

# ENZOOTIC NASAL ADENOCARCINOMA OF SHEEP

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**Summary:** Enzootic nasal adenocarcinoma (ENA) is a contagious viral disease of sheep and goats characterised by neoplastic growth of the ethmoidal mucosa in the nasal cavity. ENA is caused by retrovirus, which is closely related to the ovine pulmonary adenomatosis virus (JSRV). The disease is probably spread by respiratory route and it can be introduced into a flock by purchasing infected animals. In affected flocks, the prevalence of the disease is typically 0.5 – 2 %, although it can be as high as 15 %. The disease primarily affects young adult animals at the age of 2 to 4 years. It is usually clinically manifested by seromucous or purulent nasal discharge, dyspnoea, emaciation and less often by exophthalmus and skull deformations. A clinical diagnosis of ENA in a live animal can be confirmed by endoscopic or x-ray examination. There is no effective treatment and the majority of the animals die within 90 days after the onset of the first clinical signs. The tumour, which arises unilaterally or bilaterally from the ethmoidal area of the nasal cavity, may occlude both nasal cavities, grows into the pharynx, paranasal sinuses and skull cavity, but does not metastasise to the lymph nodes, brain or other organs. Pathoanatomic diagnosis of ENA must also be confirmed with the histopathological examination. In the latest classification of tumours, ENA is classified as a low-grade adenocarcinoma. Besides histopathological examination, Western blotting, immunohistochemical examination and electronic microscopy can be used for research purposes.

**Key words:** sheep diseases – epidemiology; nose neoplasms – pathology; adenocarcinoma – pathology – virology; diagnosis – methods; microscopy, electron; Slovenia

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## Introduction

Enzootic nasal adenocarcinoma (ENA) is a contagious viral disease of sheep and goats characterised by neoplastic growth of mucosal nasal glands (1, 2, 3, 4, 5). Synonyms for ENA are also enzootic nasal tumour, infectious adenopapillomatosis, infectious adenopapilloma and infectious nasal adenocarcinoma (1, 2, 4, 6).

In the past few years, some cases of ENA were also diagnosed in Slovenia (7). With this review article, we want to remind veterinarians, especially clinicians, to consider ENA as a possible diagnosis in the case of dyspnoea and chronic nasal discharge in sheep.

## Etiology

The disease is caused by enzootic nasal tumour virus (ENTV; also termed ovine nasal adenocarcinoma virus - ONAV). Although the first report of disease and the hypothesis of its viral etiology are a half century old, it was only in year 1978 that Yonemichi et al. (1978) for the first time demonstrated virus-like particles in the neoplastic tissue.

Owing to the progress in molecular biology, the ENT virus has been investigated comprehensively. The complete sequence of ENT virus has already been determined, and based on the sequence data the virus was classified as a type B/D retrovirus (8, 9). The ENT virus is closely related to the virus associated with the ovine pulmonary adenomatosis virus (JSRV), which causes neoplastic lesions in the lower respiratory system (9, 10).

## Epidemiology

ENA occurs in many countries all over the world. The first case of the disease was described by Cohrs in 1953, and up till now the disease has been found in all countries with well developed sheep breeding, except in Australia, New Zealand (2) and Great Britain (6). In Slovenia, the first case of ENA was diagnosed in year 2001 (7).

The disease is probably spread horizontally, most likely by the respiratory route. ENA was successfully transmitted by experimental intranasal and intrasinusoidal inoculation of the homogenate of the neoplastic tissue of sheep (11) and concentrated nasal discharge of naturally infected goats (12).

The disease can be introduced into a flock by purchasing infected animals. In affected flocks, the prevalence of the disease is typically 0.5 – 2 %, although it can be as high as 15 % (6).

On the basis of the similar prevalence of ENA in many different sheep breeds, several authors concluded that sex and breed have no influence on the occurrence of ENA (2, 3, 13). Genetic predisposition to the ENA was also rejected (1), despite Duncan et al. (1967) reporting ENA in sheep and its offspring of the second generation (4).

## Clinical signs

The ENA primarily affects young adult animals at the age of 2 to 4 years (6). The youngest sheep with ENA, described in the literature, were aged ninth months (2) and one year (4), while the oldest was nine years of age (2).

The disease is usually clinically manifested by seromucous or purulent nasal discharge, dyspnoea, like snoring (1, 2, 14), coughing, sneezing (1, 4), open mouth breathing (1, 4), and less often by exophthalmus (1, 2) and skull deformations (2, 3) (Figure 1). Depigmentation and alopecia occurs around the nostrils, as the consequence of chronic nasal discharge (6). Affected animals are anorexic, gradually lose weight (1, 2, 4, 14) and die within 90 days after the appearance of first clinical signs (3) due to pasteurellosis or other complications (1, 2, 3).

