Assiut Vet. Med. J. Vol. 58 No. 135 October 2012

CLINCOPATHOLOGICAL AND BIOCHEMICAL STUDIES ON THYMIC LYMPHOMA IN CATTLE

MOHAMED THARWAT[*] and IHA. ABD EL-RAHIM[**]

[*]Department of Animal Medicine (Internal Medicine), Faculty of Veterinary Medicine, Zagazig University, Egypt
[**]Departmant of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Assiut University, Egypt
Authors' current address: Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

ABSTRACT

This study describes the clinical manifestations, hematological and biochemical changes as well as postmortem findings of thymic lymphoma in three young cattle with cervical swelling. Anorexia, weight loss, distended jugular veins, difficult breathing, severe tympany and ataxia were the main observed clinical signs. The results of the indirect enzyme-linked immunosorbent assay indicated that all animals were seropositive for bovine leukemia virus infection. Fine needle aspiration biopsy of cervical mass revealed that numerous lymphocytes (typical and atypical) are apparent. Hematologoical examinations indicated leukocytosis with severe lymphocytosis, while biochemical analysis showed hyperglycemia, increased concentrations of non-esterified fatty acids and hydroxy butyric acid, and increased serum activity of aspartate aminotransferase and lactate dehydrogenase. Due to poor prognosis, the three cattle were euthanized and postmortem examination was thoroughly carried out and revealed presence of tumor in both cervical and thoracic thymic gland, cardiomyopathy, distended hepatic veins congested lung and hemothorax. The current study concluded that thymic lymphoma must be considered in the differential diagnosis in heifers with cervical swelling, severe tympany, and jugular venous distention.

Received at: 14/7/2012

Accepted: 25/9/2012

Keywords: *Cattle, thymic lymphoma, hematologoical examinations, biochemical analysis, postmortem findings.*

INTRODUCTION

Familial thymic lymphosarcoma was reported in calves. The calves were negative for bovine leukemia virus-specific antibodies. Most of the calves had been sired by the same bull. Morphological studies including light and electron-microscopic cytology, and serological and virological studies of 14 of the cases suggest that the disease was transmitted genetically (Parodi *et al.*, 1989; Da Costa *et al.*, 1991). In another study, familial thymic B lymphosarcoma was described in cattle. In the young females' offspring of one bull, more than 3% of animals developed a thymic lymphoblastic lymphosarcoma (Da Costa *et al.*, 1992).

Thymic lymphosarcoma was observed in a 2-year-old Holstein heifer with a swollen brisket, jugular vein distention, muffled heart sounds, tachycardia, and free gas bloat. Thymic lymphosarcoma was suspected based on a negative agar gel immunodiffusion test for bovine leukemia virus, presence of atypical lymphocytes in pleural fluid, and detection of a mass in the thoracic inlet. Right-sided cardiac catheterization was performed, and markedly increased jugular venous pressures (41 mm Hg) with a pressure gradient of 29 mm Hg immediately cranial to the heart indicated constriction of the cranial vena cava. Immunohistochemical staining of formalin fixed, paraffin-embedded tissue sections of the tumor using a rabbit anti-human T cell, CD3 polyclonal antibody confirmed that the neoplastic lymphocytes were of thymic origin (Alexander *et al.*, 1996).

Thymic lymphosarcoma with metastases was found in a heifer with spinal cord compression and pelvic limb paresis. Neurological examination of a heifer displaying pelvic limb ataxia and paresis progressing to a dog-siting position but with normal thoracic limb function indicated a spinal cord lesion in the low thoracic/high lumbar region. The progressive neurological deterioration despite normal radiological and cerebrospinal fluid findings was suggestive of a non-inflammatory, non-traumatic extradural compressive lesion; this was subsequently shown to be a lymphosarcoma (Holmes, 1990).

Retrospective case study was conducted with a search of all medical records at Cornell University for cattle diagnosed with lymphosarcoma. Categorical data were analyzed with a Wilcoxon rank-sum tests. Sensitivities of diagnostic tests were calculated. There were 106 cows and 6 bulls (median age 5 years) examined for anorexia (34%), weight loss (16%), and fever (14%). The sensitivities of ante-mortem diagnostic tests

performed were peripheral lymph node (PLN) wedge biopsy, 100%; surgical exploration and biopsy, 100%; pleurocentesis, 80%; pericardiocentesis, 67%; PLN fine-needle aspirate, 41%; abdominocentesis, 33%; and cerebral spinal fluid tap, 19%. Median peripheral blood lymphocytic count was 4,900 cells/μL, 10% of cattle were leukemic and 25% had lymphocytosis according to the Bendixen Key. The most frequently identified tumor locations (% of cattle) were the heart (66%), abomasum (61%), uterus (38%), kidney (32%), and epidural space (26%). Predilection sites were found a higher incidence of renal tumors and lower incidence of retrobulbar tumors (Burton *et al.,* 2010).

