

## CUTANEOUS LYMPHOMA IN A COW – A CASE REPORT

KURELJUŠIĆ Branislav<sup>1\*</sup>, MAKSIMOVIĆ Slobodan<sup>2</sup>, VUJINOVIĆ Slobodan<sup>2</sup>, SAVIĆ Božidar<sup>1</sup>, MILIĆEVIĆ Vesna<sup>1</sup>, VELJOVIĆ Ljubiša<sup>1</sup>, JEZDIMIROVIĆ Nemanja<sup>1</sup>, ŽUTIĆ Jadranka<sup>1</sup>

<sup>1</sup>Institute of Veterinary Medicine of Serbia, Belgrade, Serbia;

<sup>2</sup>Veterinary Specialized Institute „Šabac“, Šabac, Serbia

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### Abstract

In this paper the case of a 2.5-year-old Simmental cow, with suspicion of lumpy skin disease in mid-2017 in Serbia will be presented. Clinical examination revealed numerous nodules of varied size from a few millimeters to approximately 10 centimeters disseminated predominantly on the skin of the udder and the perineum, some of which were coalescing and exulcerated. The general condition of the affected animal was unchanged. According to the results of laboratory analysis, the cow was negative for the presence of antibodies against the bovine leukemia virus, showed a negative reaction in tuberculinization and was vaccinated against lumpy skin disease virus. After the surgical excision of one skin node, the sample tested negative for the genome of lumpy skin disease virus. In order to establish the morphology of the skin lesion, a histopathological analysis was performed. Histopathological analysis showed the infiltration of the corium and subcutaneous tissue by numerous mononuclear cells showing cellular atypia. Suspicion of cutaneous lymphoma was established. Furthermore, the immunohistochemical examination confirmed that the infiltrate contained exclusively CD3-immunopositive cells, suggesting a T-cell origin nonepitheliotropic lymphoma.

**Key words:** cow, cutaneous lymphoma, immunohistochemistry

## CASE PRESENTATION

In this paper, a case of a 2.5-year-old Simmental cow with suspicion of lumpy skin disease (LSD) in mid-2017 will be presented. In Serbia, the first case of LSD virus infection was confirmed by laboratory analysis on 7 June 2016 (Vidanović et al.,

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\*Corresponding author – e-mail: [branislavkureljusic@yahoo.com](mailto:branislavkureljusic@yahoo.com)

2016). After this case, many cases were confirmed in the laboratory, and vaccination started as a control measure. In 2017, vaccination continues as well as monitoring of postvaccinal reactions in the field. In this case, according to the previously obtained results of laboratory analyses, the cow was negative for the presence of antibodies against bovine leukemia virus. In the previous clinical anamnesis, the cow showed a negative reaction in the tuberculinization test and was vaccinated against LSD virus.

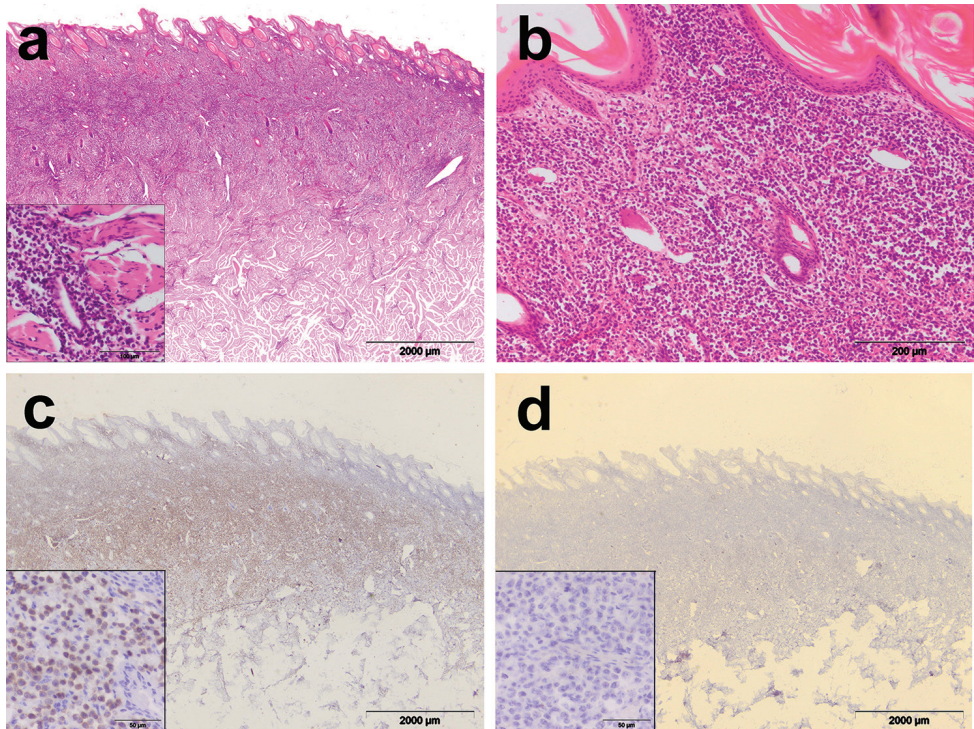
Clinical examination revealed numerous nodules of varied size, from a few millimeters to approximately 10 centimeters, disseminated predominantly on the skin of the udder and the perineum, some of which were coalescing and exulcerated and partially alopecic (Fig 1).