## Pathology

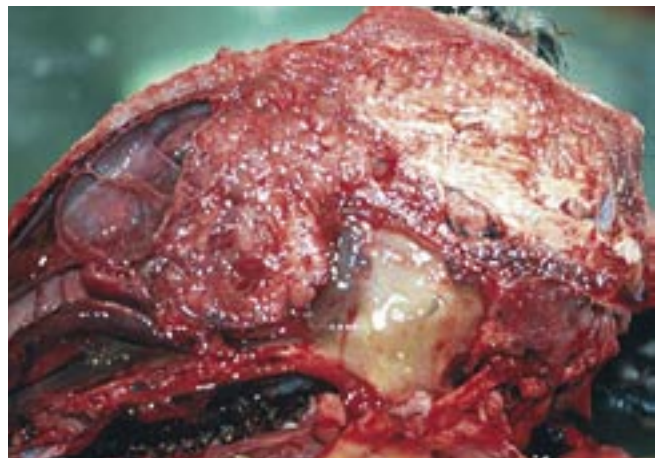
Enzootic nasal tumour virus induces neoplastic growth of mucosal nasal glands and formation of the tumour (2, 5, 6), which arises unilaterally or bilaterally from the ethmoidal area in the nasal cavity



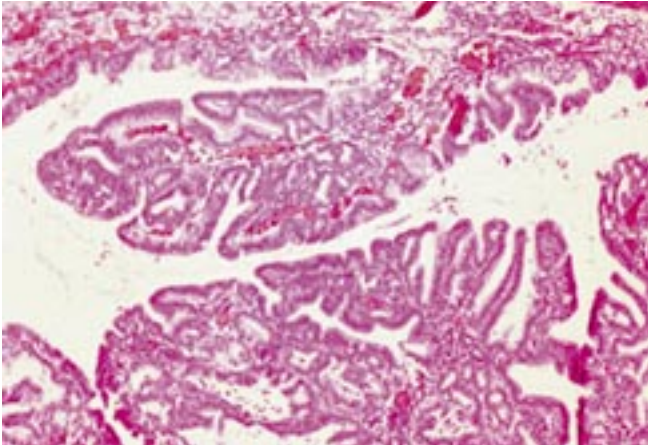
**Figure 1:** Head of sheep with enzootic nasal adenocarcinoma. Dense, mucous nasal discharge from nostrils of affected sheep. (The photo was kindly provided by Jože Starič from Clinic for ruminants)

(15). At the beginning the tumour appears as miliary protuberances and then becomes nodular and polyp-like and may occludes both nasal cavities, grows into the pharynx, paranasal sinuses (1, 4), skull cavity (4) and compresses the surrounding tissues (1, 2, 3, 13, 16) (Figure 2). The tumour surface may necrotise or exhibit secondary purulent inflammation (2). The ENA does not metastasise to the regional lymph nodes, brain or other organs (1, 2, 4, 6, 13, 14).

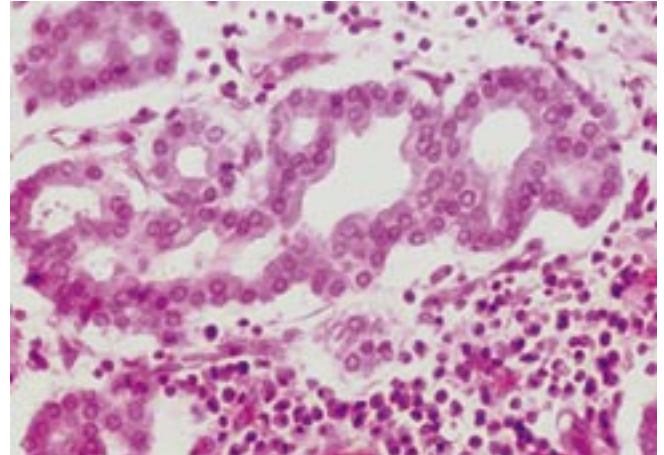
At the light microscopic level, the tumours are composed of the neoplastic epithelial cells arranged in tubular and/or papillary structures (Figure 3, 4). The neoplastic cells are mostly cuboidal and occasionally columnar. They have large round or oval nuclei with clumped chromatin pattern and they are located centrally or in the basal parts of the



**Figure 2:** Sagittal section of the head of sheep with enzootic nasal adenocarcinoma. The tumour occupies the ethmoidal area of the nasal cavity