Criteria for the differentiation of thymic lymphoma and haematoma of the ventral neck in cattle were studied (Braun *et al.,* 2007), where four cattle with thymic lymphoma and seven with haematoma of the ventral neck underwent physical, haematological, biochemical and ultrasonographic examinations as well as histological evaluation of a biopsy specimen. All of the animals had firm non-painful swellings, which were no warmer than normal and extended from the pharyngeal region to the thoracic inlet. The diagnosis could not be established based on the results of physical examination and palpation of the swellings. Animals with a haematoma had a shorter history of being ill compared to those with thymic lymphoma. In patients with a haematoma, anaemia was present, the heart rate was elevated and the haematocrit was decreased, but not in cattle with thymic lymphoma. A tentative diagnosis was done on the basis of a macroscopic evaluation of the biopsy samples, which were dark red and firm in cattle with a haematoma. All cases of malignant thymic lymphoma were confirmed by histological examination of a biopsy sample. All animals with thymic lymphoma and three of those with a haematoma were euthanased. The four remaining animals with a haematoma were treated successfully.

Bovine lymphosarcoma has multiple clinical signs, biochemical changes and postmortem findings (Radostits, 2005). So that, the current study aimed to describe the clinical manifestations, hematological changes and biochemical analysis as well as postmortem findings of thymic lymphoma in three heifers presented with cervical swelling.

MATERIALS and METHODS

Animals, history, clinical and examination

In the present study, three heifers were examined at the Veterinary Teaching Hospital, Rakuno Gakuen University, Hokkaido, Japan. Animals were aged eight months to two years and weighed 160 to 250 kg. Cases had been ill for 3 to 4 weeks before admission. All animals underwent a thorough clinical examination as described previously (Rosenberger, 1990; Radostits *et al.,* 2000), which included general behavior and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination.

Hematological examination, biochemical analyses and necropsy findings

Two blood samples were collected by puncture of the jugular vein, one on EDTA and the other without an anticoagulant. A complete blood count (hematocrit, hemoglobin, red blood cells, total and differential leucocytes) was carried out on the whole blood samples. After centrifugation of the second blood sample, serum samples were collected and then frozen for later analysis of clinical chemistries. Commercial kits were used to determine the serum concentrations of total protein, albumin, calcium, phosphorus, magnesium, glucose, total cholesterol, non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), urea nitrogen (UN), creatinine, sodium, potassium and chloride. The serum activities of aspartate aminotransferase (AST), γ-glutamyl transferase and creatine kinase (CK) were also measured in serum samples. Serum protein fractions were determined by electrophoresis. The obtained serum samples were submitted for detection of antibodies against bovine leukaemia virus (BLV) by using enzyme-linked immunosorbent assay (ELISA) (Simard *et al.,* 2000). Due to the poor prognosis, animals were euthanised and thoroughly examined postmortem.

Statistical analysis

Data of the hematological and biochemical parameters were compared between diseased and control animals using the Student's *t* test.

RESULTS

The results of the serum enzyme-linked immunosorbent assay indicated that all animals were seropositive for BLV infection. Anorexia, weight loss, cervical swelling, distended jugular veins, right-sided heart failure, difficult breathing, and ataxia. The general condition was moderate in all the 3 studied cases. Severe ruminal tympany and atony were seen in all the 3 examined heifers (Figure 1). There was enlargement of certain lymph nodes, which included prescapular lymph nodes in 2 cattle, prefemoral lymph nodes in 1 animal. Values of rectal temperature, pulse rate and heart rate were 40.5±0.6, 85±20 and 28±10, respectively. Other signs included pale mucous membranes. Fine needle aspirate of the swelling revealed the presence of numerous typical and atypical lymphocytes (Figure 2).

