

First case of nasal transitional carcinoma in a goat infected with Enzootic Nasal Tumor Virus in Belgium

Eerste geval van nasaal overgangscelcarcinoom bij een geit geïnfecteerd met het enzoötisch nasaal tumor-virus in België

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ABSTRACT

An eleven-year-old, female goat was presented for evaluation of breathing difficulties and epistaxis. Radiographs and computed tomographic (CT) examination of the head revealed the presence of a space-occupying lesion involving the right nasal passage and invading the cranial vault and retrobulbar space. Histologic examination and detection of viral genome from the nasal mass led to the diagnosis of nasal transitional carcinoma with concomitant infection with Enzootic Nasal Tumor Virus (ENTV-2). In this case report, the presence of a nasal transitional carcinoma is described in a goat; a very rare tumor which, to the authors' knowledge, has not been previously reported in goats. Reverse transcription polymerase chain reaction (RT-PCR) detected the genome of ENTV-2 within the neoplastic tissue, suggesting an infectious etiology.

SAMENVATTING

Een elf jaar oude geit werd aangeboden met ademhalingsmoeilijkheden en epistaxis. Op radiografisch en computer tomografisch (CT) onderzoek van de kop was een ruimte-innemende massa zichtbaar in de rechterneusgang met invasie in de schedelholte en retrobulbaire regio. Door middel van histologisch onderzoek werd deze massa gediagnosticeerd als een nasaal overgangscelcarcinoom. In deze massa werd echter ook viraal genoom gedetecteerd van het enzoötisch nasaal tumor-virus (ENTV-2). Volgens de auteurs is dit de eerste grondige radiografische en computertomografische beschrijving van een overgangscelcarcinoom in de neusgang van een geit geassocieerd met een ENTV-2-infectie.

CASE REPORT

Signalment, history and clinical findings

An eleven-year-old, female goat was admitted to the Veterinary Hospital of the Faculty of Veterinary Medicine of Ghent University with stertorous breathing and bilateral epistaxis, which started one week prior to the presentation. Before the referral, the goat had been treated with dexamethasone and antibiotics

without improvement. Beside the respiratory symptoms, she did not show any clinical signs. The goat had been kept as a companion animal together with a second goat for the last decade in the countryside between Brussels and Charleroi. The second goat did not show any clinical signs.

On blood examination, mild hyperbilirubinemia, lymphocytopenia, monocytopenia and eosinopenia were detected.

Medical imaging

Standard orthogonal radiographs of the skull showed a soft tissue-opacity filling the caudal aspect of the right nasal passage with a mild mass effect displacing the nasal septum slightly towards the left side. There was loss of the turbinate structure within the right nasal passage (Figure 1). There were multiple dental abnormalities unrelated to the mass, such as missing mandibular incisors and a diastema between the mandibular 08's and 09's. Based on these images, a malignant neoplasia could not be differentiated from an infectious process, such as abscess, actinomycosis, nasal aspergillosis or oestrosis. A CT-scan of the head was therefore performed under general anesthesia (LightSpeed QX/i, GE Medical Systems, Milwaukee, Wisconsin, USA). This revealed a large (approximately 6.5 x 4.5 x 3.5 cm LHW), lobulated, heterogeneous soft-tissue attenuating (35 HU), space-occupying lesion in the caudal aspect of the right nasal cavity, with destruction of the ethmoturbinates and complete obliteration of the right nasal passages. This mass had multifocal mineralized zones and demonstrated a homogeneous, strong contrast-enhancement (110 HU). It extended into the nasopharynx, invaded the right retrobulbar space and the calvarium through the cribriform plate. There was contrast-enhancing tissue in the rostral aspect of the right olfactory lobe. Furthermore, the mass also invaded the right maxillary, palatine and lacrimal sinuses, which were filled with fluid-attenuating, non-contrast enhancing material representing secondary sinusitis (Figure 2). Similarly, fluid related to the secondary sinusitis was also present in the lateral and medial parts of the frontal sinus and in the cornual diverticulum of the frontal sinus. The retropharyngeal and mandibular lymph nodes were within normal limits. In addition, several dental elements were missing and demonstrated severe apical changes; however, this was not related to the mass.

Based on these findings, a malignant neoplastic process involving the right nasal passage and invading the cranial vault and retrobulbar space was strongly suspected. Based on reported nasal tumors in goats, the main differential diagnoses were adenocarcinoma and, less likely, lymphoma (Löhr, 2013). The patient was euthanized due to poor prognosis and a necropsy was performed.

