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# Congenital cleft lip-jaw-palate and cleft palate in German Holstein Calves with Common Ancestry

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**Summary:** In this study two calves with cleft palate extending over the full length of the osseous palate and common ancestors are presented. The first case, a female German Holstein calf was one month old at examination and showed a cleft palate, a right sided cleft lip and jaw, diverging rostral cerebral hemispheres, bulbi olfactorii and chiasma opticum and in addition, a meningocele on the left side of the face. The tongue of the calf hung down on left side. The second case was a male German Holstein, calf examined with an age of two months. This calf had a cleft palate (CP) extending over the entire length of the osseous palate. Other inborn organ defects could not be found in both calves. The calves originated from different dairy farms. Both calves suffered from a bronchopneumonia and in addition, the second case had a severe diarrhea. Both calves were negative for bovine viral diarrhea (BVD) virus antibody and antigen. Both calves had four common ancestors from either parent. The common ancestry may be indicative for genetic factors involved in the malformation observed in both cases.

**Key words:** Anomaly, cattle, cleft lip-palate, congenital, Holstein

**Ortak Ataları Olan İki Alman Holştayn Buzağıda Konjenital Damak-Dudak-Çene Yarığı ve Damak Yarığı Olgusu**

**Özet:** Ataları ortak olan iki buzağıda yapılan bu çalışma da sert damak kemiğine kadar uzanan bir damak yarığı olgusu sunulmaktadır. Birinci olgu, dişi bir aylık Alman Holştayn buzağı olup; dudak-damak ve çene yarığı ile beraber sağ rostral beyin hemisferi, bulbi olfactorii ve chiasma opticum'u ayrılmış olarak görüldü. Buna ek olarak, yüzünün sol tarafında bir meningosel mevcuttu. Buzağının dili sol tarafa aşağıya doğru asılı olarak duruyordu. İkinci olgu ise erkek Alman Holştayn buzağı olup iki aylık iken incelemesi yapıldı. Bu buzağı da ise tüm damak kemiği uzunluğu boyunca uzanan bir yarık damak (CP) olgusu gözlemlendi. Buzağılarda başka organ anomalileri görülmedi. Buzağılar farklı çiftliklerden gelmiştir. Her iki buzağıda da ağır seyreden bir bronkopnömoni tablosu bulunmaktadır. Buna ek olarak ikinci erkek buzağıda şiddetli bir ishal vardı. Her iki buzağınında bovine viral diare (BVD) virüs antikoru ve antijeni yönünden negatif olduğu belirlendi. Buzağıların ikisinde hem anne hem baba tarafından dört ortak atasının olduğu belirlendi. Ortak soydan gelen genetik faktörlerin her iki buzağıda gözlenen malformasyonların sebebi olabileceği düşünüldü.

**Anahtar kelimeler**: Anomali, dudak-damak-çene yarığı, Holştayn, konjenital, sığır

## Introduction

Many different congenital abnormalities have been defined in most breeds of cattle. Reportedly 0.2 to 3.6% of all calves born have congenital defects and the frequency of cleft palate (CP) is 5.1% in all congenital defects (9). CP results due to failure of closure of left and right embryonic nasal and/or maxillary fold. The disorder may involve the soft palate or the hard palate or both. Hereditary factors (18), environmental factors such as piperidine alkaloids, wild tobacco (*Nicotiana gluaca*), intoxication with selenium and lupines species (13-15,26-28), viruses such as Cache Valley virus, Akabane virus, Aino and Chuzan viruses and bovine viral diarrhea (BVD) virus (18,34,36) may be induce cleft palates in animals. In Shorthorn calves, cleft lip (CL) has been attributed to homozygosity of a simple autosomal recessive gene (37), but the author's experience with this defect in Angus and Jersey cattle indicates that genetic transmission is most likely to be polygenic. In a calf with palatoschisis CP, chelioschisis (lip palate) and flattened face an autosomal trisomy 20 (61,XX,+20) was found (7). In humans, various mutations of the CLMPT1 and PVRL1 gene may be involved in non-syndromic CP (35). A large number of genes have been implicated in non-syndromic and syndromic CP and cleft lip palate (CLP) in human (30,39). Cleft palates have been observed as isolated congenital abnormalities (3,21) or in combination with defects in other parts of the body (25,31). CP could be