Table 1 summarizes hematological and biochemical findings in heifers with thymic lymphoma. Hematologoical examinations showed leukocytosis with severe lymphocytosis, while biochemical analysis showed hyperglycemia, increased concentrations of non-esterified fatty acids (NEFA) and hydroxy butyric

acid (BHBA), and increased serum activity of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

Postmortem examination showed tumor of the thymus gland both in the neck and inside the thoracic cavity (Figure 3), cardiomyopathy, distended hepatic veins (Figure 4), small left kidney was 270 g (Figure 5), congested lung, hemothorax and enlarged pre-scapular lymph nodes (Figure 6).

Table 1: Hematological and biochemical findings in cattle with thymic lymphoma.

Parameters	Finding (*n*=3)	Reference value
Hematocrit (%)	38±6	24-46
Hemoglobin (g/dL)	13±4	8.0-15.0
Leukocyte count (/µL)	18250±5020**	4000-12000
Neutrophils (/µL)	4653±4200	600-4000
Lymphocytes (/µL)	10676±2318**	2500-7500
Atypical lymphocyte	2464±365***	0.0-0.0
Total protein (g/dL)	7.1±0.4	6.7-7.5
Albumin (g/dL)	2.0±0.9	2.1-3.6
α-globulin (g/dL)	1.0±0.4	0.75-0.88
β-globulin (g/dL)	0.7±0.3	0.88-1.10
γ-globulin (g/dL)	3.2±0.3	1.7-2.3
Aspartate aminotransferase (U/L)	278±32*	78-132
γ-glutamyl transferase (U/L)	17±5	6.1-17.4
Glucose (mg/dL)	104±19*	45-75
Blood urea nitrogen (mg/dL)	17±8	6.0-27
Cholesterol (mg/dL)	86±20	65-220
Calcium (mg/dL)	9±0.7	9.7-12.4
Magnesium (mg/dL)	1.8±0.2	1.8-2.3
Phosphorus (mg/dL)	5.5±0.7	5.6-6.5
Sodium (mEq/L)	137±5	132-152
Potassium (mEq/L)	3.3±0.3	3.9-5.8
Chloride (mEq/L)	94 ±6	95-110
Creatine kinase (U/L)	1335±42**	35-280
Lactate dehydrogenase (U/L)	6060±4200***	692-1445
Non-esterified fatty acid (mEq/L)	0.7±0.4***	0.07-0.15
B-□ hydroxybutyric acid (µmol/L)	620±93*	100-400

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Reference values from Radostits et al., 2007.

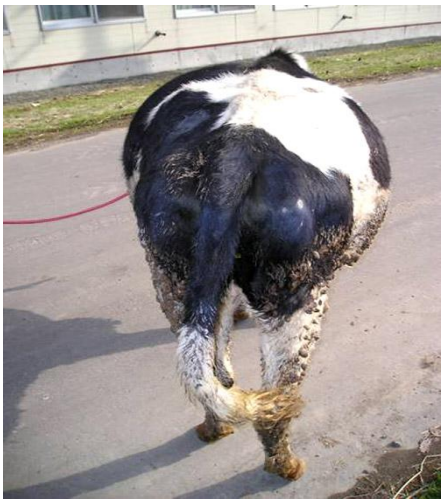

Figure 1: Severe tympany in a heifer with thymic lymphoma.

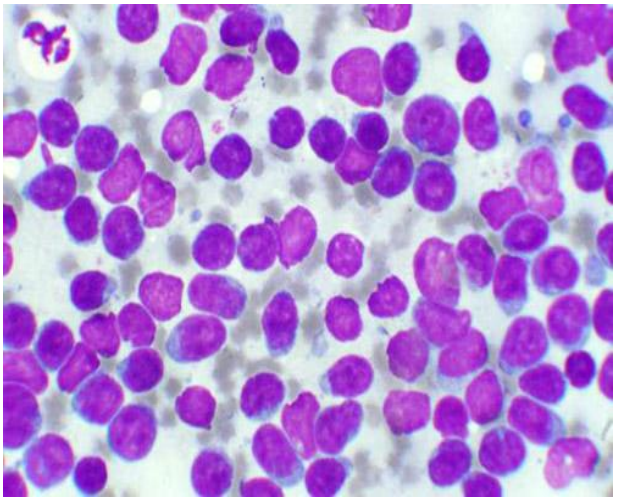

Figure 2: Fine needle aspiration biopsy of cervical mass in a heifer with thymic lymphoma. Numerous lymphocytes (typical and atypical) are apparent. Giemsa stain.

