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Case Report

A Case of Enzootic Nasal Adenocarcinoma in a Ewe

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An approximately 2-year-old open Suffolk ewe presented to the North Carolina State University College of Veterinary Medicine Veterinary Health Complex for evaluation of a left nasal mass. An ultrasound-guided aspirate and core biopsies were performed. An epithelial neoplasia with mild mixed inflammation (neutrophils and plasma cells) was diagnosed on cytology and confirmed on histopathology. Immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RT-PCR), and transmission electron microscopy were also performed. IHC and RT-PCR identified the presence of enzootic nasal tumor virus and confirmed the final diagnosis of enzootic nasal adenocarcinoma.

1. Case History and Presentation

An approximately 2-year-old open Suffolk ewe presented to the North Carolina State University College of Veterinary Medicine Veterinary Health Complex (NCSU-VHC) for evaluation of a left nasal mass, (Figure 1). Four months previously, the ewe started having clear nasal discharge. The following month, slight facial distortion was first observed by her owner. At that time, the ewe was not showing any signs of depression or illness and had a normal appetite and activity level. The facial distortion evolved into a left-sided fluid filled dorsal nasal mass with bilateral serosanguinous nasal discharge when pressure was applied to the mass. The ewe was subsequently referred to the NCSU-VHC where radiographs confirmed a left nasal mass with frontal sinus involvement. An ultrasound-guided aspirate of the mass was performed at that time.

2. Cytologic Findings

Direct smears and cytocentrifuged preparations of fluid from an ovine nasal mass aspirate produced highly cellular cytological samples with a thick pink background, mild-to-moderate hemodilution, and windrowing of RBCs. Large clusters of round to polygonal-shaped epithelial cells with minimal anisocytosis and anisokaryosis were present. Cell-cell junctions were noted within the clusters. The cells had round nuclei with ropey chromatin and occasional small round nucleoli and a small amount of medium blue cytoplasm. Scattered neutrophils and plasma cells were present. The cytologic interpretation and diagnosis was epithelial neoplasia (carcinoma) with mild mixed inflammation (neutrophils and plasma cells), (Figure 2).

3. Histologic, Immunohistochemical, Polymerase Chain Reaction, and Electron Microscopy Findings

The ewe returned 1 month later for a recheck examination and additional diagnostic tests. Ultrasound-guided core needle biopsies were performed and tissue was collected into neutral buffered formalin for histology and Trump's fixative for transmission electron microscopy (TEM). A well-differentiated, expansile epithelial neoplasm contained acini and tubules supported by scant fibrovascular stroma. The cells were tall cuboidal to tall columnar with distinct cell borders and a moderate amount of eosinophilic to amphophilic cytoplasm. The nuclei were round to oval and central to eccentrically located. Cells exhibited mild

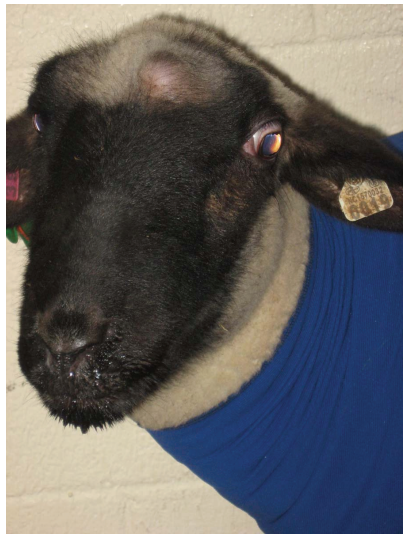

Figure 1: Picture of ewe on initial presentation with left-sided dorsal nasal mass.

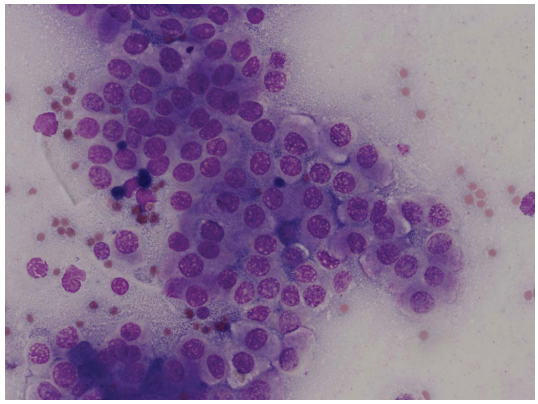

Figure 2: Initial cytology of nasal mass with large cluster of round to polygonal epithelial cells, Wright-Geimsa stain, 50x objective.

anisocytosis and anisokaryosis. Mitotic figures were rare, less than 1 per 10 high powered fields. There were multifocal mild lymphoplasmacytic and neutrophilic infiltrates. Nasal carcinoma with mild multifocal mixed inflammation was the final diagnosis (Figure 3).