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associated with the akroteriasis syndrome in Holstein cattle, chondrodysplasia in Dexter cattle, arthrogryposis (SAP) and spinal anomalies in Charolais cattle and hydrocephalus, diaphragmatic hernia, and freemartins in Simmental cattle (1,8,10,16,17,19,20,22,31,32). Successful operative treatments in affected calves for longer survival life are performed (2,23). The CP has been described in various animal species (11,12,29). The objective of the present study was to present a rare form of cleft lip-jaw-palate (CLPJN) in a 103-days-old female and CP in an 81-days-old male German Holstein calf based clinical, neurological, medical imaging, pathological, virological and cytogenetic findings as well as pedigree analysis.

**Case history**

Case 1 (CLPJN), a 21-day-old Holstein female calf was presented with a cleft-lip-jaw-palate (Figure 1A, 1B, 1C, 1D).

The CLPJN-affected-calf was born after full term gestation in July, 2009 by a Holstein heifer. All cows were bred by artificial insemination (AI). The sire of the CLPJN-affected-calf was an Italian Red Holstein AI-bull. The dairy herd was free of BVD virus and bovine herpes virus type 1 (BHV1). Case 2 (CP), a 14-day-old Holstein male calf was presented with a CP (Figure 2A). The CP-affected-calf was born after full term gestation in September, 2009 by a second-lactation Holstein cow. Other cases with CLPJN or CP were not known on both dairy farms. At the age of three weeks, the affected calves were transferred to the Institute for Animal Breeding and Genetics, Hannover, Germany.

***Clinical and neurological examination***

The animals were clinically examined. Cerebrospinal fluid (CSF) has been taken from atlantooccipital area for neurological analysis. In the clinic for small animals, Magnetic Resonance Imaging (MRI, Magnetom Impact Plus, 1.0 Tesla Siemens) and Magnetic Resonance Computer Tomography (MSCT, Brilliance CT 64, Philipps) were performed for the heads of both calves. The general condition of the CLPJN-affected-calf worsened due to a bronchopneumonia. The CLPJN-affected-calf showed abdominal type breathing with an increased respiratory rate of 44 per minute. Breath sounds were slightly aggravated at tracheobronchial inspiration and tightened in auscultation. Further, a tachycardia with a heart rate of 118 beats per minute was present. The internal body temperature was at 40.4°C. Due to the severe bronchopneumonia, poor general condition and resulting from CLPJN care and nutrition problems, the CLPJN-affected-calf had to be euthanized at the age of 103 days. The general condition of the CP-affected-calf worsened due to a pneumonia and diarrhea. The CP-affected-calf had a