**Figure 1.** Cutaneous nodules disseminated on the skin of the perineum

Some single nodules were detected on the abdominal lateral skin and femoral region. The nodules were firm in consistency and predominantly round in shape, but some of them were irregular in shape. Additionally, an edema had been observed in the chest, dewlap and anterior extremities. The regional lymph nodes were without changes. The general condition of the cow was unchanged and good body condition was established. The appetite was preserved; the body temperature, heart rate and respiratory rate were within the physiological limits. After local anesthesia by 2% lidocain hydrochloride, one skin node from the perineum was surgically excised. Although the epizootic and clinical data were not in favor of a diagnosis of LSD, pronounced skin lesions and an unfavorable epizootiological situation during 2016 were sufficient reasons for testing for the LSD virus. The sample was sent to the virology laboratory at the Institute of

Veterinary Medicine of Serbia to determine the presence of the genome of LSD virus by PCR. However, PCR was negative for the presence of LSD virus nucleic acid. After exclusion of LSD, further infectious and neoplastic diseases (e.g. papillomatosis and cutaneous lymphoma) had to be taken into consideration. General clinical symptoms of infectious diseases were absent, and for that reason it was decided to establish the morphology of the skin lesion. Histopathological analysis was performed. Briefly, the skin node was fixed for 48h in 10% buffered formalin, embedded in paraffin and 3-4  $\mu\text{m}$  sections were cut and stained with hematoxylin and eosin (Bio-Optica, Italy). The histopathological analysis showed the infiltration of the dermis and subcutaneous tissue by numerous mononuclear round cells showing cellular atypia (Fig 2a).



**Figure 2.** Microscopic and immunohistochemical findings in the skin of the cow with cutaneous lymphoma: **a)** Severe infiltration of the dermis and subcutaneous tissue by numerous mononuclear round cells, hematoxylin and eosin (HE). Insert: Lymphoblasts with scant cytoplasm, HE; **b)** Severe infiltration of the dermis by neoplastic lymphoblastic cells. Moderate epidermal hyperkeratosis, HE; **c)** Labeling of lymphocytic cells with rabbit anti-human CD3 antibody, LSAB2; Insert: Positive membranous reaction, LSAB2; **d)** Labeling of lymphocytic cells with mouse anti-human CD79a antibody, negative reaction, LSAB2; Insert: Negative reaction, LSAB2.

Cells had scant cytoplasm and mitotic figures were rare and abnormal (1-2 per high-power magnification fields). Polymorphonuclear cells were rarely present, and additionally, moderate epidermal hyperkeratosis was found (Fig 2b). Suspicion

of nonepitheliotropic cutaneous lymphoma was established. Subsequently, immunohistochemistry (IHC) was performed. After antigen retrieval, the sections were then treated with methanol containing 0.3% hydrogen peroxide for 15 minutes at  $22\pm 3^\circ\text{C}$  in order to inactivate the endogenous peroxidase. Non-specific binding of secondary antibodies was minimized by incubating with 50% normal goat serum in PBS for 20 minutes. Two primary antibodies were applied: rabbit anti-human CD3 (pan T-cell marker, DAKO A0452) and mouse anti-human CD79a (B-cell marker, DAKO M7051). Sections were incubated with appropriate primary antibodies diluted in PBS (dilution 1:50, 1 h in humid chamber at  $22\pm 3^\circ\text{C}$ ). All rinsing procedures and serum dilutions were conducted using PBS (pH 7.2-7.4). The detection kit was DAKO Cytomation LSAB2 System-HRP, Rabbit/mouse (DAKO, K0675). Positive reactions were visualized by applying DAB+ (DAKO, K3468) for 5 to 10 minutes. Counterstaining was performed using Mayer hematoxylin for 2 seconds. Aqueous medium glycerol (DAKO, C563) was used on stained sections for mounting. Sections not treated with the primary antibodies were used as negative controls. Bovine lymph nodes were used as positive controls for IHC.