Additional diagnostic tests that were performed included immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RT-PCR), and TEM. IHC was performed using a mouse monoclonal antibody against the Jaagsiekte sheep retrovirus (JSRV) envelope protein. This antibody cross-reacts with the enzootic nasal tumor virus (ENTV) envelope protein. Strong positive surface staining was observed (Figure 4). The same tissue was similarly stained with normal mouse serum as a negative control and there was no detectable staining.

RT-PCR was performed using oligonucleotide primers specific for ENTV and RNA extracted from tumor tissues; oligonucleotide primers previously were developed by Dr.

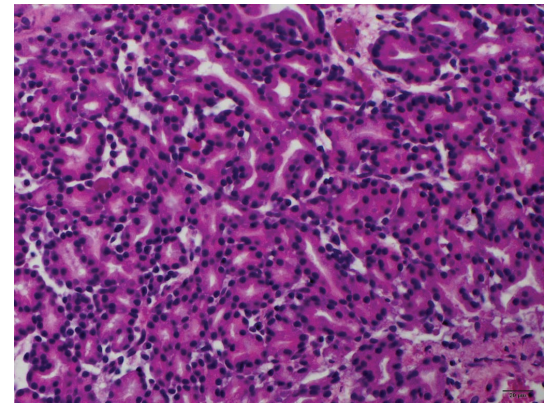

Figure 3: Histopathology of nasal mass, hematoxylin and eosin stain, 40x objective.

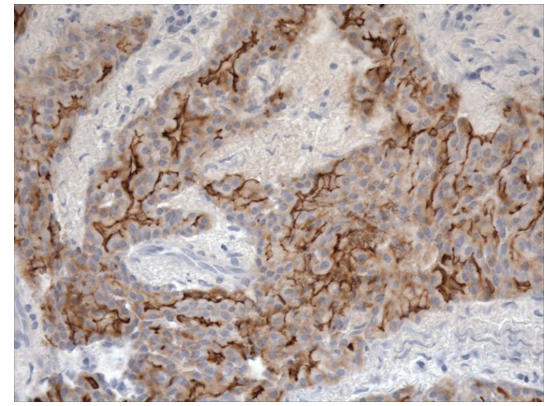

Figure 4: Immunohistochemistry of nasal mass using mouse monoclonal antibody against JSRV envelope protein. Note the positive apical staining.

Sarah Wootton, University of Guelph. The RT-PCR procedure yielded a distinct product of approximately 155 base pairs, and direct nucleotide sequencing of this product indicated 88% sequence identity with ovine ENTV [1]. The 88% identity to a previous known strain of ENTV suggests that the virus identified in this case is likely a distinct strain of ENTV. TEM results were equivocal for the presence of retrovirus. Epithelial cells contained well-formed junctional complexes at apical areas. The cytology, histology, IHC, and RT-PCR all confirmed a diagnosis of enzootic nasal adenocarcinoma (ENA).

4. Necropsy Findings

The ewe did not receive treatment and remained comfortable and active for several months in an isolated area. Eventually her clinical signs worsened and she was humanely euthanized five months after presenting to NCSU-VHC which was nine months after clinical signs were first noticed. Complete necropsy revealed a large, expansile, friable mass effacing the left and right ethmoidal conchae which was mottled tan to red and measured 11.0 cm $\times$ 11.0 cm $\times$ 1.0 cm.

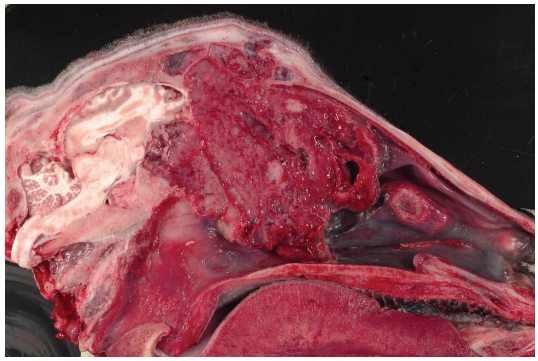

Figure 5: Gross image of the tumor invading the nasal cavity and compressing the cerebrum at necropsy (courtesy of Dr. Allison C. Boone).

The mass replaced approximately 50% of the left and right rostral frontal sinus, 50% of the dorsal nasal concha, and 100% of the middle nasal concha. The mass compressed but did not invade the ventral portion of the brain and extended ventrally to the vomer bone (Figure 5). Bony lysis caused an externally palpable, focal, soft depression of the frontal bone (3.0 cm × 3.0 cm and was 1.0 cm deep) with a small open draining track to the neoplasm in conjunction with local hemorrhage and edema. The lungs failed to collapse when the thoracic cavity was opened, contained rib impressions, and was mottled pink to dark red. The remaining viscera were grossly unremarkable. The lungs contained a multifocal neutrophilic and lymphoplasmacytic bronchointerstitial pneumonia with intralesional bacterial colonies and peribronchial lymphoid hyperplasia. A gram stain was applied to the lung tissue and no infectious agents were identified. A culture was not performed on the lung tissue. Histopathology performed on necropsy tissue samples confirmed the diagnosis of a nasal adenocarcinoma.

