

## Subcutaneous ectopic lung in the cervical region of a calf - a case report

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### ABSTRACT

This case presents a clinical, radiological and histopathological description of ectopic lung in a calf. The present case was a 45-day-old, female Simmental calf, showing a congenital mass in the lower neck region. Upon clinical inspection, it was determined that the calf was in excellent condition, but a pedunculated tumor-like mass appeared in the neck region. Palpation revealed that the mass had normal body temperature and was somewhat hard and flexible. The mass was surgically removed under inhalation anesthesia and diagnosed as an ectopic lung based on the histopathological examination. The calf was monitored for two years after the operation and was observed to be healthy and better developed than its peers. We concluded that a rare case like this should be presented as a contribution to the literature and veterinary practice.

**Key words:** ectopic lung, calf

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### Introduction

Ectopia and heterotopia are terms referring to an abnormal location and are used to describe congenital anomalies that have been reported to develop in humans, as well as in many animal species. Ectopia describes an organ that is situated or grows in an anatomically abnormal site, while heterotopia describes the same situation for a given type of tissue (HAZIROGLU and MIILLI, 1998; KOC et al., 1998; YAYLA et al., 2013). Examples of ectopia include a kidney located in the pelvis, inguinal canal or the thorax due to a diaphragmatic hernia, or a heart situated outside of the thorax, while examples of

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heterotopia include testes tissue located in the peritoneum or thyroid tissue in the tongue root (CHAUVET et al., 1994; HAZIROGLU and MIILI, 1998; KOC et al., 1998; ISLAM et al., 2011; CANPOLAT and EROKSUZ, 2007; BASSI et al., 2010).

It has been reported that congenital anomalies in calves can include cases of heterotopia, which are quite rare (HAZIROGLU and MIILI, 1998; KOC et al., 1998). These kinds of anomalies are thought to develop when an organ or a part of an organ becomes dislocated during the prenatal period and continues to grow. It has been reported that in many animal species, including pigs, cases of heterotopia have been found with abnormally small testes on the peritoneum (KOC et al., 1998; ISLAM et al., 2011). Koç et al. reported that they found a heterotopic pulmonary anomaly in the paralumbar region of a calf (KOC et al., 1998), while CANPOLAT and EROKSUZ (2007) reported a case of pulmonary sequestration and bronchogenic cyst in a calf.

In this report, we describe a case of an ectopic lung in the cervical region of a 45-day-old Simmental calf, together with an evaluation of the long-term post-operative clinical results.

### **Case presentation**

The present case was a 45-day-old Simmental calf that was presented with a mass measuring about  $15 \times 14 \times 8$  cm in dimension, located in the lower neck, that was seen at birth.

Upon clinical inspection, it was determined that the calf was in excellent condition and the pedunculated tumor-like mass appeared to be localized in the cervical region (Fig. 1). Palpation revealed that the mass had normal body temperature, was somewhat hard and flexible, and was lighter than expected. An infected site with purulent discharge was noted in the distal one third of the mass. After the region was shaved and disinfected, a surgical procedure was carried out on the mass under inhalation anesthesia. Firstly, an investigation of the relationship with the cervical region was performed, which revealed that the mass was limited to muscles at the scapulohumeral joint. An incision was made to expose the connection point, which revealed one artery 0.5 cm in diameter going toward the cranial artery and pulsing synchronously with the a. carotis communis, and one vein of approximately the same diameter leading in the caudal direction. The artery was observed to bifurcate with the carotid approximately 20 cm caudal to the mandible, while the vein ran toward the thorax inside the jugular groove parallel to the jugular vein. Direct radiography and contrast radiography (venography) were performed, which revealed that the vein in question was intrathoracic, but given the available equipment, it was not possible to make a definite conclusion about where it connected. Examination of the surrounding tissue revealed that the pre-scapular lymph node was enlarged, but no abnormality was observed in any other tissue. After conducting final checks, the mass was totally extirpated and the site was closed up with routine methods. Analgesia and

antibiotics were administered to the calf for three days following surgery. The calf was monitored for two years after the operation and no complications were observed. We learned that since the calf was in better condition when compared to other calves, and the owner used it in breeding.



