

## Case Report

# Clinical, histopathological and surgical evaluations of persistent oropharyngeal membrane case in a calf

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## Abstract

A male, 4 days old and 20 kg Simmental calf was evaluated for regurgitation and hyper salivation since birth. The mother became pregnant by artificial insemination and the pregnancy was the second of the mother. A membrane closed the pharynx and a diverticulum on dorsal of this membrane was seen during oropharyngeal examination through inspection. Membrane was also viewed by endoscopy under general anaesthesia. Larynx and oesophagus were imaged by bronchoscopy through the back side of the membrane. After these applications, it was decided that soft palate adhered firmly to the root of tongue causing congenital atresia. Surgical treatment of oropharyngeal membrane was carried out under general anaesthesia. Firstly, tracheotomy was performed for to ease breathing and membrane removed by electrocautery application. Intensive fluid accumulation and oedema formation at the incision area were detected by endoscopic examination following operation and the calf had severe dyspnoea two days after operation and died due to respiratory insufficiency. At necropsy, severe inflammatory reaction, laryngeal oedema and intensive salivation at the surgical side was determined. Direct imaging techniques should be used to determine in the closed oropharyngeal lumen. Moreover, nasopharyngoscopy should be considered to image larynx and oesophageal way. Present case is the first report with concern to pharyngeal membrane formation together with direct imaging and surgical procedures. Therefore, it was considered that this case report could be useful for colleagues and literatures.

## Introduction

Oropharyngeal membrane forms in two different layers from the embryonic stage. First, outer layer originates from ectodermal epithelium and the second is inner layer from the endodermal epithelium. These two layers differ to each other by a connective tissue (Deep stomodeum wall). Pharynx is closed by this tissue towards to anterior direction. Ectoderm of stomodeum forms *membrana oropharyngea* by fusing with blind end of foregut (*Membrana buccopharyngea*). The membrane separates stomodeum from the front bowel, which is known as draft of mouth cavity. In the progressive stage, stomodeum and pharynx or pharyngeal membrane disappears during embryonic development for many breed in the third

and fourth week of pregnancy in order to create pharynx, this provides formation of oral cavity and oropharynx following palatoglossal arch. Persistent oropharyngeal membrane separates oral cavity from oesophagus and laryngeal entrance and then stays permanent because of insufficiency of embryonic development of gastrointestinal tract. Briefly, it is a developmental anomaly of oropharynx [1-8].

## Case Presentation

### First day after administration

A male, 4 days old and 20 kg Simmental calf was referred to Erciyes University, School of Veterinary Medicine Clinics at 11 April 2016. The calf had regurgitation (milk could not

## More Information

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**Keywords:** Calf; Persistent oropharyngeal membrane; Anomaly



be swallowed so directly came out from the mouth) and hypersalivation since birth. It was second calving of the cow, which became pregnant with artificial insemination. General condition of the calf was weak but mucosal colour was normal and had mild level of dehydration. Body temperature, respiratory rate and pulse frequency were 38.7°C, 44/min and 92/min, respectively. A commercial Bovine Viral Diarrhoea Virus (BVDV) antigen kit (BVDV Ag Point-of-Care-Test, Idexx) were used for the presence of BVD virus.

In the first evaluations of the calf, there was a complaint of regurgitation and therefore, oesophageal tubing made to relieve possible obstruction via mouth but no transition observed. Therefore, detailed pharynx examination was carried out under general anaesthesia by an endoscopy device (Eickemeyer Eickview 150 LED videoendoscope). For general anaesthesia, 0,1mg/kg xylazine hydrochloride (Xylazine hydrochloride, Basilazin %10, Bavet Ilac San. and Tic. A.Ş Tepeoren-Tuzla Istanbul) injected intramuscularly for sedation, 5 minutes after 1mg/kg ketamin hydrochloride (Ketamin hydrochloride, Ketasol %10 Richter Pharma AG, Wels Austria) injection intravenously. Front side of oropharyngeal membrane, dorsal to diverticulum was viewed by endoscopy. Back side of the membrane, larynx and oesophagus were imaged by a bronchoscope (Eickemeyer Eickview 70 LED bronchoscope). At the endoscopic examination, soft palate was viewed which was adhered firmly to the base of tongue causing congenital atresia (Figure 1A). There was a blind pouch access approximately 3 mm in width at the dorsal aspect of atresia (Figure 1B). The blind pouch was enlarged by free air during endoscopy. The pouch quite narrowed and obstructed by milk clot (Figure 1C). Using a suitable size of bronchoscope, isthmus faucium was imaged entering from nostril and nasal space, larynx, oesophageal and tracheal structures were recorded (Figure 1D). Nasopharyngoscopy and endoscopy were applied together following the procedures. The catheter could be passed through the natural hole which it was 3 mm in width, located in the oropharynx, extending to *Isthmus faucium*. No important findings were encountered at mouth and oropharyngeal junction apart from atresia by physical examination.

