

Multicentric B-cell lymphoma in a pygmy goat

Multicentrisch B-cellymfoom bij een dwerggeit

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ABSTRACT

A six-year-old, male pygmy goat was referred with a sudden onset of peripheral lymphadenopathy, which initially started as enlarged inguinal lymph nodes. Clinical examination showed swollen retropharyngeal, prescapular and inguinal lymph nodes. Serologic testing for bovine leukemia, caprine arthritis-encephalitis virus and caseous lymphadenitis was negative. Fine needle aspirates of the prescapular lymph nodes were taken and revealed multiple, large lymphoblastic cells on cytology. Because of the poor prognosis and clinical deterioration, the animal was euthanized. Full necropsy was performed and showed generalized lymphadenopathy. Further histological and immunohistochemical investigation of the lymph nodes characterized this neoplasia as a multicentric large B-cell lymphoma.

SAMENVATTING

In dit artikel wordt een zes jaar oude, mannelijke dwerggeit met acute perifere lymfadenopathie beschreven die initieel begon met opzetting van de inguinale lymfeknopen. Op het lichamelijk onderzoek werd ook zwelling van de retrofaryngeale, de boeg- en de inguinale lymfeknopen vastgesteld. Serologisch onderzoek naar boviene leukemie, capriene arthritis-encefalitisvirus en caseuze lymfadenitis was negatief. Cytologisch onderzoek van een dunne naaldaspiraats van de prescapulaire lymfeknopen toonde multipiele, grote lymfoblastische cellen. Vanwege de algemene klinische achteruitgang en slechte prognose werd besloten het dier te euthanaseren. Op autopsie werd een veralgemeende vergroting van de lymfeknopen waargenomen. Na histologisch en immunohistochemisch onderzoek van de lymfeknopen werd deze neoplasie als een multicentrisch B-cellymfoom gekarakteriseerd.

INTRODUCTION

Lymphoma is a sporadic disease in ruminants. It has been described in cattle (De Vlieghe et al., 2000; Malatestinic, 2003; Schweizer et al., 2003; Braun et al., 2005; Duncan et al., 2005; Loh, 2007), sheep (Valenti and McDonough, 2003) and goats (Olson and Baumgartner, 1975; Olson et al., 1981a,b; Baker et al., 1982; Bakers and Sherman, 1982; Higgins et al., 1984; de Silva et al., 1985; Craig et al., 1986; Smith and Sherman, 1996; Puette and Lalimer, 1997), but also in deer (Madson and Opriessnig, 2009; Kleinschmidt et al., 2012; Reissig et al., 2013) and water buffalo (Vale-Echeto, 2009). In goats, as in other species, clinical presentation varies and depends on the localization of the tumor. Predilection sites are liver, spleen, lungs and peripheral lymph nodes (Rozaer et al., 1998; Matthews, 1999). The most common associ-

ated clinical signs are anorexia, weight loss, depression, fever, dyspnea and peripheral lymphadenopathy (Higgins et al., 1984; DiGrassie et al., 1997; Matthews, 1999). The involved cell type has only been reported in two goats, one T- and one B-cell (Rozaer et al., 1998; Valentine et al., 2011). In this report, the clinical, pathologic and histologic examination of a six-year-old male goat with multicentric B-cell lymphoma is described.

CASE DESCRIPTION

Case history and clinical examination

A routine consultation with preventive hoof care and deworming was performed, by the referring veterinarian, on two goats. The owner noticed that one of



Figure 1. A six-year-old, male pygmy goat with multicentric large B-cell lymphoma. Enlarged inguinal lymph nodes were noticed on clinical examination.

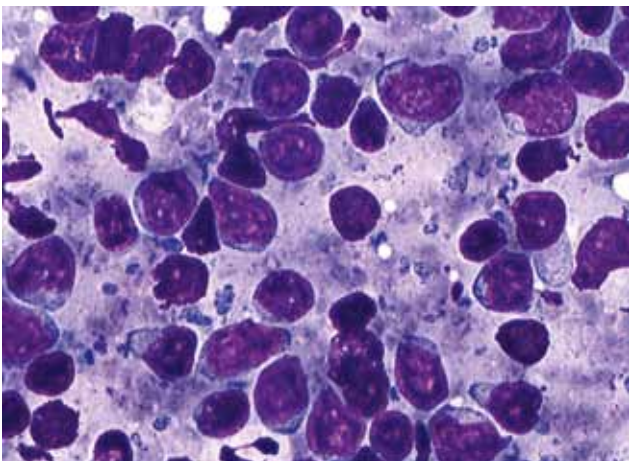


Figure 2. Cytology of a fine needle aspiration from the prescapular lymph node: monomorphic population of large round cells with a large dark blue and round nucleus and a small amount of light blue cytoplasm intermixed with some small, most likely reactive T-lymphocytes (Hemacolor®, Merck KGaA, Germany).