5. Discussion

Adenomas and carcinomas of the nasal cavity in sheep have the greatest prevalence in the United States, Canada, France, Germany, and Spain [2] and can be caused by *"enzootic nasal tumor virus-1"* (ENTV-1) infection, which is a betaretrovirus [2, 3]. ENTV-2 causes the same disease process in goats [3]. Individual or multiple animals in a flock can be infected. It is presumed that the disease is spread by contact with nasal secretions [2]. Clinical disease is usually present in adult sheep, but young lambs have also been affected [2]. Tumors originate from the ethmoid turbinates and can be unilateral or bilateral [2, 3]. The tumors can fill a large portion of the nasal cavity before clinical signs develop, which includes abundant seromucinous nasal discharge [2, 3]. Clinical signs are usually slowly progressive, with additional clinical signs being dyspnea, facial deformities, exophthalmia, and weight loss [4]. These neoplasms are typically invasive, predominantly low grade and rarely metastasize [3, 5]. When presented with a suspect case of ENA on cytopathology or histopathology, additional diagnostics such as immunohistochemistry and/or polymerase chain reaction (PCR) are required to make a definitive diagnosis. Once the diagnosis is confirmed, the patient should be separated from the remainder of the flock to avoid spread of disease.

Gross evaluation, cytology, histopathology, IHC, PCR, and TEM are diagnostics that can aid in the diagnosis of ENA. Grossly the tumor is tan to white, firm, and multinodular. The tissue may also contain red-brown areas of hemorrhage or necrosis [2]. The histologic appearance is consistent with that of an adenoma or adenocarcinoma. The classic description includes cuboidal or pseudostratified nonciliated epithelial cells that form orderly tubular, papillary, or acinar arrangements. Neoplastic cells have basal round nuclei and a variable mitotic rate. Mucus secretion is usually abundant, and the fibrovascular stroma is usually scant. Neoplastic tissue and adjacent nonneoplastic tissues often contain numerous lymphocytes [2]. A viral etiology can be definitively proven by several methods. IHC can be performed using a mouse monoclonal antibody against the JSRV envelope protein which cross-reacts with the ENTV-envelope protein. The positive staining pattern is typically apical along the cell periphery. RT-PCR can detect the exogenous ENTV-1 using virus specific primers. TEM is variably reported to reveal extracellular or budding retroviral particles in 75–100% of ovine cases [2].

Cytologic differentials include well-differentiated adenopapillomas, adenomas, and adenocarcinomas which account for the majority of nasal neoplasms in sheep [6]. Nasal epithelial hyperplasia is another consideration for this lesion. It is not possible to differentiate these three neoplasms on cytology, biopsy, and histopathology, making IHC, PCR, or TEM required to identify evidence of viral infections. Nonneoplastic differentials in sheep with gross lesions causing exophthalmia, facial deformity, nasal discharge, and dyspnea include nasal fungal or bacterial granuloma, actinobacillosis, actinomycosis, and sinusitis [6]. Conidiobolomycosis is a zygomycosis characterized by granulomatous and necrotic lesions in the ethmoidal and nasopharyngeal regions that extend into the turbinate bones and can invade the brain and orbit [7]. These patients often experience exophthalmia along with nasal discharge, dyspnea, anorexia, facial distortion, and enlargement of the anterior or posterior nasal cavity [7].

Ovine pulmonary adenocarcinoma (OPA), also known as Jaagsiekte, is caused by the betaretrovirus JSRV and is another common neoplasm of the respiratory tract in sheep. JSRV induces neoplastic transformation of alveolar and bronchial secretory epithelial cells which results in pulmonary tumors [4]. The main clinical sign is difficulty in breathing which is due to pulmonary edema that may drain from the nostrils [4]. Similar to ENA, a definitive diagnosis of OPA requires further diagnostic testing: gross and histologic evaluation, immunohistochemistry using antibodies against JSRV proteins, PCR with JSRV specific primers, and/or TEM. Grossly affected animals have a thin carcass with fluid filling the trachea and nares. Lungs are enlarged, heavy, and edematous and also contain consolidated foci or diffuse areas of tumor [3]. The tumor has a solid, grey, granular

surface, and exudes fluid when cut [3]. Similar to ENA, OPA is a contagious disease that is spread through contact of nasal drainage. It can infect sheep of any age, but is usually diagnosed in sheep between 2 and 4 years of age [4]. This disease has been reported in many sheep-rearing regions around the world including the United States. Infected animals can live with subclinical disease for months to years, which aids in spread of disease. Once clinical signs become severe, the sheep have a very poor prognosis.

Conflict of Interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this paper.

Acknowledgments

Dr. Sarah Wooton, Ph.D., at the University of Guelph Ontario Veterinary College developed the oligonucleotide primers to detect ENTV. The authors received funding from the North Carolina State University Veterinary Health Complex for diagnostic testing.

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