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Original article

Malignant thymoma – the most common neoplasm in goats

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Abstract

In the literature the occurrence of thymomas in goats varies from 0.7 to 25%, depending on the study. Therefore the current investigation was carried out to determine the prevalence of thymoma in goats in Poland. Between 2007 and 2018 at the Warsaw Veterinary Faculty 399 goat autopsies and ultrasound examinations of the chest in other 145 goats were performed.

Mediastinal tumors were diagnosed during post mortem examination in 2 goats. Additionally, ultrasound examination of the chest revealed a large mass close to the heart in the thoracic cavity in 1 case. This goat was euthanized and an autopsy confirmed a mediastinal tumor. Histopathological examination, with immunohistochemical tests to anti cytokeratin, p63 and p40 confirmed thymomas in all three cases.

In our study thymomas were found in 0.5% (95% CI: 0.1% to 1.8%) of examined goats and they represented the most common malignancy in this species.

Key words: goats, thymus, tumor, neoplasm

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Introduction

Thymomas are neoplasms derived from thymic epithelial cells that usually have mixed cortical and/or medullary differentiation (Ströbel et al. 2014). For many years, thymomas have been thought to be benign tumors both in human and in veterinary medicine. This opinion is still sustained by the latest veterinary pathology classification published in 2016 (Jubb et al. 2016). However, this classification is based on the guidelines of World Health Organization (WHO) classification established in 2004 which classed thymomas as tumors of uncertain or unknown character, while the current WHO classification defines thymomas as unequivocally malignant tumors (ICD-O code 3) due to their well-documented metastatic potential (Marx et al. 2015a).

Thymomas have been described in humans and a variety of other mammals, such as dogs and cats (Day 1997)"page":"393-403","volume":"38","issue":"9","-source":"PubMed","abstract":"Data are presented from 30 cats and 36 dogs in which thymic disease was recognised clinically or on postmortem examination. The diagnoses included thymic lymphoma (19 cats, 12 dogs, rats (Tanaka et al. 2012), rabbits (Florizoone 2005), ferrets (Taylor and Carpenter 1995), cattle (Parker and Casey 1976), tigers (Allan et al. 2014), horses (Furuoka et al. 1987), monkeys (Schwartz et al. 2011), sheep (Sandison and Anderson, 1969) and parrots (Wernick et al. 2013, Lang et al. 2017). In comparison to other neoplasms, thymomas occur very rarely in humans. However, it seems that these tumors are not uncommon in cattle (Sandison and Anderson 1969). Thymomas accounted for 9% of 102 goat tumors in one survey (Löhr 2013). This neoplasm was diagnosed in 25% of saanen goats necropsied during a study on slow viral diseases (Hadlow 1978) which may have given rise to the opinion that thymomas were prevalent in the general goat population. However, the results of another survey published so far do not corroborate this statement. Thymomas were found in 0.7% of 1146 goats in which necropsy or biopsy was performed (Löhr 2013).

In this report we present 2 cases of thymoma diagnosed during routine post mortem examination and 1 case coincidentally found in the echocardiographic examination.

Materials and Methods

Female dairy goats aged 2-10 years from six dairy farms were enrolled in the study. They belonged to two breeds – Polish Fawn Improved (PFI) and Polish White Improved (PWI).

Between 2013 and 2014, ultrasonographic examination of the chest was performed in 145 goats as a part of a study regarding echocardiographic reference values in healthy animals (Szaluś-Jordanow et al. 2017). This examination was performed without anesthesia using a Mindray M7 machine equipped with a phased array probe (2-4 MHz). In one goat (goat no 1) an intrathoracic mass was found during this study. Later, the owner reported that this goat had been infertile for 3 preceding years, but it did not manifest any other symptoms.