the goats (male and six years old) seemed more dull recently and showed a stiff gait. A thorough clinical examination was performed, but no significant abnormalities were detected, apart from mild swelling of the left inguinal lymph node. Treatment with 10 mg doramectine (Dectomax®, Pfizer AH, Belgium) and 2 mg dexamethasone (Rapidexon®, Eurovet, Belgium) was started. On a control visit one month later, there was clinical deterioration and the animal demonstrated dysphagia. Clinical examination revealed swollen retropharyngeal, prescapular and inguinal lymph nodes (Figure 1). Complete bloodwork was performed and serologic testing was done for bovine leukemia, caprine arthritis-encephalitis virus and caseous lymphadenitis; all tested negative (Table 1). Treatment was started with 1500 mg natriummetamizol en 12 mg butylhyoscine (Buscopan compositum ad us vet®, Boeinger, Belgium) and 6 mg dexamethasone (Rapidexon®, Eurovet, Belgium).

Despite treatment, the animal didn't improve and was referred the next day to the Large Animal Internal Medicine Department of the Faculty of Veterinary Medicine, Ghent University, Belgium. Complete bloodwork was repeated and revealed a mild leukocytosis with elevation of the segmented neutrophils (Table 1). Leukocyte differentiation on a blood smear (Hemacolor®, Merck KGaA, Germany) revealed 43% lymphocytes and 57% neutrophils. No abnormal cells were detected. Blood biochemistry revealed several mild abnormalities and a sharply increased creatine kinase, which was probably due to hemolysis. An abdominal and thoracic ultrasound was performed, but no internal mass or any other abnormality could be visualized. Ultrasound of peripheral lymph nodes showed sharp delineation of these lymph nodes with a homogenous echogenicity. Fine needle aspirates were taken (20G needle, 4 cm long) from the left and right prescapular lymph node for cytological evaluation and stained with Hemacolor® (Merck KGaA, Germany). Both aspirates revealed a monomorphic population of large round cells (20-35µm) with a large dark blue and round nucleus and a small amount of light blue cytoplasm (Figure 2). These cells were identified as lymphoblasts. Since these findings were indicative of lymphoma, it was decided to euthanize the animal.

Necropsy, histology and immunohistochemistry

Full necropsy was performed and confirmed the generalized peripheral lymphadenopathy primarily of the prescapular, mandibular, inguinal and popliteal lymph nodes (Figure 3). On closer inspection, enlargement of the deep cervical (Inn. cervicalis profundum, cranialis and caudalis), retropharyngeal (Inn. retropharyngealis) and thoracic lymph nodes (Inn. thoracicum dorsalis and ventralis) was noted. The abdominal lymph nodes of the rumen (Inn. ruminalis), mesentrium (Inn. mesentericum cranialis and ventralis) and aortic lymph nodes (Inn. hemales) were also enlarged. The liver was pale and showed a marked zonal pattern. Gross lesions were not detected in other organs. Within the hour after euthanasia, a bone marrow cytologic sample was taken, using a 18G needle, for a cytologic smear and stained (Hemacolor®, Merck KGaA, Germany). The number of lymphocytes was increased, but no abnormal cells were observed.

Samples of the prescapular lymph node, spleen, lung and liver were fixed in a 4% neutral-buffered formalin, routinely processed and embedded in paraffin. Five-µm-thick sections were mounted and stained with hematoxylin and eosin for histological examination. There were no significant histological abnormalities present in the spleen, lung and liver. The lymph nodes however, had completely lost their normal architecture due to massive infiltration of a monomorphic population of large (12-15µm) neoplastic lymphoblasts. There were mild anisokaryosis and anisocytosis. Mitotic numbers were less than one per ten high power field. The neoplastic population infiltrated the capsule multifocally. Immunolabelling for CD3 (T-cells) and

CD20 (B-cells) was performed as previously described (Vangeel et al., 2012). More than 95% of the cells demonstrated a membranous labeling for CD20, while only a small population of cells were positive for CD3 (Figure 4). Based on these results, this neoplasia was characterized as a multicentric large B-cell lymphoma.