**Figure 3:** Enzootic nasal adenocarcinoma of sheep. Papillary type of growth. Stroma of papillary proliferation is densely infiltrated with lymphocytes and plasma cells and well vascularised. HE staining, x 100

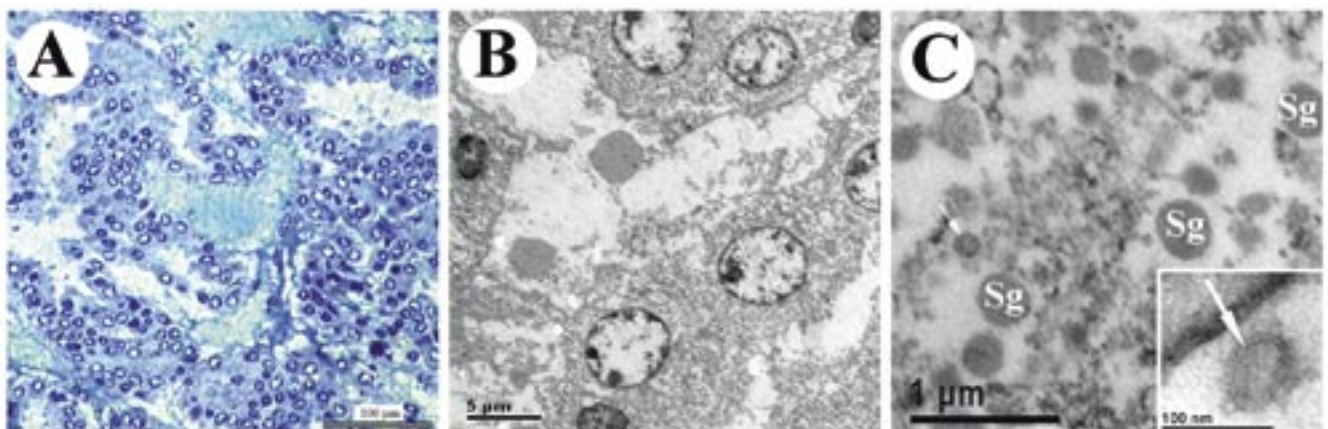


**Figure 4:** Enzootic nasal adenocarcinoma of sheep. A group of neoplastic tubules with cuboidal cells and large round nuclei with clumped chromatin and small nucleoli. Stroma is infiltrated with numerous plasma cells. HE staining, x 400

cells. The fibrous connective tissue stroma is frequently scanty, oedematous and densely infiltrated by numerous lymphocytes, plasma cells and macrophages. A fewer number of neutrophils and desquamated neoplastic epithelial cells can also be noticed in the lumina of several neoplastic tubules (1, 4).

Initially, ENA was histologically classified as a benign neoplasia. Terms used for ENA in the classifications were epithelioma (17), adenoma (13) and adenopapilloma (1, 11). Later, it was classified as a low-grade adenocarcinoma (2, 3, 5, 14). The most recent classification was based on the infiltrative growth of the neoplastic cells into the surrounding connective tissue and the absence of metastasation (2, 5).

Using electron microscopy, characteristic, round, membrane-coated secretory granules (2) of varied electron opacity and size from 0.2 to 1  $\mu\text{m}$  (13) can be demonstrated in the cytoplasm of the neoplastic cells. Extracellularly, close to the apical surfaces, and in the cytoplasmic vacuoles of the neoplastic epithelial cells, virus-like particles can also be found. The particles vary in diameter from 80 to 100 nm, and have characteristic eccentrically or centrally located electron dense nucleoid with a diameter of 47 nm. The nucleoid is bounded by electron lucent zone and spiked unit membrane (2, 13). We successfully applied rapid reprocessing of paraffin-embedded tissue for diagnostic electron microscopy (7). Semi-thin sections were examined to confirm that



**Figure 5:** (A) Semi-thin section of the reprocessed tumor tissue displaying characteristic glandular morphology; toluidine blue. (B) Low-power electron micrograph of the neoplastic tissue showing cuboidal cells with distinctly euchromatic round or oval nuclei. (C) Portion of cell's cytoplasm; secretory granules (Sg) predominate in cytoplasm matrix. (D) Virus-like particle (white arrow) found in the cytoplasm and in intracytoplasmic vacuole (insert)

tumor regions displaying typical glandular morphology were successfully reprocessed (Fig. 5; panel A). Ultramorphological appearance of the cells and the location of spherical virus-like particles were in agreement with previous reports (3, 13). Briefly, neoplastic proliferations were predominantly composed of cuboidal cells. Their nuclei (8–14 nm in diameter) were round or oval and distinctly euchromatic (Fig. 5; panels B). The cells cytoplasm had an electron lucent matrix containing numerous secretory granules that ranged in size from of 220 to 540 nm (see Fig. 5; panel C). The presence of intracytoplasmic spherical virus-like particles of 70–90 nm in diameter is shown (Fig. 5C and insert).

