with histological and immunohistochemical features.

Case history

A 7-day-old Brown Swiss female calf from a village in the region of Kars, Turkey, was presented to the Department of Veterinary Surgery, University of Kafkas, for clinical examination on 17 February, 2009. The owner reported that a large mass next to the right ear was observed at birth (Figure 1). The calf was seen to be in poor condition and had respiratory difficulty. The rectal temperature was 39 °C, the pulse was 100 beats/min, and the respiratory rate was 70 breaths/min. Upon auscultation, the increased lung sounds were heard clearly. A radiological examination showed numerous masses of varying sizes distributed in the all of the lung lobes. Our suspicion of neoplasia was communicated to the owner, and biopsy specimens were taken from the large mass for histological examination. The owner rejected euthanasia and the calf was taken to a barn. The calf died 2 weeks later due to increased difficulty in respiration, and was presented for necropsy. At necropsy, it was observed that the large tumor mass located on the head was larger than recorded at the initial examination and was measured as approximately 10 × 8 × 7 cm in diameter. There were also a few masses adjacent to the large mass, of which a surface incision revealed a small amount of pus. When the skin was peeled, tumor masses (about 1-3 cm in diameter) were seen to attach to the internal surface of the skin, and to locate on the gluteal and intercostal muscles. When the abdominal cavity was opened, approximately 1 L of yellowish colored fluid, fibrin collections, adhesions, and multiple masses (0.5-3 cm in size) were observed at the serosa of forestomachs, peritoneum, mesentery, and diaphragm. No tumor mass was seen in the liver, spleen, or kidneys. When the thorax was opened, about 0.5 L of fluid was found in the thoracic cavity. Numerous masses were distributed in all of the lung lobes and lung parenchyma was obliterated by multiple neoplastic formations (Figure 2A). The mediastinal lymph nodes were displaced by large masses. There was also involvement of the submandibular, retropharyngeal, and cervical lymph nodes by the masses, with no involvement of the thymus. Necropsy examination showed no mass in the cerebrum or cerebellum, but a mass of 1 × 1.5 cm was detected in the cerebellum following formalin fixation (Figure 2B). Tissue samples from the tumor masses were fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E).

Figure 1. Large tumor mass on the head of neonatal calf.

Sections from the masses were stained immunohistochemically using the avidin-biotin-peroxidase complex (ABC) technique, for $CD3^+$ T and $CD79\alpha cy^+$ B lymphocytes, CD45 leukocyte common antigen (LCA), lambda light chain IgG (λ IgG), and proliferating cell nuclear antigen (PCNA). Details of the primary antibodies used are given in Table 1. Paraffin sections were dewaxed and hydrated. Endogenous peroxidase activity was

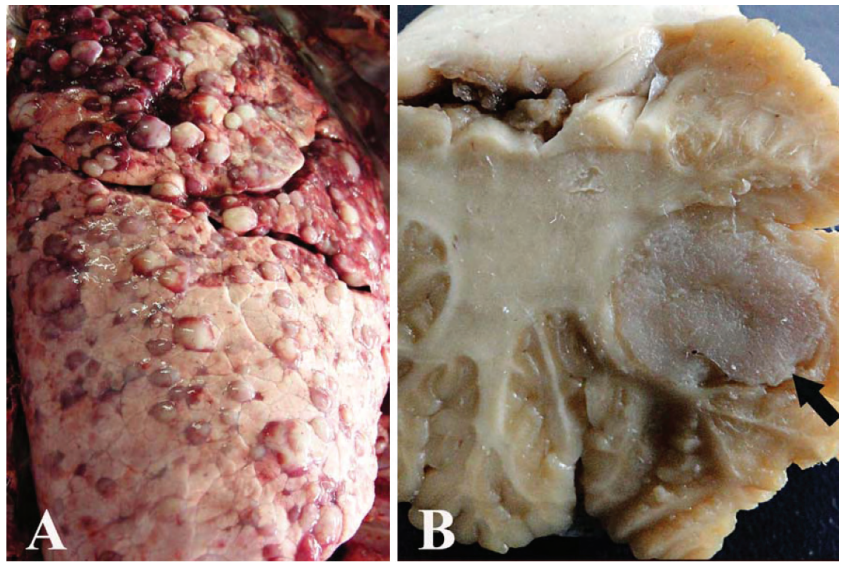

Figure 2. A) Lung. Numerous tumor masses distributed throughout all of the lobes. B) Cerebellum. Tumor mass (arrow) following the formalin fixation.