DISCUSSION

Pygmy goats are increasingly popular as companion animals, which increases their lifetime and the amount of veterinary attention demanded by their owners. Lymphoma is the most common tumor in goats, followed by cutaneous squamous cell carcinoma and thymoma (Löhr, 2012). Multicentric lymphoma is the most common type of caprine lymphoma (De Silva et al., 1985; Craig et al., 1986; DiGrassie, 1997 and Löhr, 2012). Age ranges from one to nine years and no breed or gender predilection has been detected so far (Löhr, 2012). The most common clinical sign is loss of normal body condition (Higgins et al., 1984; Craig et al., 1986 and DiGrassie et al., 1997). General peripheral lymphadenopathy, as described in the present case, is not a consistent clinical finding, but has been described in other cases (Baker and Sherman, 1982; Higgins et al., 1984; De Silva et al., 1985; Craig et al., 1986; DiGrassie et al., 1997; Guedes et al., 1998). Other clinical signs are variable and related to the localization of the tumor. Involvement of the head with mandibular and maxillary bone swelling as well as conjunctival swelling or exophthalmos have been described (Baker and Sherman, 1982; De Silva et al., 1985; Craig et al., 1986; Guedes et al., 1998; Valentine et al., 2011). Paraparesis has been reported in a case of lymphoma with involvement of the spinal cord (Gygi et al., 2004).

Coughing and dyspnea were present in a case of caprine pulmonary lymphoma (Matthews, 1999). It has been suggested that lymphoma should be considered as a differential diagnosis in any goat over two years of age with any signs of disease (Craig et al., 1986).

A retroviral cause for caprine lymphoma has been proposed because some goats infected with bovine leukemia virus develop multicentric lymphoma (Olson et al., 1981). Unfortunately, in only two cases, serological testing was performed for exposure to bovine leukemia virus and the results were negative (Valentine et al., 2011). In addition, serologic examination for bovine leukemia virus was also negative in the present case, so the role of a retrovirus in the etiopathogenesis of caprine lymphoma still needs to be demonstrated.

In this case report, fine needle aspirates of two enlarged peripheral lymph nodes were taken for cytologic evaluation and proved to be accurate for the diagnosis of lymphoma. Since fine needle aspiration is minimally invasive, does not require patient sedation, is rapidly performed and inexpensive, it is commonly used in humans and dogs for the diagnosis of non-Hodgkin's lymphoma/leukemia (Caniatti et al., 1996; Young et al., 1998; Culmsee et al., 2001; Dong et al., 2001; Zeppa et al., 2003; Bangerter et al. 2007). In cattle, it was demonstrated that fine needle aspiration of enlarged peripheral lymph nodes is more specific than core needle biopsy of enlarged peripheral lymph nodes for the ante mortem diagnosis of enzootic bovine lymphosarcoma (Washburn et al., 2007). In goats, this technique has seldom been reported (Baker et al., 1982; de Silva, 1985), but it is a useful tool for practitioners. Furthermore, it is the experience of the authors that unstained cytological specimens can be used for immunophenotyping.

Table 1. Hematology and biochemistry results in a 6 year old male pygmy goat with multicentric large B-cell lymphoma.

	Parameter	Result 28/08	Result 12/09	Reference
Hematology	Hematocrit	152 ml/l	220 ml/l	300-400 ml/l
	Leukocytes	26,9 x 10 ⁹ /l	14,0 x 10 ⁹ /l	4-13 x 10 ⁹ /l
	Neutrophils	0,2 x 10 ⁹ /l	1,3 x 10 ⁹ /l	0,6-4 x 10 ⁹ /l
	Lymphocytes	2,3 x 10 ⁹ /l	2,2 x 10 ⁹ /l	2,5-7,5 x 10 ⁹ /l
	Monocytes	0,39 x 10 ⁹ /l	/	0,025-0,84 x 10 ⁹ /l
	Eosinophils	0 x 10 ⁹ /l	/	0-2,4 x 10 ⁹ /l
	Basophils	0,001x 10 ⁹ /l	/	0-0,2 x 10 ⁹ /l
Biochemistry	Total serum protein	64 g/l	51 g/l	64-70 g/l
	Albumin	65,3%	/	25-49%
	Alfa globulins	19,7%	/	8,18 ± 3%
	Beta-globulins	7,9%	/	5,6 ± 1%
	Gamma-globulins	7,1%	/	20,4 ± 5%
	Total bilirubin	0,1 mg/dl	/	0-0.1 mg/dl
	Urea	/	14,1 mmol/l	2,16-10,5 mmol/l
	GPT	/	214 U/L	50-130 U/L
	AST	/	2500 U/L	46-161 U/L
	LDH	/	8400 U/L	123-392 U/L
	CPK	/	22500U/L	104-219 U/L
	AF	/	50 U/L	27-210 U/L
	γ-GT	/	71 U/L	34-65 U/L