Fig. 1. A view of the calf with an ectopic lung in the cervical region

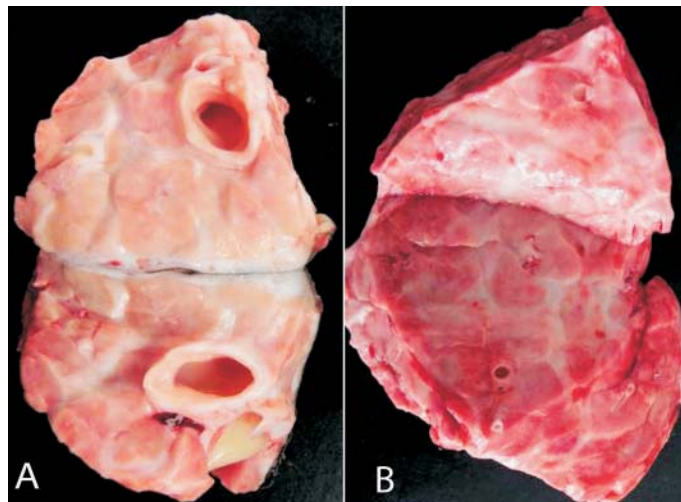


Fig. 2. The surgically removed mass (A) and its cut surface (B)

The mass was surgically removed and submitted to the Department of Veterinary Pathology for histopathological evaluation (Fig. 2). Tissue samples from the mass were fixed in 10 % buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E). Sections from the mass were also stained immunohistochemically using the avidin-biotin-peroxidase complex (ABC) technique, for thyroid transcription factor 1 (TTF-1), pulmonary surfactant protein A (SP-A) and B (SP-B) and proliferating cell nuclear antigen (PCNA). Paraffin sections were dewaxed, hydrated and endogenous peroxidase was blocked with 3 % H<sub>2</sub>O<sub>2</sub>, and the sections were placed in citrate buffer saline (pH 6.0) in a microwave oven for antigen retrieval. Detection of TTF-1, SP-A, SP-B and PCNA was performed with polyclonal rabbit anti-TTF-1, polyclonal rabbit anti-SP-A and SP-B, and monoclonal mouse anti-PCNA antibodies and incubated for 60 min at RT, diluted according to the manufacturer's recommended procedures. Detection was performed with biotinylated linked anti-mouse and anti-rabbit immunoglobulin and streptavidin HRP (Dako LSAB2™ system) for 30 min at RT. Immunostaining was obtained using 3,3' diaminobenzidine (DAB) as the chromogen. Harris' hematoxylin was used as the counterstain.

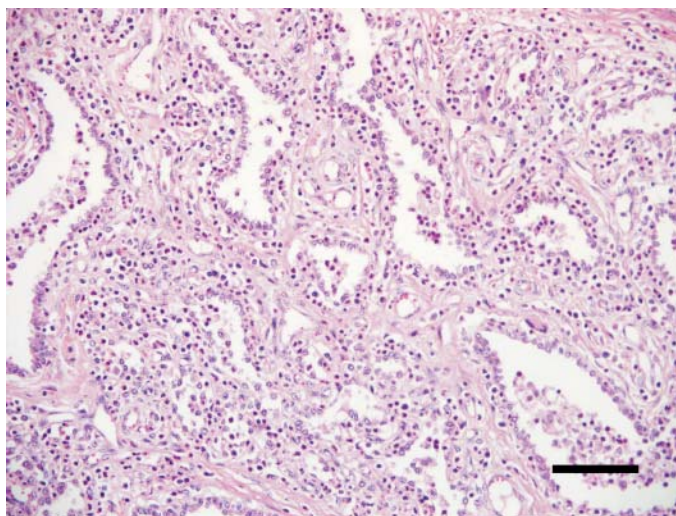


Fig. 3. Ectopic pulmonary tissue showing widespread neutrophil infiltration and stromal fibrosis with no development of alveolar structure. H&E; scale bar = 166  $\mu$ m.