Necropsy, histologic and immunohistochemical findings

On necropsy, there was a large space-occupying mass in the right caudal nasal cavity, originating from the ethmoturbinates and extending rostrally over a length of 6 cm (Figure 3) and ventrally into the right ventral meatus and the nasopharynx, thereby compressing the soft palatum downwards with partial obstruction (60-75%) of the pharynx. Caudally, it crossed the cribriform plate to invade the right rostral



Figure 1. A. Lateral and B. dorsoventral projections of the head showing soft-tissue opacity filling the caudal aspect of the right nasal passage with loss of the turbinate structure (arrows). Missing mandibular incisors and a diastema between the mandibular 08's and 09's are visible as well.

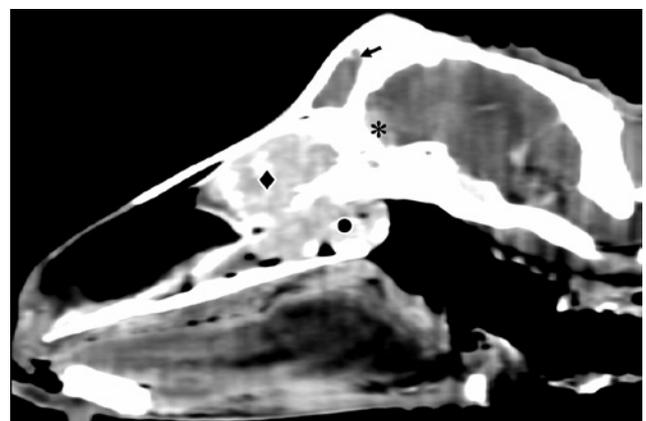


Figure 2. Postcontrast sagittal reconstruction of the nasal cavity showing a space-occupying lesion in the caudal aspect of the right nasal cavity (◆) invading the nasopharynx (●) and the cranial vault (*). The right frontal sinus is fluid-filled (arrow).

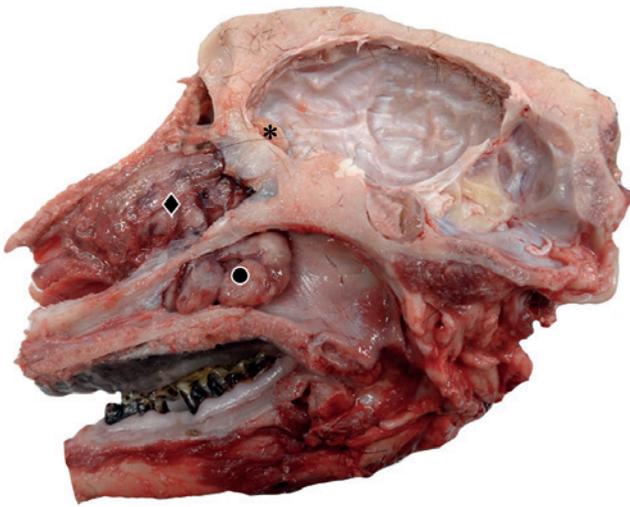


Figure 3. Sagittal section of the head showing a mass occupying the right caudal nasal cavity with destruction of the ethmoturbinates (◆) invading the cranial calvarium (*) and the nasopharynx (●).

aspect of the calvarium, in the region of the right olfactory lobe. The mass consisted of a slightly multinodular, pale pink, soft tissue admixed with bony structures (remnants of ethmoturbinates). The right frontal sinus was filled with a brown mucous content.

Histopathology showed a cell-rich, non-encapsulated, infiltrative monomorphic cell population arranged in thick stratified layers, often folded and separated by delicate fibrovascular septa (Figure 4A). The cells were pleomorphic, medium sized, with indistinct cell borders, a moderate amount of eosinophilic cytoplasm and a large, round, light basophilic nucleus with fine chromatin and an inconspicuous nucleolus. Within the tumor, there were multifocal areas with an acinar growth pattern. These acini sometimes contained erythrocytes and were lined by a single layer of high cylindrical cells, sometimes ciliated, with a moderate amount of eosinophilic cytoplasm and a basally oriented, round, basophilic nucleus (Figure 4B). There was moderate anisocytosis and anisokaryosis. Mitotic figures were rare. There were small aggregates of hemosiderin laden macrophages and oligofocal areas of old hemorrhage with chronic granulation tissue, fibrosis and hematoidin pigment and some subepithelial aggregates of lymphocytes. The intracranial mass showed the same appearance, with predominance of a lobular growth pattern.