Figure 3. Necropsy of a six-year-old, male pygmy goat with multicentric large B-cell lymphoma. Bilateral enlarged mandibular lymph nodes.

The cell origin of caprine lymphoma has been poorly studied and in only two cases of caprine lymphoma, immunophenotyping has been reported. In one case, a diagnosis of T-cell lymphoma was made (Rozaer et al., 1998), whereas the second case was a multicentric B-cell lymphoma (Valentine et al., 2011). Since in the present case, a B-cell origin was also demonstrated in the multicentric form, it would be interesting to immunophenotype further cases. Improved knowledge of phenotype could be useful to evaluate therapies and/or to predict prognosis.

Treatment of lymphoma in goats is not routinely performed, but since more and more pygmy goats are kept as companion animals, it is likely that owners will demand it in the future. Currently, there are no reports of effective treatment. Only administration of glucocorticoids might cause transient improvement because of lympholysis (Radostits, 2007). Treatment has been extensively studied in small companion animals and the treatment options vary from inexpensive single-agent prednisolone to aggressive chemotherapy, radiation therapy and even bone marrow transplantation. In dogs and cats, the backbone of the current therapy for B and T-cell lymphoma is systemic multi-agent chemotherapy, using drugs, such as vincristine, cyclophosphamide and doxorubicin, with or without L-asparaginase (Chun, 2009; Marconato, 2010).

The prognosis for dogs with multicentric lymphoma is influenced by the clinical stage and substage of disease according to WHO classification at the time of diagnosis. Immunophenotyping is also clinically relevant, because T-cell immunophenotype is a significantly adverse prognostic factor (Teske et al., 1994; Ruslander et al., 1997). For both B- and T-cell lymphoma's, an important factor influencing survival time is the presence or absence of complete remission during chemotherapy (Jagielsky et al., 2002). In goats, this disease has a very rapid course, with most animals dying within one to two months after clinical diagnosis (Jubb and Kennedy, 2007).

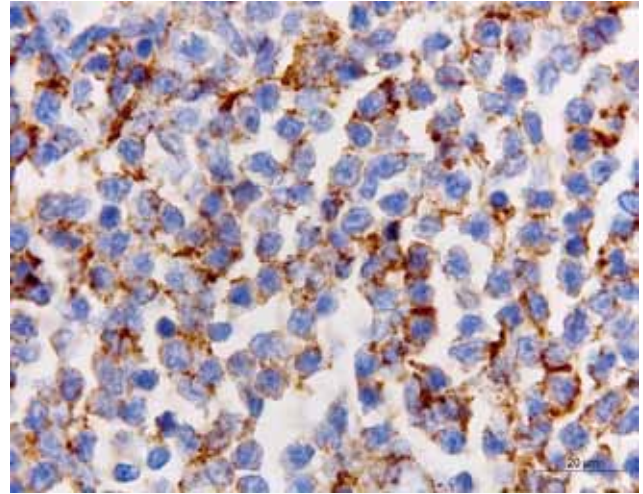


Figure 4. Immunolabeling for CD20 of a sample of the prescapular lymph node. More than 95% of the cells demonstrated a positive membranous labeling. (bar: 20µm).

In conclusion, clinicians should keep caprine lymphoma as a differential diagnosis in mind in many rather vague clinical presentations. Careful inspection and palpation of the peripheral lymph nodes during clinical examination are crucial. In this case report, cytologic evaluation of fine needle aspirates proved to be a simple and useful method to confirm the diagnosis in vivo. Immunophenotyping could be useful to predict prognosis.