The surgically removed mass was about 15 × 14 × 8 cm in diameter and grayish in color. Cross-section revealed that the mass was composed of soft to moderately firm tissue, contained small lobules with a slimy consistency, and had small ducts that resembled bronchi.

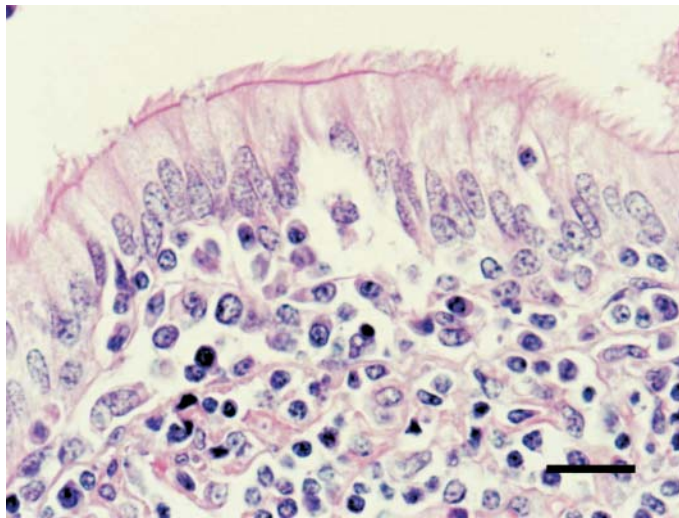


Fig. 4. Ciliated mucosal epithelium of bronchi and submucosal lymphoid cell infiltration. H&E; scale bar = 11  $\mu$ m.

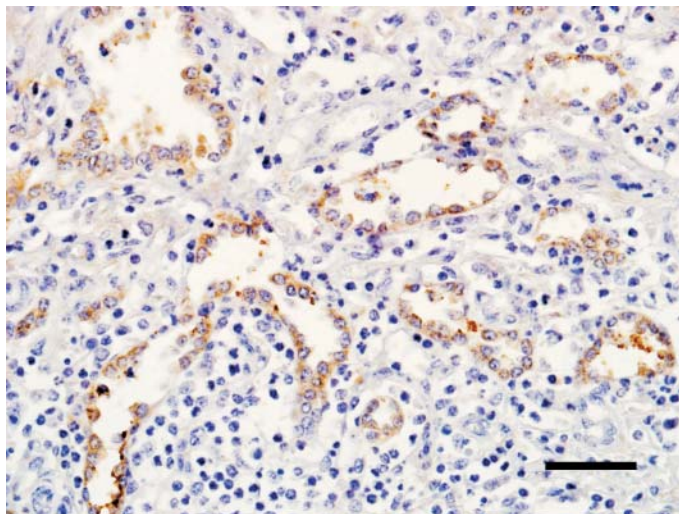


Fig. 5. SP-A cytoplasmic positive reaction in gland-like proliferated epithelial cells. IHC; scale bar = 51  $\mu$ m.

It was determined that ectopic pulmonary tissue was separated from the dermis with collagenous connective tissue, and that the connective tissue formed a lobular septa

that extended into the lung tissue in places. The ectopic pulmonary tissue was noted to have terminal bronchiole structures, containing a small number of normal bronchi and bronchioles, but it did not have normal alveolar structures (Fig. 3). In the bronchi and bronchioles, the mucosal epithelium was observed to be lined with ciliated cells, as seen in normal pulmonary tissue (Fig. 4). Hyaline cartilage was only detected within the walls of a few larger bronchi. Widespread neutrophil leukocyte exudation was observed on the interstitium and the lumen of the bronchi and bronchioles, and numerous clumps of lymphoid cells were also found around the airways. Numerous mitotic figures were also observed at the center of the lymphoid follicles. Severe hyperplasia of mucosal epithelium was observed in some bronchi, and severe fibrotic tissue, consisting of fibroblasts and fibrocytes, was found throughout the parenchyma, along with papillary proliferations in the lumen of the airways.