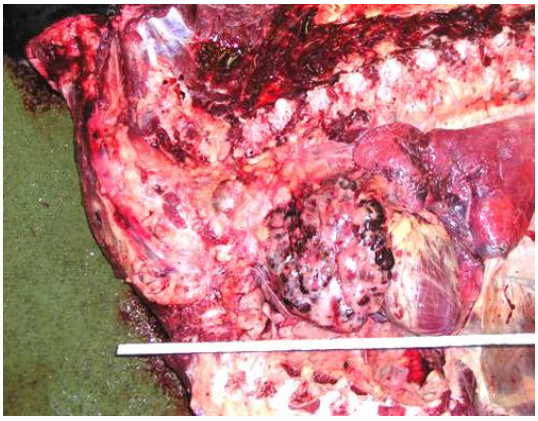

Figure 3: Postmortem examination in a heifer with thymic lymphoma. Both the cervical and thoracic parts of the thymus are enlarged.

Figure 4: Postmortem examination in a heifer with thymic lymphoma. The liver is enlarged and the hepatic veins are severely distended.

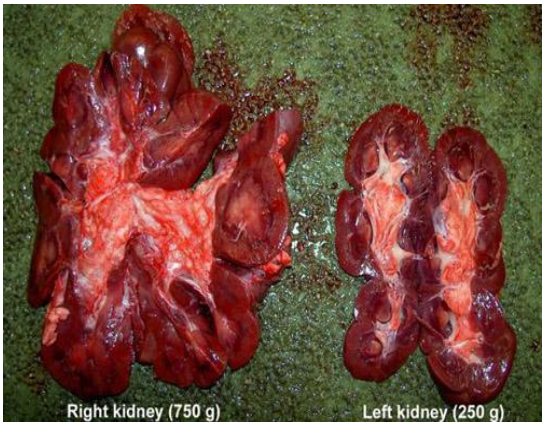

Figure 5: Postmortem examination in a heifer with thymic lymphoma. The right kidney is enlarged and the left kidney is smaller than normal.

Figure 6: Postmortem examination in a heifer with thymic lymphoma. The pre-scapular lymph node is enlarged.

DISCUSSION

The current study recorded thymic lymphoma in three heifers aged eight months to two years. The three heifers were presented because of a severe swelling in the thymic region. Due to the poor prognosis, animals were euthanised and thoroughly examined postmortem. Radostits (2005) stated that thymic lymphosarcoma is a common finding in animals 1-2 years of age. In a case report, De Vliegher *et al.* (2000) described thymic lymphoma in a Red Holstein heifer. The heifer was presented because of a severe swelling at the throat and ventral neck region. Based on the clinical examination the diagnosis of thymic neoplasia was made. Because of the poor prognosis the heifer was euthanized, autopsied and histopathological examination revealed a thymic lymphoma.

Thymic lymphosarcoma must be considered in the differential diagnosis in heifers with fever, bloat, and jugular venous distention (Hatfield *et al.*, 1986). In the present study anorexia, weight loss, cervical swelling, distended jugular veins, right-sided heart failure, difficult breathing, severe tympany and ataxia were the most common reported clinical manifestations. Radostits (2005) reported that thymic lymphosarcoma is characterized by massive thymic enlargement in the brisket area and lesions in bone marrow and regional lymph nodes. Jugular vein engorgement and marked brisket edema extending to the submandibular region are common. Moderate bloat due to inability to eructate because of esophageal compression may occur. The thymic mass is usually not palpable. In another study, ventral edema, dyspnea, fever, tachycardia, bloat and muffled heart sounds were identified in a 3-year-old heifer suffering from thymic and mammary lymphosarcoma (Matthews *et al.*, 1992).

Metastatic thymic lymphosarcoma was diagnosed in a 16-month-old mixed-breed heifer with a history of progressive weight loss. Physical examination revealed cachexia, pale mucous membranes, large peripheral lymph nodes, and a 15 X 40-cm mass in the ventral portion of the neck, extending cranially from the thoracic inlet. Neoplastic lymphocytes were identified in aspirates of pleural effusion and bone marrow. Histopathological examination of necropsy specimens substantiated metastatic dispersal of the tumor into lymphoid tissue, liver, intestine, heart, and kidney. This case differs from other reported cases of thymic lymphosarcoma because of the involvement of organs other than the thymus and lymph nodes (Angel *et al.*, 1991). While, the observed clinical signs of thymic lymphoma in the present study were similar to the most common previously reported one.