The cutaneous infiltrates became labeled with rabbit anti-human CD3 antibody, but not with mouse anti-human CD79a (Fig 2c, Fig 2d). This confirmed that the infiltrate contained exclusively CD3 immunopositive cells and lymphoblastic cells of T-cell origin. Therefore, the final diagnosis of a cutaneous form of sporadic bovine leukosis was reached.

## DISCUSSION

Lymphoma in cattle is usually linked with infection by enzootic bovine leukemia virus which causes enzootic bovine leukosis (Murayama et al., 2011). Cutaneous lymphoma is a form of sporadic leukosis of cattle that is not etiologically linked to the infection with bovine leukosis virus. Sporadic leukosis of cattle is rarely diagnosed, having an incidence of 1 in 100,000 (Oliver-Espinosa et al., 1994). In addition to the cutaneous form, there are two other forms of sporadic leukosis in cattle: juvenile and thymic bovine leukosis. The cutaneous form is most common in cattle aged from one to three years (Stober, 1981; Schweizer et al., 2003; Harbo et al., 2004; Klinkon & Cerne, 2006; Radostits et al., 2007), which was also the animal age in this case. Unlike sporadic leukosis, enzootic leukosis occurs most commonly in animals aged three to eight years (Lucas, 1992; Theilen & Madewell, 1987).

Severe LSD is highly characteristic morphologically, but milder forms can be confused with other infectious or parasitic or degenerative diseases such as: bovine herpes virus mammillitis, bovine papular stomatitis, pseudocowpox, vaccinia virus and cowpox virus, dermatophilosis, demodicosis, insect or tick bites, besnoitiosis, *Hypoderma bovis* infestation, onchocercosis, photosensitization, urticaria or cutaneous tuberculosis (OIE, 2017).

Further differential diagnosis of LSD, in addition to the above listed diseases, could include cutaneous lymphoma. Although the epizootic and clinical data were not in favor of a diagnosis of LSD, pronounced skin lesions and an unfavorable epizootiological situation during 2016 were sufficient reasons for testing for the LSD virus.

In this case, information about case history is scant. The local veterinarian did not observe any other clinical symptoms, such as pyrexia, rhinitis, conjunctivitis, reduced milk yield or abortions, which are characteristic for LSD. The regional lymph nodes were not involved. It is known that cutaneous lymphoma is characterized by skin lesions, and sometimes regional superficial lymph nodes can be involved. Metastases to the internal organs can be detected (Stober 1981; Schweizer et al., 2003). However, this case was confirmed negative for LSD, and therefore, its health status was not monitored further. From those reasons, we can only discuss whether this case was a primary cutaneous neoplasm or whether the skin lesions could be a part of a multicentric lymphoma.

Histological investigation revealed severe infiltration of the dermis and subcutaneous tissue by neoplastic mononuclear round cells. This picture was suggestive of a cutaneous lymphoma. Immunophenotyping of the infiltrates showed CD3-positive and CD79a-negative cells. This finding is consistent with a lymphoma of T-cell origin and proves that these markers can be used in the diagnosis of cutaneous lymphoma in cattle. Similar findings were observed by Otrocka-Domagala et al. (2012) and Loh (2007), suggesting that cutaneous lymphomas are usually of T-cell origin. However, there are some literature reports of cutaneous neoplasms of B-cell origin in cattle (Burg et al., 2006; Yamamoto et al., 2007). In humans, the most common form of cutaneous lymphoma is mycosis fungoides, usually derived from clonal expansion of T helper cells and rarely from T suppressor/killer or NK cells (Burg et al., 2006).

The final diagnosis of nonepitheliotropic cutaneous lymphoma was established by histopathological and immunohistochemical examination. Involvement of the regional lymph nodes was not observed. This case suggests that differential diagnosis of LSD, in addition to other diseases, should include bovine cutaneous lymphoma.

## REFERENCES

- Burg G., Kempf W., Cozzio A., Dobbeling U., Freit J., Golling P., Michaelis S., Scharer L., Nestle F., Dummer R. 2006. Cutaneous malignant lymphomas: Update 2006. *Journal der Deutschen Dermatologischen Gesellschaft*, 4:914-933. <https://doi.org/10.1111/j.1610-0387.2006.06069.x>.
- Harbo S.J., Barrington G.M., Allen A.J., Sample G.L., Parish S.M., Hamilton M.J., Davis W.C. 2004. Characterization of lymphocyte populations by flow cytometry in a calf with sporadic juvenile lymphoma. *Veterinary Clinical Pathology*, 33:163-167.
- Klinkon M., Cerne M. 2006. Cutaneous T-cell lymphoma in heifer with increased serum lactate dehydrogenase activity. *Veterinary Clinical Pathology*, 35:231-234. <https://doi.org/10.1111/j.1939-165X.2006.tb00120.x>.