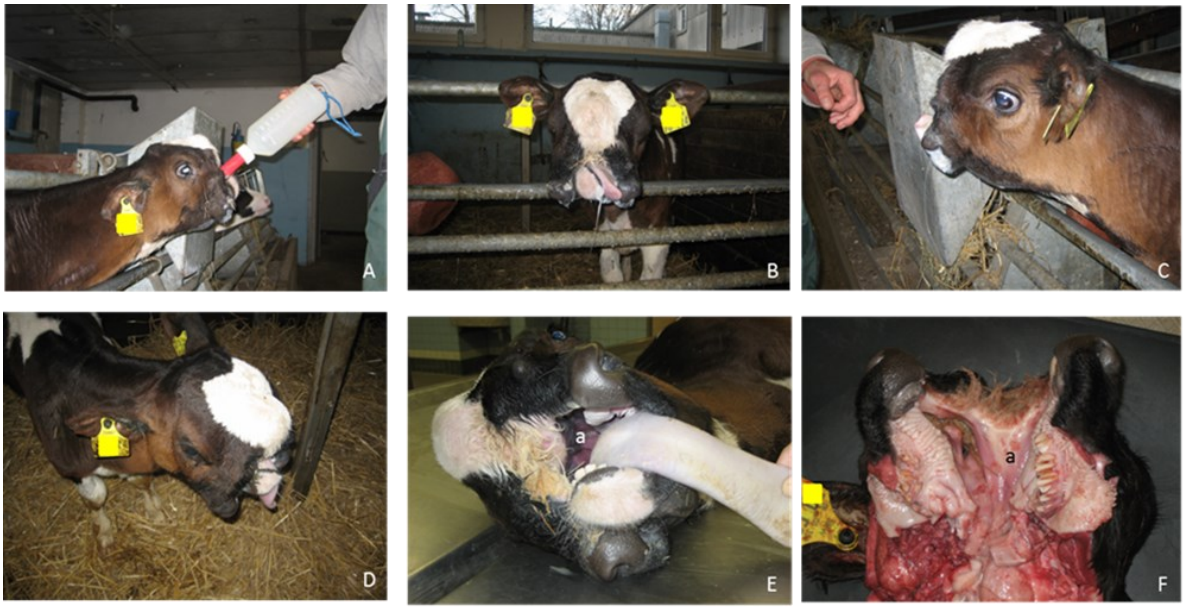


**Figure 1**: Holstein calf affected by cleft-lip-jaw palate (CLPJN) from the (A) right side, (B) front side, (C) left side, (D) upper side, (E) front side and (F) nasopharynx after necropsy.