In this goat X-ray and CT scans were further made under general intravenous anesthesia using ketamine (Vetketam) at dose of 10 mg/kg and xylazine (Xylapan) at dose of 0.05 mg/kg. Right and left lateral and dorso-ventral radiographs of the thorax were performed. A non-contrast CT examination of the thorax was conducted with the animal in right lateral recumbency, stabilized with multiple foam blocks, using a 2-slice CT scanner (HiSpeed CT / e Plus GE). The technical settings for the scan were 120 kV, 350 mA, 1s tube rotation, 3 mm slice collimation and a pitch of 1.2 and 5. Three-dimensional reconstructions were obtained using a graphical desktop program (Philips). Cytological examination of the mass performed after an ultrasound-guided fine-needle biopsy suggested a thymoma (a differential diagnosis also included a lymphoma). The animal was euthanized due to this neoplasm and anatomopathological examination was performed.

In addition, between 2007 and 2018, autopsies of 399 adult dairy goats (other than the above 145) culled mainly due to emaciation, low milk yield or progressive arthritis were performed. All animals were euthanized under general anesthesia (10 mg/kg ketamine + 0.05 mg/kg xylazine) by overdose of barbiturates (pentobarbital 30 mg/kg). According to the European Legal Regulations ethics approval is not required when tissues are collected during post mortem examination. Samples of macroscopically altered organs were fixed in 10% buffered formalin, embedded in paraffin, cut in sections (3 μm) and stained with haematoxylin and eosin. For immunohistochemical tests, the samples were processed in the same way, and immunostaining was performed using commercially available antibodies (Dako® Denmark): anti-cytokeratin (Monoclonal Mouse Anti-Human, clone AE1/AE3; concentration 1:100)and anti-vimentin (Monoclonal Mouse Anti--Human, clone V9; concentration 1:100). Other stainings for pancytokeratin (clone AE1AE3), p40 (clone BC28) and p63 (4A4) were performed in BenchMark GX automated immunostainer (Ventana Medical System Inc., Tucson, Arizona, USA) according to producer's recommendation. Cytological slides were fixed in 70% methanol and stained with Giemsa solution.

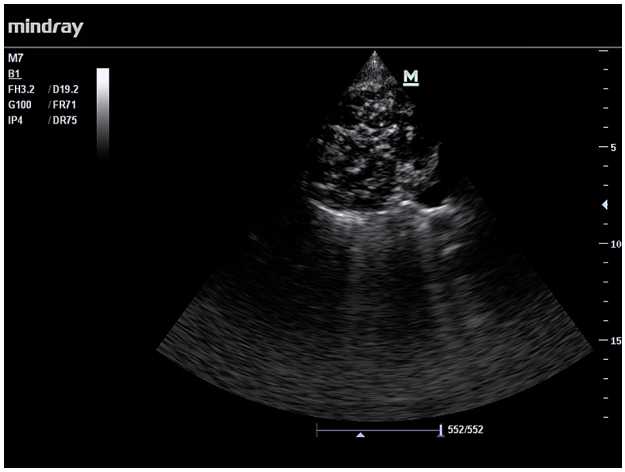

Fig. 1. Ultrasound examination of the thorax revealed a mass.

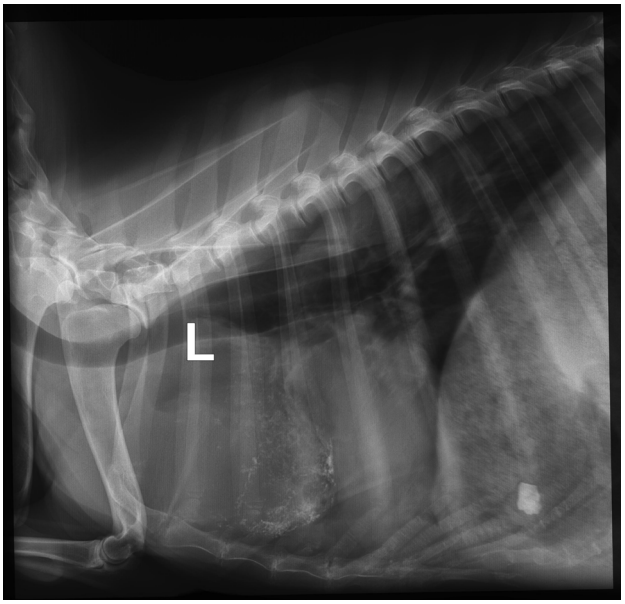

Fig. 2. The lateral X-ray – the mass is displacing the heart caudally and the trachea dorsally.