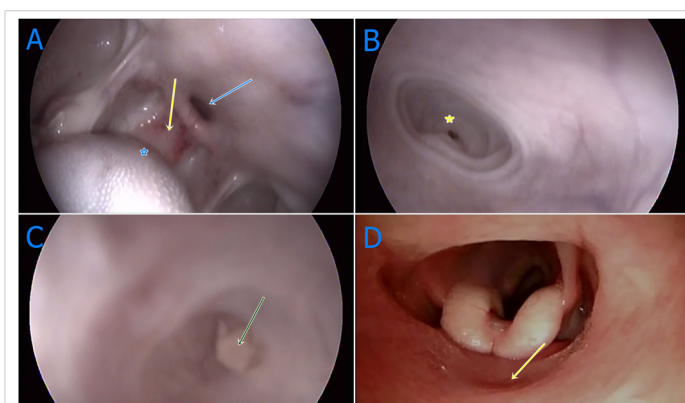
There was a swallowing reflex during catheterisation when a metal forceps touched to the membrane (Figures 2A-F).

Blood sample with ethylenediaminetetraacetic acid (EDTA) was used for hematologic analyses via a hematology analyzer (Mindray BC 2800 Vet. Auto Hematology Analyzer).

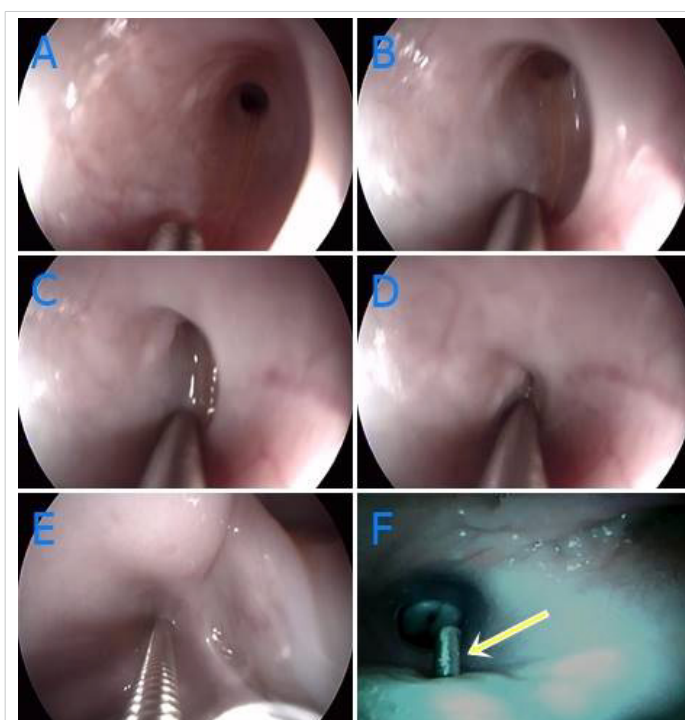
After diagnostic imagings, an oesophageal feeding tube was placed to feed the animal and to help the animal to get better its general condition before operation. For this purpose; sedation (Xylazine 2% 1 ml / 100 kg IM) was applied. The calf was then laid in the right lateral position and local anesthesia (Lidocaine 2%, 10 ml) was infiltrated from the left sulcus jugularis. Then, 5 cm skin incision was made

and the surrounding tissues and trachea were retracted and the esophagus was observed. Approximately 1 cm vertical incision was made in the esophagus, then, esophageal tube (Properties) was placed and fixed, the skin was closed with simple separate sutures. The calf was then fed through this tube. In addition, coagulated milk pieces detected during tubing which considered originated from natural hole.