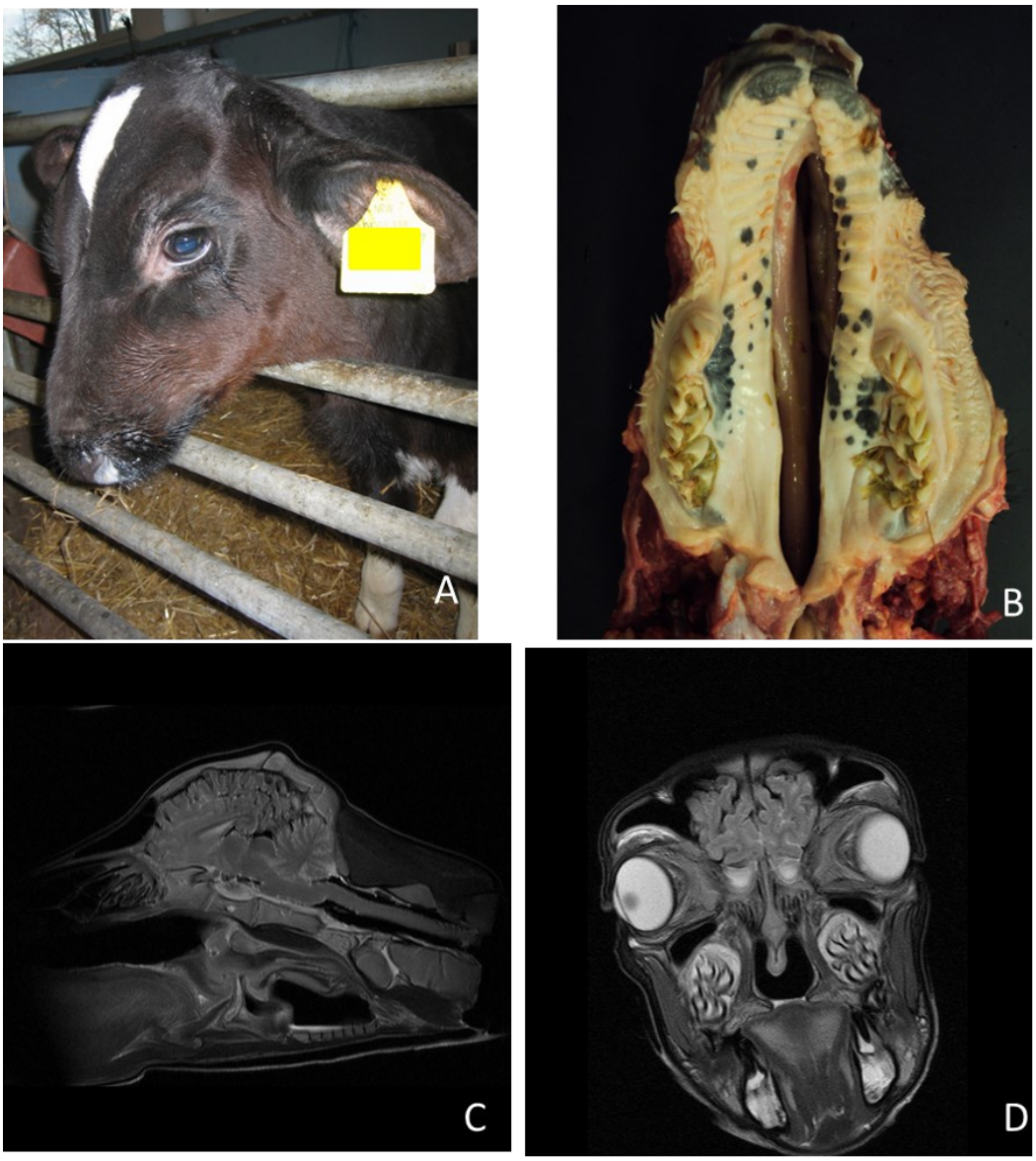


**Figure 2**: Holstein calf showing a median cleft palate (CP) (A, B). Images using computer tomography (CT) of the CP-affected calf from the (C) sagittal and (D) transversal side

rectal temperature of 38.1°C, a respiratory rate of 41 per minute and a heart rate of 105 beats per minute. Due to the pneumonia, severe diarrhea, and generally weak condition resulting from nutrition problems due to the CP, the calf had to be euthanized at the age of 81 days. Neurological examination showed normal awareness, behavior and attitude. A mild tetraparesis with partial-mouth grinding, very wide apart and uncertain (ataxia) were observed in the CLPJN-affected calf. The nose including maxillary bone and mandibular angle from right down to left up were split (Figure 1B). Therefore, jaw closure was impossible (Figure 1A, C, D). There was a bony exostosis in the left frontal area of the face with a soft and compressible central area with a dimension of 6x7x8 cm. Because of that, a rotation of both eyes to the lateral sides of the CLPJN-affected calf was observed. The corneal reflex (N. opticus and N. facialis) was highly reduced on the right side but was inconspicuous