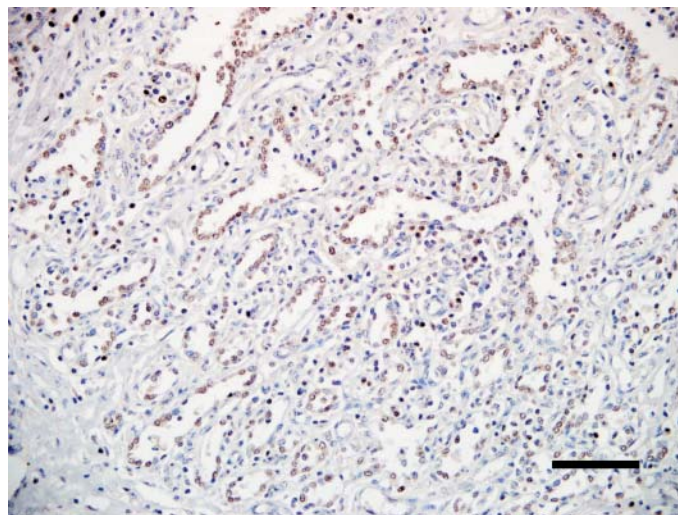


Fig. 6. Weak TTF-1 positive reaction in the nuclei of epithelial cells within the tubular-like structures. IHC; scale bar = 166  $\mu$ m.

Immunohistochemically, SP-A and SP-B positive reaction was detected, particularly in the cytoplasm of the epithelial lining cells of the tubular-like structures. The positive reaction was of a fine granular type, usually weak and was in limited areas. The SP-A positive staining was stronger and more prevalent (Fig. 5), while the SP-B reaction occurred in more limited areas. TTF-1 positive staining was observed to occur in the nuclei of epithelial lining cells within the tubular-like structures (Fig. 6). PCNA staining

was used to assess cellular proliferation, and was seen in the nuclei of the epithelial lining cells of the tubular-like structures and connective tissues of the septa (Fig. 7).

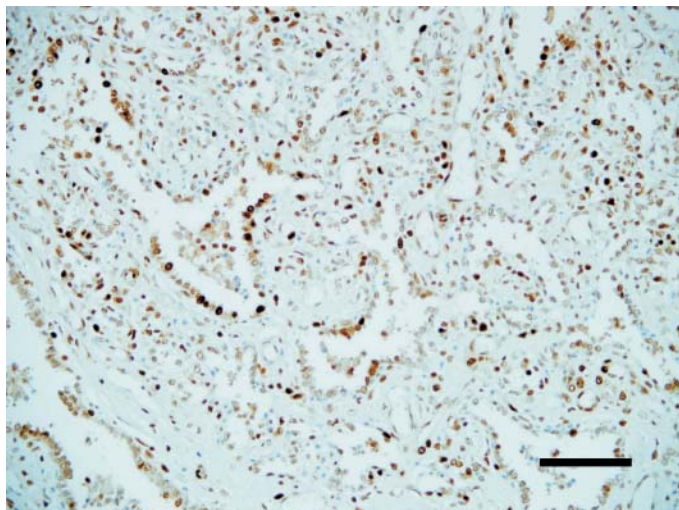


Fig. 7. PCNA nuclear positive reaction in the epithelial and connective tissue cells. IHC; scale bar = 166  $\mu$ m.