Additional cytological smears were collected for immunocytochemistry. They were dried, fixed in acetone at 4°C for 5-10 minutes, and stored at -20°C. Immunocytochemical staining was performed using the primary antibodies mentioned above, at the same concentrations.

Results

A mediastinal tumor was diagnosed ante mortem in a mixed breed doe, aged 6 years, from a small dairy farm. The ultrasound examination (Fig. 1), X-ray (Fig. 2) and CT scan (Fig. 3) revealed a giant mass in the cranial mediastinum at the height of the cardiac silhouette. According to all three imaging methods the dimension of the tumor was 11.1 cm. Radiographic examination revealed that the mass was extending from

Fig. 3. A reformatted sagittal CT image – the mass in the thorax.

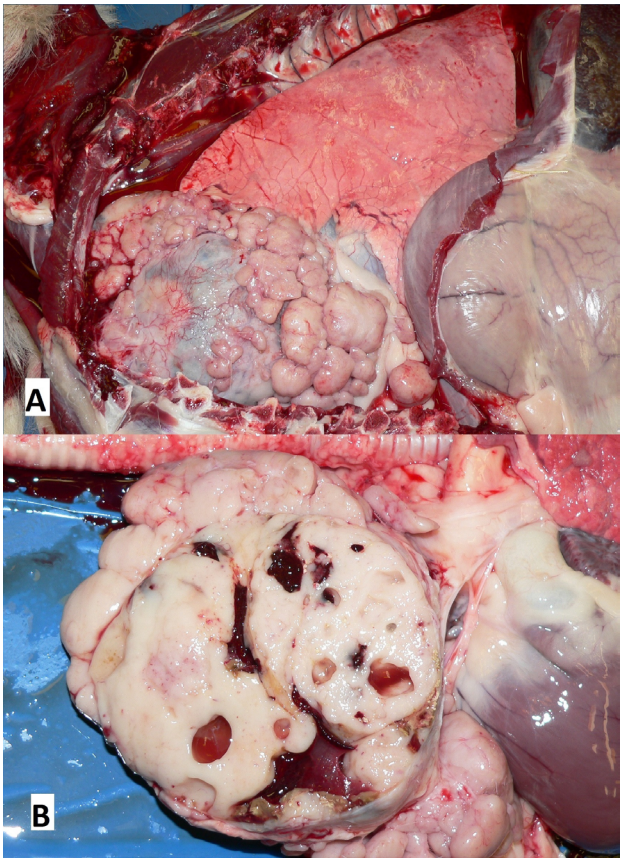

Fig. 4. (A) The neoplastic mass located in the cranial mediastinum (B) the heterogeneous tumor with numerous cyst-like spaces.

the thoracic inlet to half of the length of the thorax, and compressed the heart pushing it caudally, mainly pressing the right atrium and the base of the aorta. The mass displaced the trachea dorsally and to the right but the lung parenchyma appeared normal. The CT-scans revealed that the tumor deformed

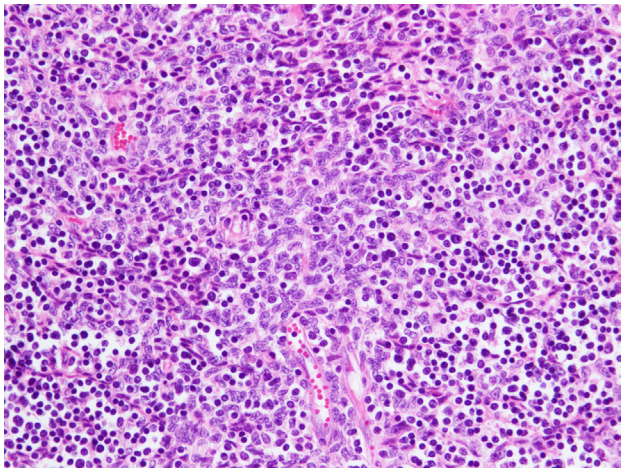

Fig. 5. The epithelial, neoplastic cells with elongated nuclei accompanied by numerous small lymphocytes (type AB thymoma) (HE staining; x400).