Table 1. Details of primary antibodies used for immunohistochemical analysis.

Primary antibodies	Pretreatment	Primary antibody dilution	Incubation condutions	Origin (commercial reference)
Polyclonal rabbit anti-human CD3‡	Microwave oven	1 in 150	Room temperature	Dako (Catalog no. N 1580)
Polyclonal rabbit anti-human IgG lambda light chain (λ IgG)‡	Microwave oven*	1 in 1500	overnight at 4 °C	Novocastra (NCL-LAMp)
Monoclonal mouse anti-human CD79αcy‡	Microwave oven	1 in 25	Room temperature	Dako (Catalog no M 7051)
Monoclonal mouse anti-rat PCNA‡	Microwave oven	1 in 2000	overnight at 4 °C	Chemicon (Clone PC10)
Monoclonal mouse anti-human CD45 leukocyte common antigen (LCA)	Microwave oven	1 in 200	Room temperature	NeoMarkers/LabVision MS-355-S (clones PD7/26/16+2B11)

‡: The antibodies have been shown, by the manufacturer's data sheets, to cross-react in cattle, except λ IgG, for which we found to have an intense reaction in plasma cells.
*: Microwave oven pre-treatment consisted of immersion of the sections in 10 mM sodium citrate buffer pH 6.0 and irradiation in a 800 W microwave oven for 20 min.

blocked with 3% H_2O_2, and the sections were placed in citrate buffer saline (pH 6.0) in a microwave oven for antigen retrieval. The slides were incubated with normal rabbit (CD79αcy, CD45 LCA, PCNA) or goat (λ IgG) serum at room temperature (RT) for 60 min, and then incubated with monoclonal mouse anti-human CD79αcy and CD45 LCA, polyclonal rabbit anti-human λ IgG and monoclonal mouse anti-rat PCNA primary antibodies, according to the manufacturer's recommended procedures. The sections were incubated with biotinylated rabbit anti-mouse IgG (CD79αcy, CD45 LCA, PCNA) and biotinylated goat anti-rabbit IgG (λ IgG), at a dilution of 1/200 in TBS for 60 min at RT. The sections were treated with streptavidin peroxidase complex, at a dilution of 1/300 for 30 min at RT. Detection of $CD3^+$

T cells was undertaken with polyclonal rabbit anti-human CD3 antibody and with biotinylated linked anti-mouse and anti-rabbit immunoglobulin and streptavidin HRP (Dako LSAB2™ system) for 30 min at RT. Immunostaining was obtained using 3,3 diaminobenzidine (DAB) as the chromogen. Harris' hematoxylin was used as the counterstain. Apoptotic cells were evaluated using the DeadEnd Colorimetric TUNEL system (Promega, Madison, WI, USA). The sections following deparaffinization and rehydration were permeabilized with proteinase K for 30 min at ambient temperature, incubated with equilibration buffer for 10 min and then terminal deoxynucleotidyl transferase reaction mixture was added to the sections, which were incubated at 37 °C for 60 min. The reaction was stopped by immersing the sections in 2 × SSC buffer for 15 min. Thereafter, the sections were quenched of endogenous peroxidase activity using 0.3% H_2O_2 for 10 min, and then treated with streptavidin (1:500) for 30 min at RT, and were incubated with DAB for color development.

Results and discussion

Tumor cells were arranged in irregular lobules separated by thin fibrovascular connective tissue (Figure 3A). The predominant cells in the neoplastic growths were large, atypical lymphoid cells, which were 2-3 times larger than normal lymphocytes. The nuclei of the tumor cells were round to oval with finely stippled chromatin. The number of nucleoli varied between 1 and 3, and they were located centrally. Mitotic figures were numerous, with up to 11 figures per high-power field (×40). Tumor cells often revealed apoptotic bodies. The primary tumor mass also frequently contained central necrosis, hemorrhages, and thromboses. In the lungs, multiple unencapsulated tumor masses were distributed to all of the lobes, causing compression of adjacent parenchyma. Venules and dilated lymphatics often contained tumor cell embolus incorporated with blood cells. Alveoli often contained neutrophilic collections and edema. In the mediastinal lymph nodes, neoplastic growths wholly obliterated normal architectures and the nodes could be distinguished by the presence of a few lymphoid follicles. Capsular and perinodal invasion of tumor cells was common. All lymphatics contained tumor embolus containing various numbers of tumor cells. An unencapsulated tumor mass was detected in the cerebellum, showing similar histological features as in other sites. Tumor cells invaded the cerebellar layers and perivascular sheaths, along with degeneration of numerous purkinje cells.