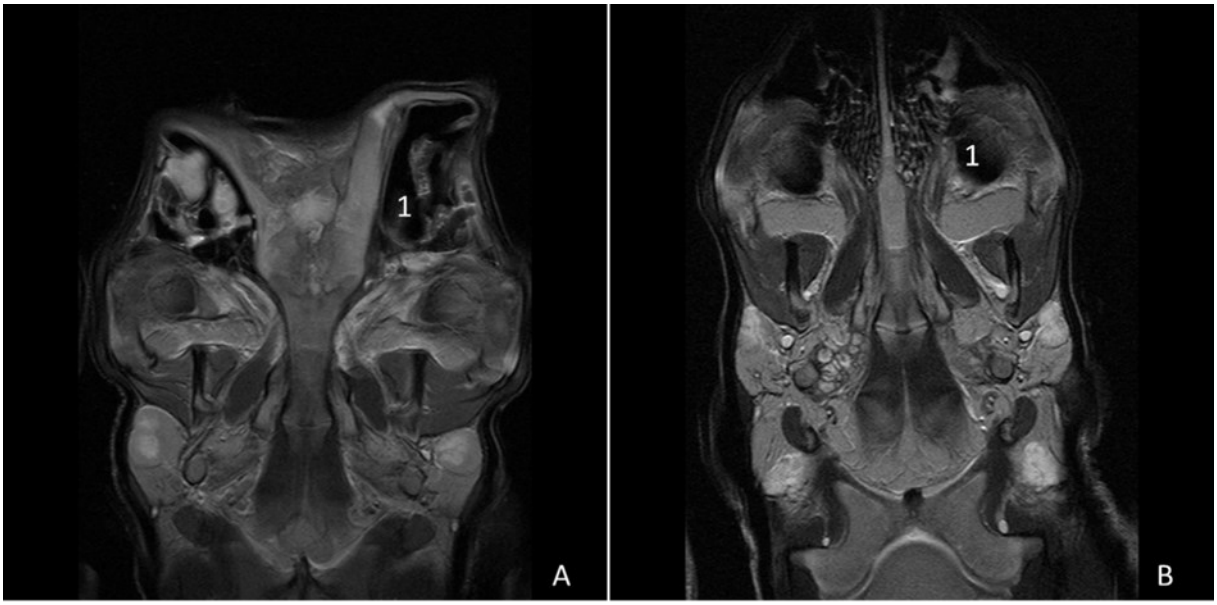


**Figure 3:** Comparison of both cases, cases 1 with a cleft-lip-jaw and cleft palate(CLPJN) and 2 with a cleft palate(CP), at the same intersection using computed tomography (CT). **A:** Dorsal CT-section of the CLPJN-affected calf, **B:** Dorsal CT-section of CP-affected calf. **1:** Same CT-section of the nostrils.

on the left side. The sensitivity, lid reflex and physiological nystagmus of both sides were inconspicuous. The pupils of both sides were rather small. Swallowing reflex was slightly reduced. Glucose (73 mg/dl), UCSF-TP (total protein) (19 mg/dl), counts for erythrocytes (0) and leukocytes (0) in CSF were within the normal range for cattle. Neurological examination of CP -affected-calf showed normal visual awareness, behavior, attitude, walk and head nerves. In addition swallowing reflex, flexor reflex of four limbs and cutaneous trunci were inconspicuously. CT of the CLPJN-affected-calf showed a cleft extending from the middle of the lip to the caudal end of the bony palate. In addition, the cleft also involved the median axis of the rostral parts of the brain (Figure 4A, 4B). The cleft divided the cerebral hemispheres at a length of 5 cm and width of 1.5 cm starting rostral to the hypophysis and ending caudally to the chiasma opticum. Thus, septum pellucidum became visible between the two cerebral hemispheres. Instead of the bulbi olfactorii each one meningocele filled by a yellowish to brownish fluid with a volume of 30 ml was found. CT of the CP-affected calf confirmed the cleft palate in the median of the palate (Figure 2C, 2D).

***Pathological examination***

The present cases were submitted for necropsy and histopathological examinations at the Institute for Pathology, University of Veterinary Medicine Hannover. All organs were subjected to pathohistological examinations. Tissue sample of the organs were fixed for ten days in 10% neutral buffered formalin (NBF). Then the tails of organs were laminated and the macroscopically altered areas were dehydrated in an ascending alcohol series and embedded at 56°C in a paraffin-parablast mixture (Histo-Comp, Vogel, Giessen, Germani). The paraffin block sections (2-3µm) were prepared in a colour machines (Leica ST 4040, Nussloch, Germani) with Hemotoxylineosin (HE). The CLPJN-affected calf showed a complete right sided cleft of the lip, jaw and palate. The left lip and the muzzle were separated through a broad cleft and thus, there was an opening of the oral cavity to the nasal meatus. The widest extension of the cleft was 8.2 cm in length and 7.4 cm in width (Figure 1F). A meningocele with a dimension of 6x7x8 cm was located on the left dorsal side of the face including the os frontale. The borders of the meningocele were bony and congenital cleft lip-jaw and cleft palate the central area consisted of soft connective tissue. The