Immunohistochemistry was negative for synaptophysin but strongly positive for cytokeratin, indicating an epithelial origin of the neoplastic cells. Immunohistochemistry for Ki67 highlighted 30 to 50 nuclei per high powered field (HPF), depending on the slide/localization within the tumor.

Based on the histopathologic findings, a nasal transitional carcinoma was diagnosed with multifocal adenocarcinomatous pattern arising from the ethmoturbinates with invasion through the cribriform plate

into the right rostral calvarium and partial obstruction of the nasopharynx.

Enzootic Nasal Tumor Virus 2 (ENTV-2) detection

Viral genome was detected using ENTV-2 specific primers on reverse transcribed RNA extracted from the tumoral tissue. Sequencing of a short sequence in the 3' end of the genome confirmed the presence of ENTV-2 in this symptomatic goat.

DISCUSSION

This is the first case report with thorough radiographic and CT-description of a transitional carcinoma of the nasal passages in a goat associated with ENTV-2 infection.

ENTV infections are typically associated with the induction of nasal adenocarcinomas (enzootic nasal adenocarcinoma, ENA) (Özmen et al., 2010), although some authors use the broader term 'enzootic intranasal tumor' (De las Heras et al., 1995). Even though the nasal transitional carcinoma has not yet been linked to ENTV-infection, the presence of viral genome within the pathologically affected tissue is suspicious for a causal relationship. Another hypothesis is that this rare tumor occurred in the goat that was coincidentally infected with ENTV-2. The coincidental combination of these two very rare entities is therefore thought to be unlikely, but not impossible.

Enzootic nasal tumor is a chronic and contagious tumor in sheep and goats, induced by ENTV (De las Heras et al., 2003). ENTV is an oncogenic retrovirus belonging to the genus Betaretrovirus, which is closely related to Jaagsiekte Sheep Retrovirus (JSRV). While ENTV induces neoplastic transformation of nasal epithelial cells, JSRV is responsible for transformation of epithelial cells of the distal lung (Monot et al., 2015). ENTV-1 and ENTV-2 have been isolated, respectively from sheep and goats. They are highly homologous, but can be differentiated by some unique genomic sequences (De las Heras et al., 2003).

In goats, the disease has been described in France (Lombard et al., 1966) Spain (De las Heras et al., 2003), Italy (Vitelozzi et al., 1993), Greece (Giadinis et al., 2013), Turkey (Özmen et al., 2010) Iran (Namjou et al., 2018), India (Rajan 1987), China (Ye et al., 2019), Algeria (Sid et al., 2018) and Canada (Pringle et al., 1989). Ovine enzootic nasal tumors have been recorded worldwide, with the exception of Australia and New Zealand (De las Heras et al., 2003). The prevalence of ENTV-2 in Belgium is unknown, but no cases of ENA have been reported in Belgium up till now.

In most of the countries, ENA is a sporadic disease, but enzootics causing considerable economic losses have been reported (Giadinis et al., 2013; Huang et al., 2019; Özmen et al., 2010).

ENTV is thought to be spread horizontally, most likely by the respiratory route. Enzootic nasal tumor has been successfully reproduced in goats by experimental nebulization with concentrated nasal discharge of naturally infected goats (De las Heras et al., 1995). Other routes of transmission should not be excluded, such as milk, colostrum or intra-utero or perinatal infection (Sid et al., 2018).

Clinical signs of enzootic nasal tumors include continuous nasal discharge, respiratory distress, exophthalmos and skull deformations. The animals usually remain active with a good appetite and normal body temperature. Nevertheless, body condition is gradually lost. The animals most commonly die in a few weeks, often due to bacterial or toxemia complications. Adult sheep and goats between two and four years of age are preferentially affected, but in the authors' experience, very young individuals of five to twelve months of age can develop the disease as well. Several cases are often observed within the same flock. No genetic, sex or breed predisposition has