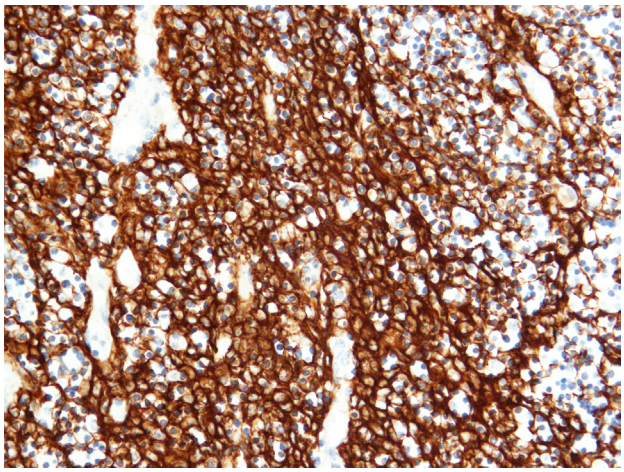

Fig. 6. Strong and diffuse cytoplasmic immunoreactivity for pancytokeratin (Anti-AE1AE3 reaction; x400).

the trachea. A substantial part of the mass was adjacent to the chest wall, but no evidence of bone destruction or infiltration into large blood vessels was detected. The density of the mass was mixed, with numerous very small, irregular foci of mineralization. Areas of fibrosis were seen in the caudal lung fields.

In goat no. 1 cytological samples of both fine-needle aspirates and imprints were abundant in lymphoid cells with predominance of small lymphocytes and lower number of medium-sized and large blast cells. Only a few cells present in the cytological samples were identified as thymoma cells based on their epithelial appearance. The subtype of the thymoma was not established.

In 2 other goats with tumors of cranial mediastinum found during autopsy the main lesions were irregular (Fig. 4) and measured 17.2 cm and 7.3 cm, respectively. Each of the tumors consisted of a large main mass with numerous smaller protrusions on its surface. In one goat

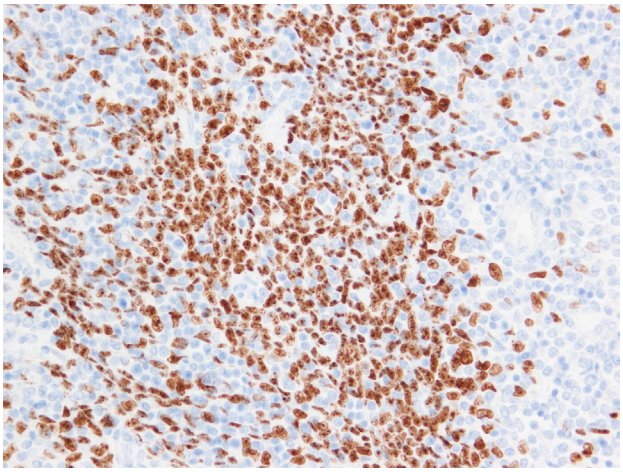

Fig. 7. Strong and diffuse nuclear immunoreactivity for p40 (Anti-p40 reaction; x400).

the tumor was firmly fixed to the cranial part of the pericardial sac and a few smaller satellite nodules were also present at the cardiac apex on the pericardium. On the cut surface, the mass was heterogeneous with numerous cavities filled with serous or sero-hemorrhagic fluid.

Microscopically, the tumors consisted of a mixed population of blind lymphoid cells (predominantly small mature lymphocytes, with smaller number of blast cells) intermingled with less apparent spindle or ovoid neoplastic epithelial cells with minor nuclear atypia, usually indistinct cell borders and small and inconspicuous nucleoli (Fig. 5). In one tumor there was also a population of larger and polygonal cells dispersed among lymphocytes or agglomerated into small, loosely formed groups; those cells showed vesicular nuclei and pronounced eosinophilic nucleoli. Both tumors were separated into lobules by fibrous septa. Perivascular spaces were not seen. Neoplastic cells revealed strong cytoplasmic immunostaining for cytokeratin (Fig. 6) and nuclear reaction with squamous differentiation markers p63 and p40 (Fig. 7).

The features of the first tumor corresponded with type AB thymoma. In the second case a diagnosis of a type B2 thymoma seems to be more appropriate.