In the current study, necropsy showed tumor of the thymus gland both in the neck and inside the thoracic cavity, cardiomyopathy, distended hepatic veins, small left kidney (270 g), congested lung, hemothorax. In a previous study, lymphosarcoma of the pharyngeal region was diagnosed in a 7-month-old beef steer presented for retropharyngeal swelling and dyspnea. Biopsy of the swelling indicated lymphoblastic lymphosarcoma, and necropsy revealed involvement of regional veins and arteries (Ivany *et al.*, 2000).

In the present study ELISA was used for detection of the BLV antibodies in the sera of the investigated heifers and the results indicated that all animals were seropositive. It was concluded that ELISA can fully replace radioimmunoassays in the serodiagnosis of enzootic bovine leukosis (Ressang *et al.*, 1981). Thymic form of lymphosarcoma was recorded in three heifers. Detectable antibody to bovine leukosis virus was not found on agar gel immunodiffusion testing in any of the cases. Thymic lymphosarcoma was confirmed by necropsy and histologic examination (Hatfield *et al.*, 1986). As far as in the present study, hematologoical examinations indicated leukocytosis with severe lymphocytosis in the three examined animals.

The laboratory abnormalities of lymphocytosis were compatible with lymphosarcoma. The increased AST and LDH activity and creatinine concentration could be attributed to hepatocellular, renal dysfunction and necrosis and due to neoplastic infiltration into the hepatic and renal parenchyma, respectively (Braun *et al.*, 2007). Elevated NEFA and BHBA concentrations could be attributed to anorexia. Elevated activity of LDH could also be attributed further due to tumor cell infiltration into muscular and other body systems (Braun *et al.*, 2007).

In conclusion, thymic lymphoma must be considered in the differential diagnosis in heifers with Anorexia, weight loss, cervical swelling, tympany, and jugular venous distention.

REFERENCES

Alexander, A.N.; Constable, P.D.; Meier, W.A.; French, R.A.; Morin, D.E.; Lowry, J.E. and Hoffman, W.E. (1996): Clinical and immunohistochemical characterization of thymic lymphosarcoma in a heifer. Journal of Veterinary Internal Medicine, 10: 275–278.

Angel, K.L.; Stott, J.; Tyler, J.W. and Groth Jr., A.H. (1991): Metastatic thymic lymphosarcoma in a calf. J. Am. Vet. Med. Assoc; 198: 1771-1773.

Burton, A.J.; Nydam, D.V.; Long, E.D. and Divers, T.J. (2010): Signalment and clinical complaints initiating hospital admission, methods of diagnosis, and pathological findings associated with bovine lymphosarcoma (112 cases). J. Vet. Intern. Med., 24: 960-964.

Braun, U.; Hauser, B.; Meyer, S. and Feller, B. (2007): Cattle with thymic lymphoma and

haematoma of the ventral neck: A comparison of findings. Vet. J., 174: 344-350.

Da costa, B.; Djilali, S.; Kessler, J.-L.; Sacré, B.; Femenia, F. and Parodi, A.L. (1991): Epidemiological and pathological studies of a familial thymic lymphosarcoma in bovine species. Leukemia, 5, (5): 420–424.

Da Costa, B.; Djilali, S.; Levy, D.; Kessler, J.L.; Cribiu, E.P. and Parodi, A.L. (1992): Partial characterization of a familial B lymphosarcoma with a thymic localization in cattle. Leukemia; 6 (7): 696-702.

De Vliegher, S.; Opsomer, G.; Hoflack, G.; De Bosschere, H.; Deprez, P. and De Kruif, A. (2000): A thymic lymphoma in a heifer Vlaams Diergeneeskundig Tijdschrift, 69: 44–46.

Hatfield, C.E.; Rebhun, W.C. and Dill, S.G. (1986): Thymic lymphosarcoma in three heifers. J. Am. Vet. Med. Assoc; 189: 1598-1599.

Holmes, L.A.; Scott, P.R. and Aldridge, B.M. (1990): Thymic lymphosarcoma with metastases causing spinal cord compression and pelvic limb paresis in a heifer. Br Vet. J.; 146: 91-92.