- Loh C.C. 2007. Atypical presentation of cutaneous T-cell lymphosarcoma in a 19-month-old Holstein heifer. *Canadian Veterinary Journal*, 48:309-311.
- Lucas M.H. 1992. Enzootic bovine leukosis. In *Bovine Medicine*, eds. Andrews, A. H. et al., Blackwell Science, Oxford, 530-537.
- Murayama S., Sato K., Ikehata T., Wada Y., Ishikawa Y., Kadota K. 2011. Cytologic and immunophenotypic investigation of lymphohematopoietic neoplasms in cattle. *Japan Agricultural Research Quarterly*, 45: 225-231. <https://doi.org/10.6090/jarq.45.225>.
- OIE, 2017. Lumpy Skin Disease. Etiology, Epidemiology, Diagnosis, Prevention and Control References. *Terrestrial Animal Health Code*. OIE, Paris.
- Oliver-Espinosa O., Physick-Sheard P.W., Wollenberg G.K., Taylor J. 1994. Sporadic bovine leukosis with ataxia and tibiotarsal joint swelling: A case report. *The Canadian Veterinary Journal*, 35:777-779.
- Otrocka-Domagala I., Procajlo Z., Pazdzior K., Gesek M., Rotkiewicz T., Szweda W. 2012. Immunohistochemical profile of multicentric cutaneous epitheliotropic T-cell lymphoma with generalized lymphadenopathy in a Holstein-Friesian cow: a case report. *Veterinarni Medicina*, 57 (5):251-257.
- Radostits O.M., Gay C.C., Hinchcliff K.W., Constable P.D. 2007. *Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Saunders, 10th Edition.
- Schweizer G., Hilbe M., Braun U. 2003. Clinical, haematological, immunohistochemical and pathological findings in 10 cattle with cutaneous lymphoma. *Veterinary Record*, 153:525-528. <http://dx.doi.org/10.1136/vr.153.17.525>.
- Stober M. 1981. The clinical picture of the enzootic and sporadic forms of bovine leukosis. *Bovine Practitioner*, 16:119-129.
- Theilen G. H., Madewell B. R. 1987. Bovine. In *Veterinary Cancer Medicine*, 2nd ed., eds., Lea & Febiger, Philadelphia, 408-430.
- Vidanović D., Šekler M., Petrović T., Debeljak Z., Vasković N., Matović K., Hoffmann B. 2016. Real-time PCR assays for the specific detection of field Balkan strains of lumpy skin disease virus. *Acta Veterinaria-Beograd*, 66(4):444-454. DOI: 10.1515/acve-2016-0038.
- Yamamoto S., Wada Y., Ishikawa Y., Kadota K. 2007. Precursor B-1 B cell lymphoma in a newborn calf. *Journal of Veterinary Diagnostic Investigation*, 19: 447-450. <https://doi.org/10.1177%2F104063870701900422>.

## KUTANI LIMFOM KOD KRAVE – PRIKAZ SLUČAJA

KURELJUŠIĆ Branislav, MAKSIMOVIĆ Slobodan, VUJINOVIĆ Slobodan,  
SAVIĆ Božidar, MILIĆEVIĆ Vesna, VELJOVIĆ Ljubiša,  
JEZDIMIROVIĆ Nemanja, ŽUTIĆ Jadranka

### Kratak sadržaj

U ovom radu prikazan je slučaj krave simentalske rase, starosti dve godine i šest meseci kod koje je postojala sumnja na nodularni dermatitis, sredinom 2017 godine. Kliničkim pregledom ustanovljeni su brojni noduli veličine od nekoliko milimetara do približno 10 centimetara diseminovani dominantno po koži vimena i perineuma, od kojih su neki konfluirali i egzulcerisali. Opšte stanje krave je bilo nepromenjeno. Krava je prema

rezultatima laboratorijskih analiza bila negativna na prisustvo antitela protiv virusa leukoze goveda, pokazala je negativnu reakciju pri tuberkulinizaciji i bila je vakcinisana protiv nodularnog dermatitisa. Nakon hirurške ekscizije kožnog čvora, uzorak je bio negativan na prisustvo genoma virusa nodularnog dermatitisa. Kako bismo ustanovili morfologiju kožne lezije, urađena je histopatološka analiza. Histopatološka analiza je pokazala infiltraciju dermisa i subkutisa brojnim mononuklearnim ćelijama koje pokazuju ćelijsku atipiju. Tada je postavljena sumnja da se radi o kutanom limfomu. Nadalje, imunohistohemijsko ispitivanje je potvrdilo da se u infiltratu nalaze isključivo CD3 imunopozitivne ćelije što sugerise da se radi o T-ćelijskom neepiteliotropnom limfomu.

**Ključne reči:** krava, kutani limfom, imunohistohemija