Thymomas were diagnosed in 2 of 399 goats (0.5%, CI 95% from 0.1% to 1.8%), necropsied over the last decade and in one goat accidentally diagnosed during thoracic ultrasound examinations. No other tumors of internal organs were found.

Discussion

Even though thymomas are primarily epithelial neoplasms, they are always infiltrated with the various number of lymphocytes (Olchowy et al. 1996). The current WHO histological classification lists 5 main types

of thymomas: A, AB, B1, B2 and B3 depending on the cytological and structural features and epithelial cell-to-lymphocyte proportion (Marx et al. 2015a). If the proportion of lymphocytes is high, the tumor must be differentiated from lymphoma. For this purpose, immunohistochemical test for cytokeratin is helpful, as even small number of epithelial cells evenly dispersed in the tumor mass strongly indicates thymoma (Meuten 2016). In all 3 goats described in this study neoplastic epithelial cells reacted with anti-cytokeratin antibodies in immunochemical analysis. Furthermore, the lack of vimentin expression suggested the epithelial origin of neoplastic cells and nuclear immunostaining for p40 and p63 confirmed squamous cell differentiation characteristic for thymomas. Thus, the location of the tumors, the histological characteristics and the immunohistochemical features supported the diagnosis of thymomas in these goats.

The dimension of caprine thymomas described in the literature usually ranges between 2 and 10 cm (Löhr 2013), although a huge tumor measuring 30 cm in diameter has also been reported (Olchowy et al. 1996). On gross examination these neoplasms are usually lobulated and those measuring up to 4 cm are presented as tumors with a smooth border, while larger thymomas have usually more irregular shape. Sometimes necrotic foci and/or thin-walled cysts containing fluid may be seen. Many goats with thymoma do not show any clinical symptoms and the tumor is detected incidentally during diagnostic imaging performed for other reasons or more likely, during necropsy (Hadlow 1978, Löhr 2013). However, some goats with thymoma may manifest weight loss and dyspnea (Braun et al. 2009). Occasionally, compression of the lungs or heart by the expanding mass may lead to dysfagia, cough or even the superior vena cava syndrome may develop due to significantly impaired blood return through the cranial vena cava. Having compressed the heart, large tumors can even result in congestive heart failure and clinical signs such as dyspnea, jugular distension, cool extremities and accumulation of fluid in body cavities may occur. Large tumors can also displace the esophagus dorsally and lead to achalasia and regurgitation (Parish et al. 1996). None of the goats in our study had such symptoms. One goat had been infertile for 3 preceding years. In humans, about 30% of patients suffering from thymoma manifest thymoma-associated myasthenia gravis, a condition characterized by autoantibodies directed to different targets at the neuromuscular junction, thereby evoking muscle fatigability and weakness, dyspnea, tachypnea and dysphagia (Marx et al. 2015b). This condition occurs also in roughly one third of thymoma-affected dogs, with megaesophagus as a common result (Aronsohn et al. 1984, Atwater et al. 1994), as well as in cats (Hague et al. 2015). Thymoma associated myasthenia gravis has also been documented in a horse (Furuoka et al. 1987) and in a Siberian tiger (Allan et al. 2014), but there are no reports about this condition in goats. Neither did our patients have any visible signs of neuromuscular abnormalities.

According to the latest WHO guidelines, thymomas are malignant tumors as they are able to develop metastases (Marx et al. 2015a). Metastases were also found in one of our cases, and in other goats described in the literature (Olchowy et al. 1996, Braun et al. 2017, Hill et al. 2017).

In humans, thymomas follow slow clinical course – recurrences and metastases often develop long after resection of a primary tumor. In animals metastases are considered to be uncommon. It cannot be excluded that most of animals usually do not live long enough to develop metastases.

Our results seem to substantiate previously published surveys (Löhr 2013), which showed that the prevalence of thymomas in the general goat population was 0.7%. However, the fact that these were the only internal tumors found in 399 necropsied goats, appears to confirm goats' predilection for this disease, whose mechanism still awaits elucidation. Our findings also support the opinion that thymomas in animals should be regarded as malignant tumors due to their metastatic potential.

Acknowledgements

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