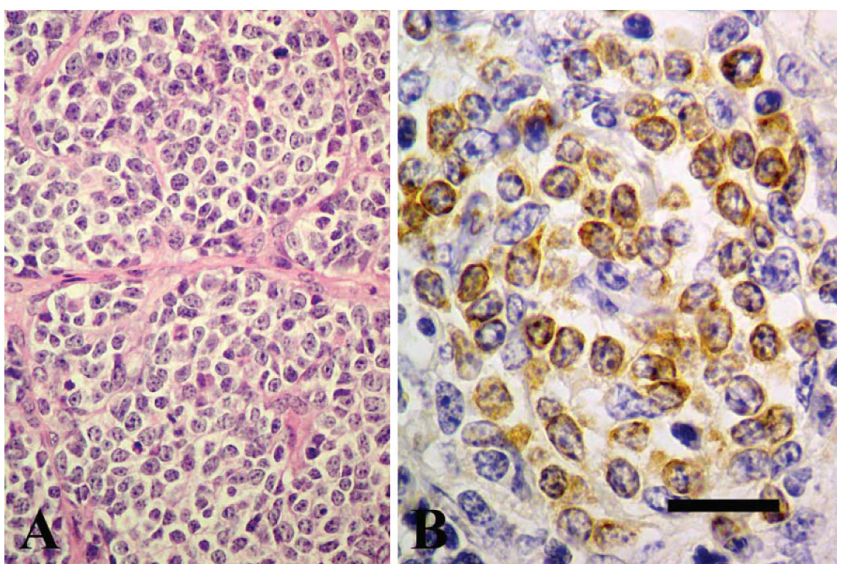

Figure 3. A) Mediastinal mass. Tumor cells of varying shapes and sizes separated by connective tissue bundles. H&E. Bar = 51 μm. B) Mediastinal mass. Positive immunolabeling of tumor cells for CD79αcy. ABC. Bar = 11 μm.

Immunohistochemical staining did not detect a $CD3^+$ reaction in the tumor cells, despite positive reactions in the peribronchial lymphoid follicles and mediastinal lymph nodes. A moderate number of tumor cells showed positive immunolabeling for CD79αcy in the masses from the lungs and mediastinal lymph nodes (Figure 3B). $CD79\alpha cy^+$ labeled cells were randomly distributed or detected as small groups in the masses. Tumor cells revealed a predominantly cytoplasmic staining pattern. $CD79\alpha cy^+$ B lymphocytes were also detected in the follicles of the nodes. CD45 LCA positive tumor cells were frequently detected, showing a cytoplasmic and membranous staining pattern. Tumor cells revealed strong immunoreactivity for λ IgG (Figure 4A). Staining was mainly cytoplasmic and immunoreaction products were often large granules in the cells. Plasma cells were also immunopositive for λ IgG in the peribronchiolar aggregates. The nuclei of many tumor cells were immunolabeled for PCNA expression. Many apoptotic cells were detected using TUNEL staining (Figure 4B). Positive labeling was strong in the apoptotic bodies.

It is reported, that as the fetal period is much shorter than the overall lifespan of an individual, it can therefore be expected that genetic factors rather than environmental factors play a role in the development of such tumors (7). In this case, the calf was presented at 1 week of age with a large mass on the head, and genetic factors might have played a role in the occurrence of the neoplasm. Lymphoid tumors have commonly been assigned as multicentric malignant lymphoma in newborns (6-8) and in calves older than 6 months (5,9). Misdorp (7) also reported that the congenital form of the neoplasm is most commonly multicentric, unlike the juvenile form. In the present case, a large mass was localized on the head, and multiple masses of varying sizes were detected in subcutaneous tissues, superficial and internal lymph nodes, abdomen, and the diaphragm and lungs, as reported by others (2,10,11). It was reported that nervous system lesions of lymphomas can easily be overlooked since masses grossly appear indistinguishable from fat (10). Consistent with this suggestion, no tumor metastasis was observed in the cerebrum or cerebellum at necropsy, but a mass was detected in cerebellum following fixation.