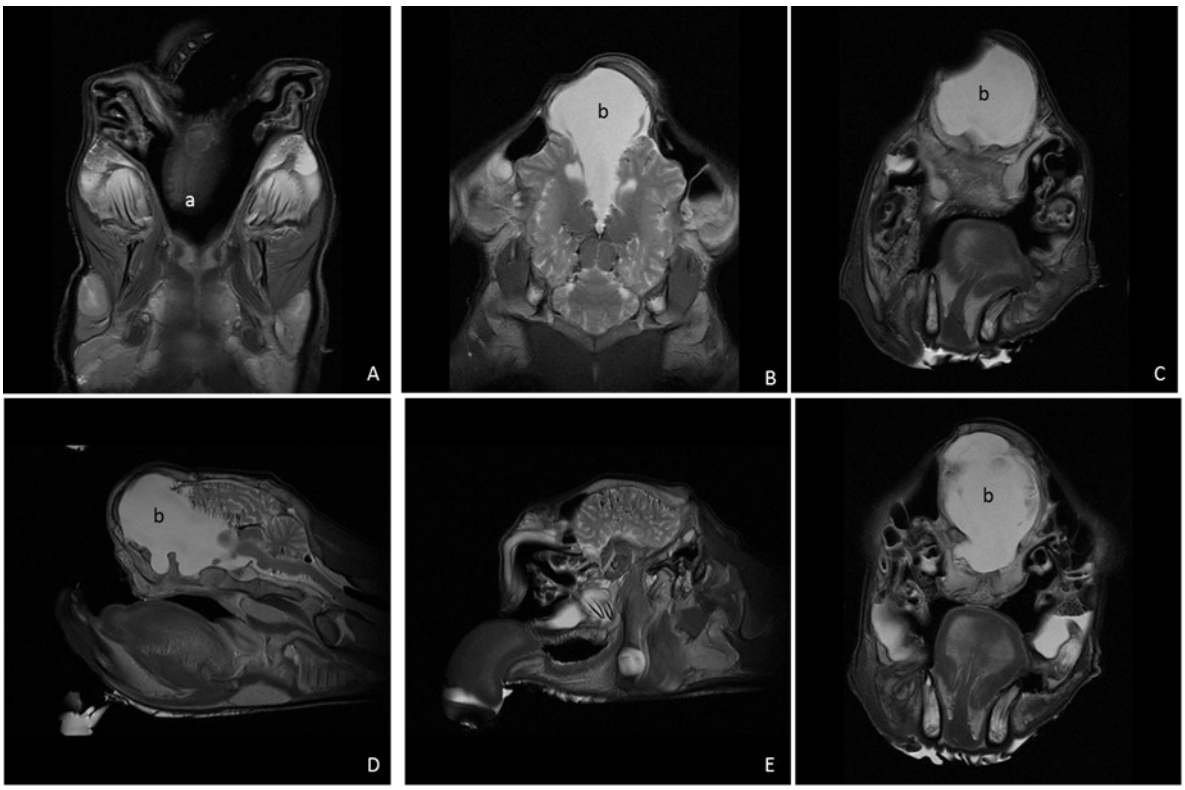


**Figure 4 A:** Dorsal section of the palate of the calf affected by a cleft-lip-jaw and cleft palate(CLPJN) using computed tomography. **B, C, D:** (b) Change of *os frontale* of the CLPJN-affected calf. **E:** Sagittal section of the CLPJN-affected calf.