Radiographic examination was carried out to see the oropharyngeal membrane (Fuji Film Computed Radiography CR-IR 392). But oropharyngeal membrane was not seen on plain X-ray. The oesophageal tube was also viewed in the radiographic examination (Figure 3A).



**Figure 1 A-D:** Oropharyngeal and nasopharyngeal endoscopic images of oropharyngeal membrane, A: General appearances of oropharynx with congenital anomaly. Yellow arrow; congenital oropharyngeal membrane, blue arrow; diverticulum dorsal to oropharyngeal membrane, blue star; caudal of tongue. B: Near plan image of diverticulum inflated with air. Yellow star; blind sac at the end of diverticulum, C: Coagulated milk accumulation at the end of blind sac (green arrow), D: Bronchoscope application via nasal approach, larynx imaging at back of oropharyngeal membrane (yellow arrow), intermediate wall of oropharyngeal membrane.



**Figure 2 A-F:** Examination of diverticulum by endoscopy and bronchoscopy, A-E: Contraction occurred by contacting of guide to mucosa, F: Imaging of guide protruding from intermediate of blind sac (yellow arrow).

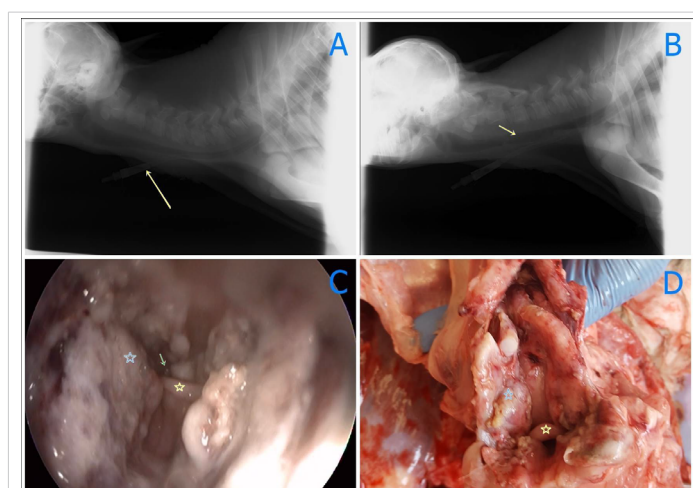
Before making operation, fluid disturbances were corrected by 0.9 % NaCl<sub>2</sub>.

Second Day after administration, a temporary tracheotomy was applied to relieve respiration before operation.

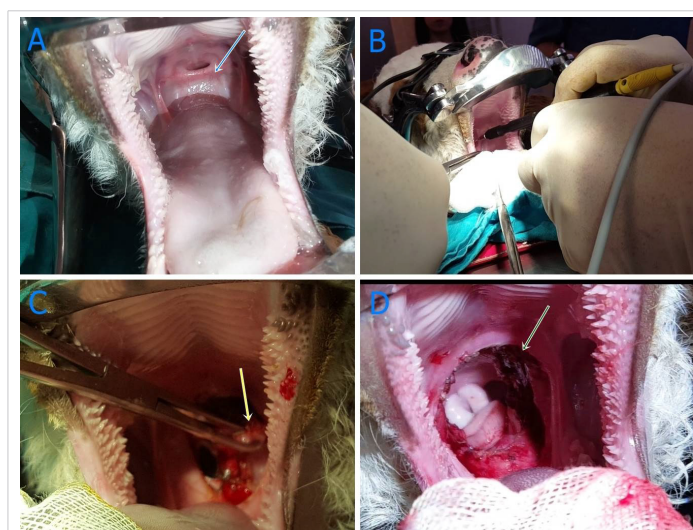
For general anaesthesia; 0,1 mg/kg xylazine hydrochloride injected intramuscularly for sedation, 5 minutes later, 1mg/kg ketamin hydrochloride injected intravenously and anaesthesia maintained by 2% sevoflurane (Sevorane, Aesica Quccnborough Ltd. Quccnborough Kent ME 11 5HL, England). After general anesthesia, the calf was turned to the ventrodorsal position for tracheotomy, approximately 5 cm of skin incision was made at the level of the 6-8 tracheal rings from the ventral line of the neck, subcutaneous connective tissue and the region was seen. The annular ligament between ring 7 and 8 was then incised transversally and the intubation tube (12 mm, Kruss, Germany) was inserted. The calf was connected to the general anesthesia device. After the operation, the intubation tube was removed and the operation site was closed by known routine technique.