The neoplasm was characterized with large, atypical lymphoid cells separated by connective bundles, consistent with those reported by other researchers (11,12). Tumor cells revealed vesicular nuclei, oval or round, with small to medium-sized nucleoli. The high mitotic rate of the tumors, where multiple tumor cell nuclei were positive with PCNA, indicated severe

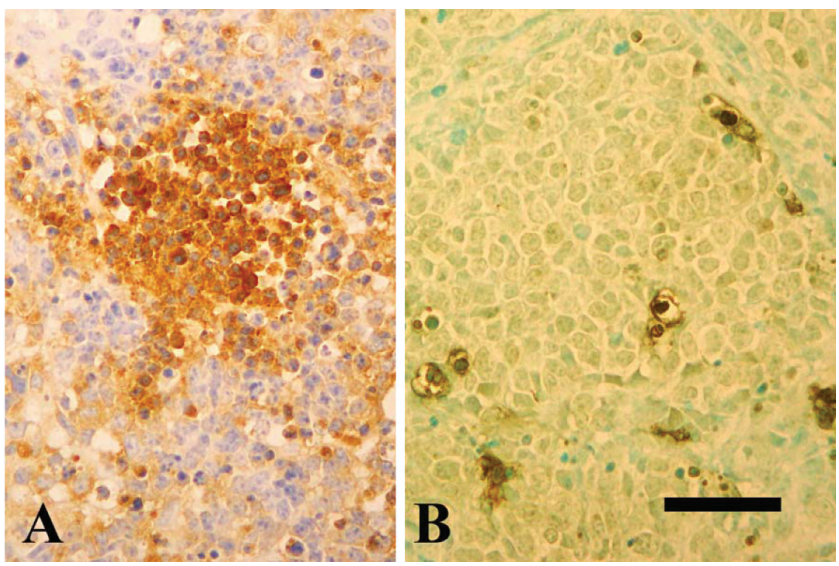

Figure 4. A) Abdominal mass. Positive reaction of tumor cells for λ IgG. B) Abdominal mass. Apoptotic cells in tumor parenchyma. TUNEL staining. ABC. Bar = 166 µm.

malignancy of the congenital form of lymphoma. Similar histological features have frequently been reported in bovine lymphomas in previous studies (2,6,8-13). These histological features are compatible with those of large B-cell lymphoma, as reported previously in calves (8,11). The lungs were severely affected by multiple metastatic tumors and normal architecture was obliterated by neoplastic growths, compatible with earlier reports (2,11). Tumor embolus in the veins and lymphatics of the lungs and mediastinal lymph nodes evidently confirmed distant metastases. Likewise, cerebellar involvement of tumors was detected, where tumor cells invaded cerebellar layers and caused degeneration of purkinje cells, as previously reported by Braun et al. (13).

CD3 and CD79αcy antibodies have been documented as very useful reagents for the immunohistochemical assessment of T and B-cell lineage lymphomas in animals, as in humans. In this case, positive staining for CD45 LCA suggested a hematopoietic origin of the neoplasm. CD79αcy$^+$ immunolabeling indicated that tumor cells might originate from the immature B-lymphocytes in the congenital type lymphoma, as documented in earlier studies (1,4,11). Peribronchial lymphocytes and a small number of plasma cells were also found to be positive for the CD79αcy marker. Immunolabeling of tumor cells for CD3 antibody was not detected. However, CD3$^+$ T cells were detected in normal lymphoid follicles. CD3$^+$ T and CD79αcy$^+$ B-cells in the lymphoid follicles may be explained by the presence of residual lymphoid tissue, or may be due to chemotaxis induced by cytokines secreted from tumor cells, as stated by others (3,10). Tumor cells and plasmacytes were also positive for λ IgG, consistent with previously reported cases (6,14,15). It was reported that the best method for identification of B-cells is the demonstration of IgG and IgM on the surface or in the cytoplasm of tumor cells. Likewise, even though the primitive B-lymphocytes and plasma cells lack surface immunoglobulins, pre B-cells and plasmacytes have cytoplasmic immunoglobulins (1,14,15). Therefore, demonstration of cytoplasmic immunoglobulins may helpful in the identification of the tumor cell lineage in newborn calves (14). Numerous apoptotic bodies were also found in the tumor cells by using TUNEL staining. It was documented that a failure of tumor cells to respond to stimuli that would lead to apoptosis might enhance tumor growth. If the apoptosis pathways are disrupted in some tumor cells and these cells fail to die, tumor growth is facilitated by the accumulation of cells (10).

In conclusion, based upon the microscopic characteristics of tumor cells and the immunopositive reaction of the cells for CD79αcy and λ IgG markers associated with the age of the calf, this congenital neoplasm was diagnosed as lymphoma with B-cell lineage. Metastases in the lungs and cerebellum, along with widespread lymph node involvement, atypical lymphoid cells, and high mitotic rate were compatible with a severe malignancy of the congenital neoplasm.

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