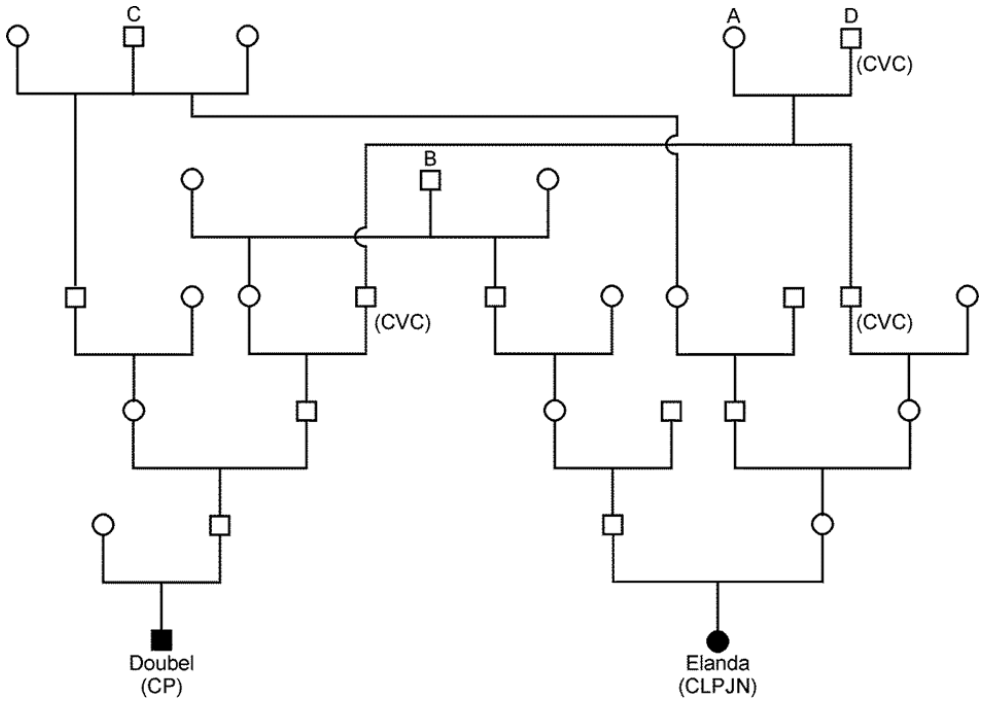


**Figure 5:** Pedigrees of the calves affected by cleft palate (CP) and cleft-lip-jaw-palate (CLPJN) CVC= tested carrier of complex vertebral malformation

content of the meningocele was fluid. The left lower jaw showed a deviation to the right side on the whole length and the right lower jaw a deviation to the right only at the rostral part. All teeth were present (Figure 3). A catarrhal purulent bronchopneumonia and a granulomatous pneumonia were observed. Mesenteric and pulmonic lymph nodes were enlarged. The CP-affected calf had a median cleft palate with an extension of 19x2 cm (Figure 2B). An ulcerative rhinitis, a follicular hyperplasia of the intestinal and retropharyngeal lymph nodes, a bronchopneumonia and catarrhal enteritis were found.

***Other findings***

Both cases had a normal karyotype of a female with 2n=60,XX (CLPJN) and a male with 2n=60,XY (CP). The blood examples for virological study was tested from BVD virus antigen (Ag) and BVD virus antibody (Ab) using ELISA tests. Virological findings in both cases, BVD virus antigen and antibody were not found. Pedigree information was recorded for ten ancestral generations. Pedigree data was analyzed using the program Opti-Mate, version 3.81 (38). Pedigree analysis the inbreeding coefficient of the CLPJN-affected calf was 1.514% and of the CP-affected calf 2.1%. The two cases shared four common ancestors, three Holstein AI-bulls (B, C and D) and a common grand-granddam (A) (Figure 5).

## Discussion

The two cases had a common ancestry and a CP in common. We could rule out environmental factors including intoxication with teratogenic substances of tobacco, lupines and selenium (13-15,26-28) and infectious agents including viruses such as Cache Valley virus, Akabane virus, Aino and Chuzan viruses and BVD virus (18,34,36). Also, chromosomal abnormalities seemed unlikely to have caused CP in these two calves (7). Autosomal trisomy 20 may be associated with multiple severe malformations (7). Charolais calves with a 1/29 translocation were affected with severe arthrogryposis besides CP (6). Genetic factors may be considered as possibly responsible due to the common ancestry and previous reports assuming hereditary factors involved in CP (18,35,37). Congenital cleft lip-jaw-palate and cleft palate bronchopneumonia and food aspiration are common signs in calves with large CP (5,33). Weight gain is usually reduced, particularly, with an increasing age of the animals (5). In the present cases, weight loss was not as severe as in older animals and in-between the range for Holsteins (4). An association of a CP with a meningocele has not yet been reported, whereas a median cleft lip and jaw can be accompanied by a CP (24). Inspection of the pedigrees for the two calves supports the assumption of an autosomal recessive inheritance. Further conclusions on the mode of the inheritance and the origin of a possible mutation cannot be made from the limited data available. In human, inheritance is assumed to be involved in CL/P and several genes and pathways have been identified to be implicated in the pathogenesis (39). However, for the majority of human patients with CP or CLP the underlying causes remain unknown. Genes with identified mutations include interferon regulatory factor 6 (IRF6), forkhead box E1 (FOXE1), muscle segment homeobox, drosophila, homolog of, 1 (MSX1), special AT-rich sequencebinding protein 2 (SATB2), sonic hedgehog (SHH) and transforming growth factor, beta-3 (TGFB3), Rho GTPase activating protein 29 (ARHGAP29) and distal-less 1, 2, 4, 5 and 6 (DLX1, DLX2, DLX4, DLX5, DLX6) (30,39). In conclusion, hereditary factors may be assumed to be involved in the two cases with CP and CLPJN. Identification of responsible mutations requires whole genome re-sequencing of the cases and their parents as well as collection of further cases.

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