### Discussion

Heterotopia is defined as tissue that is outside its normal anatomic location. Various researchers have reported ectopia of different organs, including kidneys, testes, hearts and lungs (BUNDZA and DUKES, 1978; HAZIROGLU and MIILI, 1998; KOC et al., 1998; CANPOLAT and EROKSUZ, 2007; BASSI et al., 2010; MEDEIROS et al., 2011). Similarly, the congenital mass that was localized in the cervical region of this Simmental calf was diagnosed as ectopic pulmonary tissue, based on the histopathological features. The ectopic tissue was confirmed to be a piece of pulmonary tissue because it showed bronchi and bronchioles, and ciliated mucosal lining epithelium like normal lung tissue. However, although the tissue had bronchi and bronchioles, it did not show normal alveoli and instead had tubular-like structures covered with cuboidal epithelium, that might be fetal bronchioles, that has also been reported in calves by other researchers (KOC et al., 1998; CANPOLAT and EROKSUZ, 2007). A limited amount of hyaline cartilage was observed within the wall of a few bronchi, as reported by CANPOLAT and EROKSUZ, 2007. To the best of our knowledge, immunohistochemistry for TTF-1 or surfactant proteins in ectopic lung tissues in animals has not previously been performed. Therefore, in this case, we aimed to evaluate the epithelial lining cells by using the immunohistochemical staining technique for some surfactant proteins (SP-A and SP-B) and TTF-1 commercial

markers. Thus, the fact that the nuclei of the lining cells of the tubular-like structures exhibited a TTF-1 positive reaction confirmed that these cells might most probably be lung epithelial cells, as seen in normal pulmonary tissue. Furthermore, the fact that an SP-A and SP-B positive reaction was found in the mucosal lining the epithelial cells of the tubular-like structures indicates that the ectopic lung tissue had characteristics similar to pulmonary tissue, but also shows that the alveolar type II pneumocytes were not sufficiently developed. In consistence with our results, SEZİK et al. (2012) documented a surfactant protein positive cytoplasmic reaction in the alveolar epithelial cells of fetal kid lungs.

Additionally, the occurrence of severe neutrophil exudation and lymphoid cell infiltration in the ectopic pulmonary tissue indicated that the ectopic tissue was infected, and showed that inflammation probably caused diffuse fibrosis in the septum and within some airways. Even though a microbial culture was not made from the ectopic mass, bacterial contamination might have been caused by injury to the skin covering the mass. Likewise, upon clinical examination of the calf, less purulent discharge was noticed on the distal third of the mass. Based on the location of the mass, it can be assumed that the infection was caused by the chafing that occurred during the calf's normal movements over the previous 45 days.

KOC et al. (1998) reported a case of pulmonary heterotopia and they suggested that it caused severe life-threatening complications because it was located in the paralumbar region. In view of the locality of the heterotopic pulmonary anomaly in the case we evaluated, it is clear that no life-threatening complication developed. This is why it was possible to successfully extirpate the mass surgically. Furthermore, there was no significant connectivity related to the location of the mass, other than two veins and an artery that beat synchronously with the arteria carotis communis. Checkups performed for two years after the operation indicated that the animal continued to live a healthy life.

In conclusion, we are of the opinion that the report of this rare case is a valuable contribution to the literature.

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**KILIÇ, E., S. YAYLA, E. BEYTUT, V. BARAN, C. Ş. ERMUTLU: Supkutana ektopija pluća u vratnom području u teleta - prikaz slučaja. Vet. arhiv 85, 701-709, 2015.**

**SAŽETAK**

Prikazan je klinički, radiološki i histopatološki opis ektopije pluća u teleta. U ženskog teleta simentalne pasmine, u dobi od 45 dana, primijećena je kongenitalna masa u ventralnom području vrata. Tele je klinički bilo u odličnoj kondiciji s visećom masom nalik na tumor u području vrata. Palpacijom je ustanovljena masa normalne tjelesne temperature, ponešto tvrda i pomična. Masa je kirurški bila uklonjena pod inhalacijskom anestezijom. Dijagnosticirana je ektopija pluća na osnovi histopatološke pretrage. Tele je bilo promatrano tijekom dvije godine nakon operacije. Ostalo je zdravo i bolje se razvijalo od ostalih iste dobi. Prikaz ovog slučaja koristan je prilog literaturi i veterinarskoj praksi.

**Ključne riječi:** ektopija pluća, tele

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