REFERENCES

- Baker J.C., Sherman D.M. (1982). Lymphosarcoma in a Nubian goat. *Veterinary Medicine Small Animal Clinician* 77, 557-559.
- Bangerter M., Brudler O., Heinrich B., Grieshamner M., (2007). Fine needle aspiration cytology and flow cytometry in the diagnosis and subclassification of non-Hodgkin's lymphoma based on the World Health Organization classification. *Acta Cytologica* 51, 390-398.
- Braun U., Jehle W., Soldati G. (2005). Malignant cerebellar lymphoma in a calf. *Veterinary Record* 156, 215-216.
- Canniatti M., Roccabianca P., Scanziani E., et al. (1996). Canine lymphoma: immunocytochemical analysis of fine-needle aspiration biopsy. *Veterinary Pathology* 33, 204-212.
- Chun R. (2009). Lymphoma: which chemotherapy protocol and why? *Topics in Companion Animal Medicine* 24 (3), 157-162.
- Craig R., Roth L., Smith M.C. (1986). Lymphosarcoma in goats. *Compendium on Continuing Education for the Practicing Veterinarian* 8, 190-197.
- Culmsee K., Simon D., Mischke R., et al. (2001). Possibilities of flow cytometric analysis for immunophenotypic characterization of canine lymphoma. *Journal of Veterinary Medicine Series A* 47, 199-206.
- de Silva L.N.A., Winter M.H., Jackson P.G.G., Bostock D.E. (1985). Lymphosarcoma involving the mandible in two goats. *Veterinary Record* 117, 276.
- De Vlieghe S., Opsomer G., Hoflack G., De Bosschere H., Deprez P., de Kruijff A. (2000). Een thymus lymfoma bij