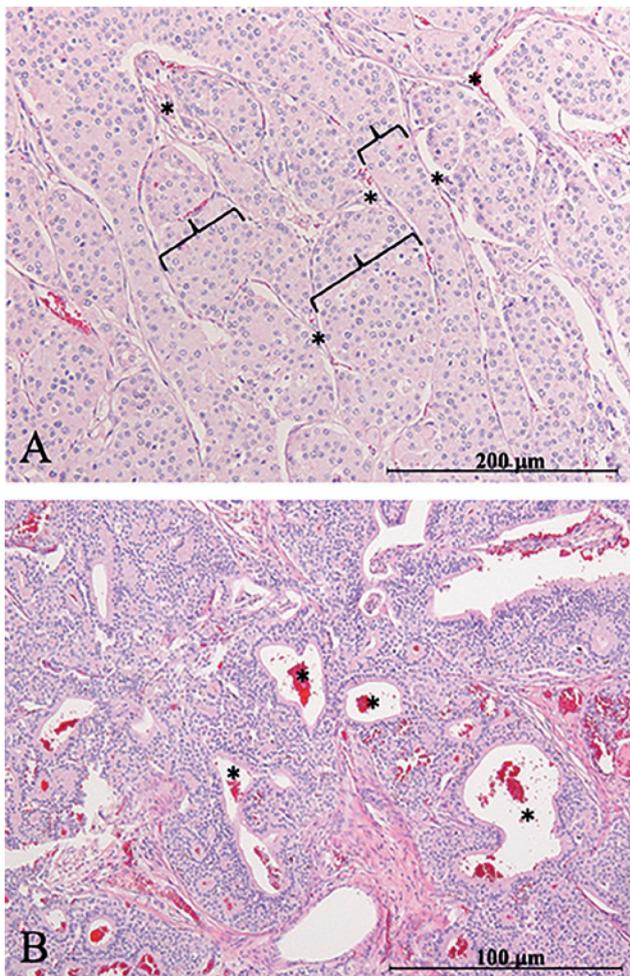


Figure 4. Histopathology of the mass. **A.** The tumor consists of thick stratified layers of cells (braces), often folded and separated by delicate fibrovascular septa tissue (*). **B.** Within the tumor, there are multifocal areas displaying an acinary growth pattern. The acini are often filled with erythrocytes (*). HE stain, (A: 200x magnification; B: 100x magnification).

been detected. Enzootic nasal tumors can be confused clinically with other diseases, such as chronic bacterial and fungal infections, dust allergy or nasal myiasis caused by *Oestrus ovis*. The clinical differentiation can be difficult, in particular in case of oestrosis, which leads to chronic catarrhal rhinitis that is sometimes hemorrhagic with production of a generous mucus-rich exudate (De las Heras et al., 2003). Infection by *oestrus ovis* has been thought to be a risk factor for the development of ENA, but a study conducted on French sheep indicates that there is no relationship between nasal myiasis and neoplastic transformation (Dorchies et al., 2003).

Currently, there are no commercially available diagnostic tools providing a non-invasive antemortem diagnosis of the disease; a RT-PCR on RNA extracted from nasal fluids has recently been developed (Huang et al., 2019). Due to the lack of circulating antibodies, serological screening for the virus seems impossible and thus the prevalence and genotypic distribution of ENTV are still unknown (Huang et al., 2019).

There is no therapy available for this disease. Surgical excision has been attempted in animals of high reproductive value, but is usually ineffective. Therefore, euthanasia of all affected individuals is recommended (Giadinis et al., 2013).

The radiographic and computed tomographic appearance of the lesion described here is similar to that of sinonasal tumors in dogs, cats and horses (Cissell et al., 2012; Saunders et al., 2003; Tromblee et al., 2006). In these species, the presence of a soft tissue attenuating mass, aggressive pattern of osteolysis, erosion of the cribriform plate and invasion of the paranasal cavities and of the calvarium are common features of malignant processes of the nasal cavities.

Although infrequently reported, transitional carcinoma represents the second most common sinonasal tumor in dogs (Ninomiya et al., 2008; Sako et al., 2005) and cattle (Rajan, 1987) and is usually locally aggressive (Paiva et al., 2013).

Enzootic nasal tumor is a contagious neoplasia. Although sporadic, it should be included as differential diagnosis for aggressive bone lesions and mass lesions in the nasal cavities of goats and sheep, in order to detect this disease in an early state and limit the spread between animals.

To the authors' knowledge, this is the first case report with thorough radiographic and CT- description of a transitional carcinoma of the nasal passages in a goat associated with ENTV-2 infection. It is the first time as well, that an ENTV-2 positive goat is detected in Belgium.

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