### Diagnosics of ENA

The above cited clinical signs are specific enough to suspect ENA. At live animal, a diagnosis can be confirmed by demonstration of the neoplasia in the caudal parts of the nasal cavity, using fiberoptic endoscopy (14) or x-ray examination (13, 14). Currently, there is no laboratory test available to confirm the clinical diagnosis of ENA as the virus has rarely been detected in the blood and due to the lack immunological response in affected animals antibodies to ENT virus have not been detected in the sera of affected animals (18).

The easiest and most reliable way to confirm the diagnosis is the examination of dead or sacrificed animal. The neoplasia can be easily noticed by pathoanatomic examination of sagittal section of the head, and it is seen as a white-grey mass of soft to firm consistency, which occludes the caudal part of one or both nasal cavities. To confirm the pathoanatomic diagnosis of ENA histopathological examination must be performed (6).

There are also a few laboratory methods for the demonstration of ENT virus in the dissection samples. The virus has been detected in neoplastic parenchyma and nasal exudates of the affected animals by Western blotting (2, 15). Using primary antibodies raised against ovine pulmonary adenomatosis virus (JSRV), a positive immunohistochemical reaction was demonstrated in the apical parts of singular neoplastic cells (2). Characteristic viral particles with diameter from 80 do 100 nm, can be showed in neoplastic tissues by electronic microscopy (2, 3, 5, 13). ENT virus can not be grown in vitro conditions, so the isolation of the virus in cell culture is not applicable (8).

### Control of the disease

At the moment, there is no efficient treatment nor vaccine against ENA. There are only two reports of unsuccessful attempts at treatment. Duncan et al. (1967) reported temporary improvement of clinical status in two sheep after irradiation of the nasal region (4). Rings and Robertson (1981) surgically removed a part of the neoplasia, but despite that, the sheep died 12 hours after (14).

### Conclusion

ENA is a contagious, retroviral disease with fatal outcome. It always should be included in a list of the differential diagnoses, when dyspnoea and chronic nasal discharge are noticed in the sheep or goat. The suspicion of ENA can be easily confirmed by pathoanatomic examination of sagittal section of the head and histopathologic examination of the tumour. The only way to control ENA is the culling of the affected animals. Delay in the recognition of ENA will lead to delay in the control of the disease, to its further spreading and to an increase in deaths in the flock and consequently to financial damage for the farmer.

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## ENZOOTSKI NOSNI ADENOKARCINOM OVC

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**Povzetek:** Enzootski nosni adenokarcinom (ENA) je nalezljiva virusna bolezen ovc in koz, pri kateri se v nosni votlini, na vohalni sluznici sitke, razvije novotvorba. ENA povzroča retrovirus, ki je soroden povzročitelju pljučne adenomatoze pri ovcah. Bolezen se širi horizontalno, v rejo se lahko vnese z nakupom nove živali. V okuženih čredah je prevalenca bolezni od 0,5 do 2 %, lahko pa doseže tudi 15 %. Učinkovitega zdravljenja bolezni zaenkrat ni, večina živali pogine v 90 dneh po pojavu prvih znakov bolezni. Zbolevajo predvsem mlade živali, v starosti med 2. in 4. letom. Najpogostejši klinični znaki so seromukozni ali gnojni nosni izcedek, oteženo dihanje, hujšanje, redkeje pa se pojavijo eksoftalmus in deformacije lobanje. Tumor se lahko razvije v eni ali v obeh nosnih votlinah, vrašča v žrelo, obnosne votline in v lobanjsko votlino, vendar v literaturi ni opisano, da bi metastazirala v bezgavke, možgane ali druge organe. Makroskopsko diagnozo potrdimo s patohistološko preiskavo. Po zadnji klasifikaciji tumorjev ENA uvrščajo med adenokarcinome nizke malignosti. V raziskovalne diagnostične namene pridejo v poštev tudi metoda western blot ter imunohistokemične in elektronskomikroskopske preiskave.

**Ključne besede:** ovce, bolezni – epidemiologija; nosne novotvorbe – patologija; adenokarcinom – patologija – virologija; diagnostika – metode; elektronska mikroskopija; Slovenija