Ivany, J.M.; Kersting, K.W. and Thompson, J.R. (2000): Lymphosarcoma of the pharyngeal region in a 7-month-old beef steer. Can. Vet. J., 41, (6), 486-488.

Kaneko, J.J.; Harvey, J.W. and Bruss, M.L. (1997): Clinical Biochemistry of Domestic Animals, 5th ed., New York, Academic Press.

Matthews, H.K.; Hunt, E. and Duncan, D.E. (1992): Thymic and mammary lymphosarcoma in a three-year-old heifer. J. Am. Vet. Med. Assoc; 200: 699-701.

Parodi, A.L.; Da Costa, B.; Djilali, S.; Michel, B.; Alogninouwa, T.; Femenia, F.; Crespeau, F.; Fontaine, J.J. and Thibier, M. (1989): Preliminary report of familial thymic lymphosarcoma in Holstein calves. Vet Rec.; 125: 350–353.

Radostits, O.M. (2005): Bovine Leukosis. In: Merck Veterinary Manual, Kahn, C.M. (Ed.). 9th Edn., Whitehouse Station: Merck and Co., New Jersey, pp. 593-597.

Radostits, O.M.; Mayhew, I.G. and Houston, D.M. (2000): Veterinary Clinical Examination and Diagnosis. W.B. Saunders, London.

Radostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2007): In: Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W. (Eds.), Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. W.B. Saunders, London.

Ressang, A.A.; Gielkens, A.L.; Quak, J. and Mastenbroek, N. (1981): Studies on bovine leukosis. VII. Further experience with an ELISA for the detection of antibodies to bovine leukosis virus. Vet Q.; 3 (1): 31-33.

Rosenberger, G. (1990): Die Klinische Untersuchung des Rindes. 3rd edn. Berlin and Munich, Paul Parey.

Simard, C.; Richardson, S.; Dixon, P.; Belanger, C. and Maxwell, P. (2000): Enzyme-linked immunosorbent assay for the diagnosis of bovine leukosis: comparison with the agar gel immunodiffusion test approved by the Canadian Food Inspection Agency. Can. J. Vet. Res. 64, 101–106.

دراسات إكلينيكية ومرضية وكيميائية على مرض سرطان الغدة الدرقية في الأبقار

محمد ثروت ، إبراهيم حسين أحمد عبد الرحيم

هذه الدراسة تصف الأعراض الأكلينيكية، والتغيرات الدموية والكيميائية الحيوية، بالإضافة إلى نتائج التشريح المرضي لسرطان الغدة الدرقية في ثلاثة من الماشية الصغيرة كانت تعاني من تورم بمنطقة الرقبة. وكان قلة الشهية، وفقدان الوزن، وتمدد الوريد الودجي، وصعوبة التنفس، والأنتفاخ الشديد الكرش، والترنح هي العلامات السريرية الرئيسية الملحوظة. وأشارت نتائج فحص أختبار الاليزا الغير مباشر أن كل الحيوانات كانت إيجابية لعدوى فيروس اللوكيميا (فيروس السرطان الدموي الليمفاوي). وكشفت خزعة الإبرة من الورم بمنطقة العنق أن الخلايا الليمفاوية (التقليدية وغير التقليدية) عديدة وواضحة. وأشار فحص عينات الدم إلى زيادة عدد الكريات البيضاء مع زيادة شديدة في عدد الخلايا الليمفاوية، في حين أظهر التحليل الكيميائي الحيوي ارتفاع السكر في الدم، وزيادة تركيزات الأحماض الدهنية غير الاستيريه وهيدروكسي حامض البيوترك، وزيادة النشاط المصلي لأنين الأمينوترانسفريز ونازعة هيدروجين اللاكتات. ونظراً لقلة الأمل في الشفاء، تم أعدام الحالات الثلاثة وأظهرت نتائج التشريح المرضي الدقيق ورم في الغدة الدرقية في كل من الرقبة وداخل التجويف الصدري، وتضخم القلب، وتمدد الأوردة الكبدية، وأحتقان الرئتين، ووجود سائل مدمم بالتجويف الصدري. وخلصت الدراسة أنه يجب وضع سرطان الغدة الدرقية في الأعتبار عند التشخيص التفريقي في الأبقار التي تعاني من تورم في منطقة العنق، وأنتفاخ شديد بالكرش، وتمدد بالوريدي الوداجي.