- een vaars. *Vlaams Diergeneeskundig Tijdschrift* 69, 44-46.
- DiGrassie W.A., Wallace M.A., Sponenberg D.P. (1997). Multicentric lymphosarcoma with ovarian involvement in a Nubian goat. *The Canadian Veterinarian Journal* 38, 383-384.
- Dong H.Y., Harris N.L., Preffer F.I., Pitman M.B. (2001). Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: a retrospective analysis of the utility of cytomorphology and flow cytometry. *Modern Pathology* 14, 472-481.
- Gygi M., Kathmann I., Konar M., Rottenberg S., Meylan M. (2004). Paraprese bei einer Zwergziege: Abklärung mittels Magnetresonanztomographie. *Schweizer Archiv für Tierheilkunde* 146, 523-528.
- Guedes RMD., Facury Filho E.J., Lago L.A. (1998). Mandibular lymphosarcoma in a goat. *Veterinary Record* 143, 51-52.
- Hemsley S., Bailey G., Canfield P. (2002). Immunohistochemical characterization of lymphosarcoma in two alpacas (*Lama pacos*). *Journal of Comparative Pathology* 127, 69-71.
- Higgins R.J., Poole A., Wilson K.E. (1984). Multicentric lymphosarcoma in a Saanen Goat. *Veterinary Record* 114, 170.
- Irwin J.A. (2001). Lymphosarcoma in an alpaca. *The Canadian Veterinary Journal* 42, 805-806.
- Jagielski D., Lechowski R., Hoffmann-Jagielska M., Winiarczyk S. (2002) A Retrospective Study of the Incidence and Prognostic Factors of Multicentric Lymphoma in Dogs (1998-2000) *Journal of Veterinary Medicine Series*, 49 (8), 419-424.
- Kleinschmidt S., Peters M., Wohlsein P. (2012). Presumptive primary central nervous system T cell lymphoma in a deer (*Capreolus capreolus*). *Research in Veterinary Science* 93, 1334-1336.
- Löhr C.V. (2012). One hundred two tumors in 100 goats (1987-2011). *Journal of Veterinary Pathology* 50 (4), 668-675.
- Madson D.M. and Opriessnig T. (2009). Multicentric T-cell lymphosarcoma in a White-tailed Deer. *Journal of Wildlife Diseases* 45 (3), 791-794.
- Malastestic A. (2003). Bilateral exophthalmos in a Holstein cow with lymphosarcoma. *The Canadian Veterinary Journal* 44, 664-666.
- Matthews J. (1999). External swellings. In: *Diseases of the Goat*. Second edition. Blackwell Science, Paris, France. 122-132.
- Marconata L. (2011). The staging and treatment of multicentric high-grade lymphoma in dogs: A review of recent developments and future prospects. *The Veterinary Journal* 188, 34-38.
- Olson C. (1981a). Lymphosarcoma. In: *Current Veterinary Therapy Food Animal Practice I*. Saunders, Philadelphia: 640-642.
- Olson C., Baumgartner L.E. (1975). Lymphosarcoma of cattle. *Bovine Practice* 10, 15-22.
- Olson C., Kettmann R., Burny A., Kaja R. (1981b). Goat lymphosarcoma from bovine leukemia virus. *Journal of the National Cancer Institute* 67, 671-675.
- Puette M., Latimer K.S. (1997). Acute granulocytic leukemia in a slaughter goat. *Journal of Veterinary Diagnostic Investigation* 9, 318-319.
- Radostits O.M., Gay C.C., Hinchcliff K.W., Constable P.D. (2007). Diseases of the hemolymphatic and immune systems. In: *Veterinary Medicine, a Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. Tenth edition, Saunders Elsevier, Spain: 462-463.
- Reissig E.C., Valli V.E., Pesavento P., Massone A.R., Iovanitti B., Gimeno E.J., Uzal, F.A. (2013) Extranodal follicular lymphoma in the lung of a free-ranging red deer. *Journal of Veterinary Diagnostic Investigation* 25 (1), 158-161.
- Rozear L., Love N.E., Van Camp S.L. (1998). Radiographic diagnosis: pulmonary lymphosarcoma in a goat. *Veterinary Radiology and Ultrasound* 39, 528-531.
- Ruslander D.A., Gebhard D.H., Tompkins M.B., Grindem C.B., Page R.L. (1997) Immunophenotypic characterization of canine lymphoproliferative disorders. *In Vivo*. 11(2), 169-72.
- Smith J.A. (1989). Noninfectious diseases, metabolic diseases, toxicities, and neoplastic diseases of South American camelids. *Veterinary Clinics of North America: Food Animal Practice* 5, 101-143.
- Smith M.C., Sherman D.M. (1996). Lymph node enlargement. In: *Goat Medicine*. Lea & Febiger, Philadelphia, 197.
- Teske E., Wisman P., Moore P.F., van Heerde P. (1994). Histologic classification and immunophenotyping of canine non-hodgkins lymphoma: unexpected high frequency of t cell lymphomas with B cell morphology. *Experimental hematology* 22, 1179-1187.
- Underwood W.J., Bell T.G. (1993). Multicentric lymphosarcoma in a lama. *Journal of Veterinary Diagnostic Investigation* 5, 117-121.
- Vale-Echeto O.E., Montiel-Urdaneta N., Simoes D., Vale-Oviedo O.R., Parra-Maldonado O., Oviedo de Vale M.G., García A. (2009). Multicentric lymphoma or multicentric lymphosarcoma in water buffalo (*Bubalus bubalis*): pathologic anatomy. Report of one case. *Revista Científica, Facultad de Ciencias Veterinarias, Universidad del Zulia* 19 (3), 257-263.
- Valentine B.A., Stieger-Vanegas S., Brown S. R., Tornquist S. J., Young K. (2011). Exophthalmos due to multicentric B-cell lymphoma in a goat. *Canadian Veterinary Journal* 52 (12), 1350-1352.
- Vangeel L., Houf K., Geldhof P., Nollet H., Vercruyse J., Ducatelle R., Chiers K. (2012). Intramuscular inoculation of cattle with *Sarcocystis* antigen results in focal eosinophilic myositis. *Journal of Veterinary Parasitology* 183 (3-4), 224-230.
- Washburn KE., Streeter RN., Lehenbauer TW., Snider TA., Rezabek GB., Ritchey JW., Meinkoth JH., Allison RW., Rizzi TE., Boileau MJ. (2007). Comparison of core needle biopsy and fine-needle aspiration of enlarged peripheral lymph nodes for antemortem diagnosis of enzootic bovine lymphosarcoma in cattle. *Journal of American Veterinary Medicine Association* 230, 228-232.
- Young NA., AL-Saleem TI., Ehya H., et al. (1998). Utilization of fine-needle aspiration cytology and flow cytometry in the diagnosis and subclassification of primary and recurrent lymphoma. *Cancer Cytopathology* 84, 252-261.
- Zeppa P., Picardi M., Marino G., Troncone G., Fulciniti F., Vetrani A., Rotoli B., Palombini L. (2003). Fine-needle aspiration biopsy and flow cytometry immunophenotyping of lymphoid and myeloproliferative disorders of the spleen. *Cancer* 99, 118-127.