Just after Tracheotomy, the membrane was removed surgically by making circumferential incision by an electrocautery (Elektro-Mag M 20-40, electrosurgical unit monopolar and bipolar) under general anaesthesia. Circumferential resection of oropharyngeal membrane was carried out (Figure 4A-D). Tracheal tube was removed following operation since a smooth respiration was monitored. Radiography was taken following operation and endoscopy and nasopharyngoscopy made one day after operation (Figure 3B). The contrast plain X-Ray film showed the some opacity on pharynges and pus aspirated in the trachea. Intensive fluid accumulation and oedema were recorded on the operation area by endoscopy (Figure 3C). After operation, white blood cells (WBC) count (10<sup>6</sup>/l), lymphocyte (10<sup>9</sup>/l), monocyte (10<sup>9</sup>/l), red blood cells (RBC) count (10<sup>9</sup>/l), haemoglobin concentration (g/dl) and haematocrit (PCV) (%) values decreased compared to those of initial values, which were also within normal reference ranges (Table 1). Two days after surgery, *dyspnoea* occurred and animal died on 2 days after operation.

Four days after administration, the animal died and immediately necropsy was performed. In necropsy, severe inflammatory reaction, oedema and excessive salivation at the side of operation area were noticed. Because of this, it was believed that the animal died due to asphyxia and sepsis depending on laryngeal oedema observed at the tracheal entrance (Figure 3D). In pathological findings, macroscopic inspection of the oral cavity showed a fold of tissue extending from the soft palate to the tongue, because of this, a complete partition between the oral cavity and oropharynx found to be formed. No other grossly observable congenital defects or lesions were present (Figure 3D). Tissue samples obtained during surgery and autopsy at oropharyngeal area were fixed in formaldehyde for 24 hours and blocked in paraffin



**Figure 3 A-D:** Radiographic, endoscopic and necropsy findings before surgery (yellow arrow), A: Radiographic findings following surgery: some pus in the trachea (yellow arrow) B: Endoscopic imaging after one day of surgery (blue star); Incised part of oropharangeal area (yellow arrow); Larinx (green arrow); fluid in larynx, D: View of pharengeal side in necropsy (yellow star), larinx (blue star), incised part of oropharangeal area.



**Figure 4 A-D:** Imaging recorded during surgery. A: Before surgery (blue arrow); oropharangeal membran, B: Extraction by electrocoater (yellow arrow); oropharangeal membran, C: Removing of membran by electrocautery, D: complete removing of membran following surgery (green arrow).

**Table 1:** Haematological findings before and after operation.

Haematological Findings	Units	Before operation	After operation	Reference Interval [13-16].
WBC	×10 <sup>9</sup> /l	4.4	3.2	3.4-9.6
Lymphocytes	×10 <sup>9</sup> /l	2.4	0.9	1.9-5.9
Monocyte	×10 <sup>9</sup> /l	0.4	0.2	0.2-0.8
Granulocyte	×10 <sup>9</sup> /l	1.6	2.1	3.80±0.77
RBC	×10 <sup>12</sup> /l	7.29	6.33	1.49-7.63
HGB	g/dl	10.8	8.9	2.21-10.41
PCV	%	31.4	27.8	23-31
MCV	fL	43.2	44.0	3.5-42.8
MCH	pg	14.8	14.0	11.0-17.0
MCHC	g/dl	34.3	32.0	36.0-40.0
PLT	10 <sup>9</sup> /l	832	454	212-451

**Abbreviations:** (HGB) haemoglobin, (MCH) mean corpuscular haemoglobin, (MCHC) mean corpuscular haemoglobin concentration, (MCV) mean corpuscular volume, (MPV) mean platelet volume, (PCV) Haematocrit, (PLT) platelet, (RBC) Red blood cells, (WBC) White blood cells.

after exposed to alcohol and xylene. Four to five  $\mu\text{m}$  thick sections were stained with haematoxylin and eosin (H&E) and examined microscopically for histopathological changes. Digital camera (Olympus DP71) and digital programmers (DP Controller and the DP Manager) fitted to a microscope (BX-51, Olympus) (minimum of 4 fields per slide, using  $\times 10$ ,  $\times 40$ ) were used to photograph all slides.

The abnormal membrane that lies between the mouth and the soft palate found to be covered with stratified squamous epithelium. The mucosa of soft palate coated with stratified flattened and non-cornified epithelium. The soft palate mucosa cell layers were in lower numbers at oropharyngeal area. The surface was covered with non-cornified, pluristratified irregular surface and flattened epithelial cells having papillary extensions. The stratum basale layer consisted of cells having 1 or 2 eccentric nuclei, condensed chromatin clusters, round, oval shape. Stratum spinosum, elongated more towards superficial epithelium had different numbers of cells, stained lightly and had big nuclei. Cytoplasm of the elongated and degenerated cells were strongly eosinophilic, and their nuclei stained darkly. Mucous glands in the propria and in the lymphoid tissue and submucosa muscle layer were present (Figure 5A-H).

## Discussion

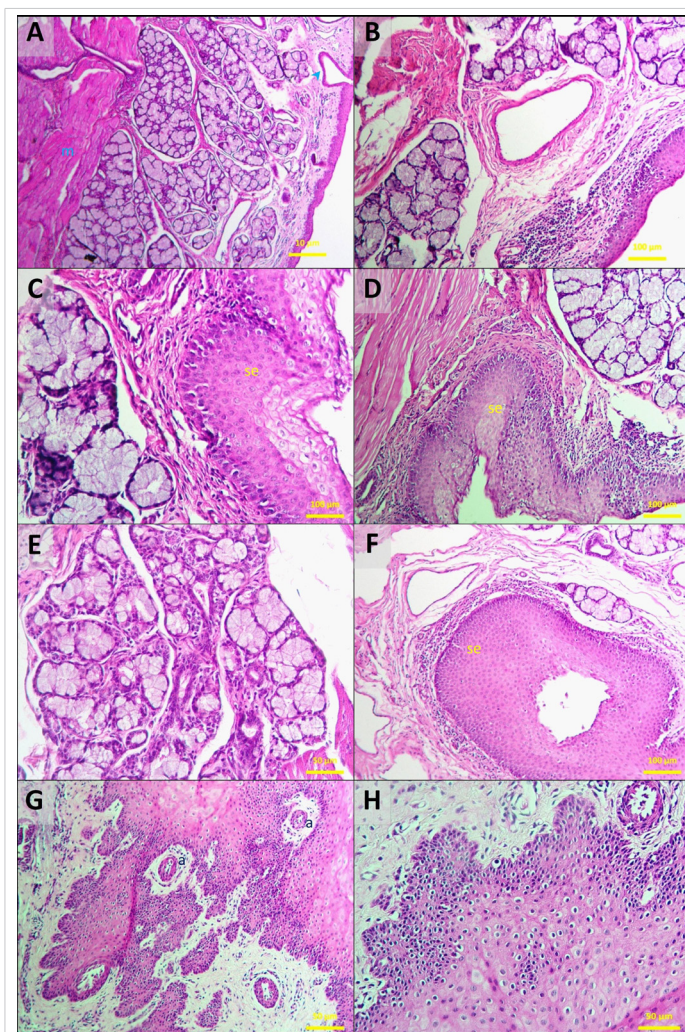
Permanent status of oropharyngeal membrane is a rare defect that defined in a human patient [8,9]. Such anomaly is also reported first time in a Hereford calf in 1996 by Smoak and Hudson [8]. In this case, the calf was 4 days old which had a tissue covering caudal of oral cavity was encountered and the case evaluated by imaging techniques and necropsy [8]. Literatures regarding to the oropharyngeal membrane in veterinary medicine are insufficient. It has been reported in brachycephalic dogs due to similarity of choanal atresia, moreover, a permanent membrane covering oropharynx also defined [10]. This kind of congenital anomaly prevents passage of milk and the other foods to oesophagus.

The present study indicates different clinical finding from that of the reported by Smoak and Hudson [8]. The calf in the present study defecated and regurgitated milk in contrary to reported by Smoak and Hudson [8]. Furthermore, a small amount of milk came out following oesophagostomy in the present case. A significant loss of body condition was also reported for the animal with oropharyngeal membrane. The birth weight and body condition of the calf in our study was also low as reported in the literature [8]. Although, aetiology of persistent oropharyngeal membrane has not been known completely yet. Pregnancy, some uncertain effects or viral infection agents may lead to the disorder [8,9]. Thus, a commercial Bovine Viral Diarrhoea Virus (BVDV) antigen kit (BVDV Ag Point-of-Care-Test, Idexx) were used for the presence of BVD virus but the result was negative.

Hematological analyses results obtained after initial

examination were evaluated within normal references ranges (Table 1). In studies on normal hematologic profile of neonatal calves, it is observed that their values are quite different and in wide ranges [11-16]. In this case, preoperative and postoperative hematological values were found to be very close to the reference values obtained from previous studies on calves of different breeds and genders. In a recent study by Panousis et al. (2018) investigated Median hematological profiles of 254 Holstein calves aged between 1-9 days and in the present study both pre- and post-operation haematological values were within the Min-Max values of above study [16]. However, postoperative WBC, lymphocyte, monocyte, RBC, hemoglobin, PCV, MCHC and PLT values were found to be lower than the same values obtained before operation. It was supposed that the decrease in the blood values might be the result of bleeding during and after the operation.

Oropharyngeal membrane may occur due to genetic or tetragenic reasons [8]. However, this hypothesis is still not



**Figure 5 A-H:** Oropharynx. Mucous membrane with stratified squamous epithelium. This is the mucosa of the oropharyngeal aspect of the soft palate. the connective tissue papillae (a) are of moderate/high height and the stratified squamous epithelium (se) is not keratinized. Mucus secreting glands open onto the surface (arrowed). Propria-submucosa contains mucous glands (B) and lymphoid tissues. Muscularis externa (m), formed by skeletal muscle fibers. H&E stain; bar = 10  $\mu\text{m}$  (A), bar = 50  $\mu\text{m}$  (E-H), bar = 100  $\mu\text{m}$  (B,C,D,F).



clear due to unproved evidences. The most common face and mouth anomalies in animals are; agnathia, brachygnathia, choana atresia or stenosis, naso lacrimal atresia, aglossia and abnormal teeth developmental disorders [2].

Oropharyngeal membrane is an uncommon type of congenital defect. Membrane resection has been made for the treatment in human and dog [12], but no treatment has been reported in farm animals. Surgical intervention was experienced first time in this case.

Radiographic examination revealed restricted clues in this case. Oropharyngeal cavity could not be observed clearly by X-ray, but the opacities due to pus in the trachea and postoperative prognosis after operation was determined. Endoscopic examination of diverticulum should be made in the case of persistent oropharyngeal membrane via the mouth. Nasopharyngoscopy application may also be required to image larynx-oesophagus entrance, epiglottis and back of closed oropharyngeal cavity. It helps to provide valuable information for evaluation of multiple anomalies and detailed image of anatomic structures. This types of anomalies cause difficulty for veterinarian regarding management and treatment. Calf may prone to infection and die quickly because of having insufficient colostrum and passive transfer insufficiency. Moreover, in the present case report, although all precautions {hygienic precautions and antibiotic (Reptopen S, 1ml/10 kg IM, CEVA, Turkey) and Nonsteroidal Antienflammatory Drug (Fulimed, 2ml/45 kg IM, Alke, Turkey) for 2 days after operation} for the health of the calf were taken, the animal had diarrhoea and local infection following operation, indicating passive transfer insufficiency.

Supporting of immune system should be maintained before operation for such animals. It was supposed that another reason of death was tracheal aspiration of exudates (Figure 3B). Therefore, tracheal tube placement should be left postoperatively until improvement of general body condition.

In conclusion, oropharyngeal atresia encountered in this study differed from that of the previous reported case [8]. Imaging, surgery and histopathological examination were carried out first time in this report on contrary to the other study [8]. The study was considered useful for veterinarian and literature. Electrocautery, cryosurgery and laser surgery should be used to minimize possible bleeding.

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