Pakistan J. Zool., vol. 54(3), pp 1345-1354, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20200713110751

Anatomical and Pathological Evaluation of Congenital Anomalies in Calves: 6 Cases

Gülseren Kırbaş Doğan[1,*], Emin Karakurt[2], Mushap Kuru[3] and Hilmi Nuhoğlu[2]

[1]*Department of Anatomy, Faculty of Veterinary Medicine, Kafkas University, Kars-36100, Turkey*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars-36100, Turkey*
[3]*Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kafkas University, Kars-36100, Turkey*

ABSTRACT

Developmental anomalies can be caused by defects in bone tissue, cartilage tissue, or primitive mesenchymal tissue. Genetic, environmental, teratogenic, faulty breeding selection, or feeding related anomalies can be observed either locally or systemically. This study aimed to evaluate in detail the various anomalies in six calves according to pathological and anatomical investigations. Six calves were delivered to the Department of Pathology at the Kafkas University Faculty of Veterinary Medicine between 2017 and 2019. These calves comprised one with anencephaly, one with diencephalic syndrome, one with schistosoma reflexum, two with anasarca, and one with nasal and calvarium openings. After necropsy, samples were taken from the organs, foreseen and routine pathological examinations were performed. Following these procedures, the calves were brought to the anatomy laboratory and anatomically examined. The most important result; in the mid-section of the sections taken from the existing structure other than the normal formation at the medulla oblangata and pons level in the brain, it was found that in the outer part of the meninges, an inversion of the substantia alba was formed in an outer layer, as if the substance was invaginated. As a result, various anomalies in 6 calves were evaluated according to pathological and anatomical investigations. These findings are believed to contribute to the literature.

Article Information
Received 13 July 2020
Revised 22 November 2020
Accepted 01 February 2021
Available online 24 June 2021
(early access)
Published 26 February 2022

Authors' Contribution
GKD took the measurements and examined anatomical structure. GKD and MK made a dissection by obtaining materials from the dead animal. EK and HN did pathohistological examination.

Key words
Anatomy, Anomaly, Calf, Case, Histopathology.

INTRODUCTION

Developmental anomalies can be caused by defects in bone tissue, cartilage tissue, or primitive mesenchymal tissue (Milli and Hazıroğlu, 2000). Genetic, environmental, teratogenic, faulty breeding selection, or feeding related anomalies can be observed either locally or systemically (Dittmer and Thompson, 2015; Milli and Hazıroğlu, 2000; Özfiliz, 1993). In one study, it was reported that 47.2% of calves had abdominal wall and gastrointestinal system, 26.6% had musculoskeletal system, 14.6% had head region, 4.1% had urogenital system and 7.3% had multiple anomalies (Aksoy *et al*., 2006). In another study, congenital anomalies were mostly seen in musculoskeletal system (59.79%), followed by digestive (27.84%), urinary (7.22%) and nervous (3.09%) systems. In the same study, anomalies were mostly seen in Swiss Brown calves (Kaya *et al*., 2011). Anasarca (Yüksel *et al*., 2019), anencephali (Zachary *et al*., 2013), diprosopus (Salami *et al*., 2011; Sharma *et al*., 2010), palatoschisis (Atasever and Ekebaş, 2016), schistosoma reflexum (Buck *et al*., 2009), brachygnathia inferior (Taşal and Aytekin, 2015), and perosomus elumbus (Jones, 1999) are the anomalies reported in calves.

Anasarca is an anomaly characterized by the accumulation of fluid between all the soft tissues of the calf. So there is excessive subcutaneous edema (Yüksel *et al*., 2019). Calf is a hippopotamus or leather bottle view. It is generally seen in multiple pregnancies (Kaya *et al*., 2015). There are also cases where anasarca is seen with palatoshysis (Yüksel *et al*., 2019). Congenital anomalies in the nasal region are mostly accompanied by craniofacial defects. Calves born with defects in the nose are usually still born dead or die immediately after birth. It can be seen with palatoshysis. These animals usually die as a result of aspiration pneumonia (Milli and Hazıroğlu, 2000). Anencephali is a case when the brainstem is preserved, the rostral part of the brain absent or rudimentary (Mahabady and Barati, 2012; Zachary *et al*., 2013). Some cases of where there are no bulbus olfactorius (arrhinocephaly) and hemispherium cerebri (inepisencephaly) from the forebrain formations have been reported (Mahabady and Barati, 2012). Diprosopus is an anomaly characterized by varying degrees of duplication of the face in animals with

* Corresponding author: glsrn36@gmail.com
0030-9923/2022/0003-1345 $ 9.00/0

a single trunk and limb system. Diprosopus is associated with anomalies such as cleft palate, unilateral agnati, pseudohermaphrodism and arthrogriposis (Atasever and Ekebaş, 2016). While diprosopus refers to all duplications in the head, dicephalus identifies duplications in the brain. Dublications are more common in the cranial part of the fetüs (Robert, 2004). In cases with diprosopus there are four eyes (tetraophtalmus), two pairs of nostrils, 2 mouths, 4 auricles (Salami *et al*., 2011). Schistosoma reflexum is a development anomaly characterized by the don't closure of the chest and abdominal cavities. Sacrum, coxae and hind legs go forward front and sides as a result of changing the direction of columna vertebralis (Semacan *et al*., 2012).

This study aimed to evaluate in detail the various anomalies in six calves according to pathological and anatomical investigations. Six calves were delivered to the Department of Pathology at the Kafkas University Faculty of Veterinary Medicine between 2017 and 2019.

MATERIALS AND METHODS

A total of 6 calves were brought to the Department of Pathology, Faculty of Veterinary Medicine, Kafkas University and prepared for systemic necropsy. These calves comprised one with anencephaly, one with diencephalic syndrome, one with schistosoma reflexum, two with anasarca, and one with nasal and calvarium openings. After necropsy, samples were taken from the organs, foreseen and routine pathological examinations were performed. Following these procedures, the calves were brought to the anatomy laboratory and anatomically examined.

Histopathological investigations

Tissue samples from animals were fixed in 10 % buffered formaldehyde solution. After routine tissue procedures, paraffin blocks were prepared and sections with a thickness of 5 µm were taken for Hematoxylin Eosin (H&E) staining. Sections were examined with H&E in the light microscope to determine histopathological subtypes and photographed with Cell^P Program (Beytut, 2017).

Immunohistochemical investigations

Avidin-Biotin peroxidase method was used as immunohistochemical method. For immunohistochemical staining, the sections of 4 µm in thickness taken to poly-L-lysine coated slides were deparaffinized and rehydrated in graded alcohols. In order to prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution in phosphate buffered saline (PBS) for 15 min. For antigen retrieval, the sections were boiled in citrat buffer solution (pH 6) for 25 min in the microwave oven (at 800 watt). In order to prevent nonspecific staining, the sections were incubated for 30 min with non-immune serum (Genemed Biotechnologies REF 54-0003) at room temperature. Primary antibody (Synaptophisin, Thermo Fischer, SP11, Ready to use) were incubated for one hour at room temperature. The sections were washed 3 times in PBS solution for 5 min, and the biotinylated secondary antibody (Genemed Biotechnologies REF 54-0003) were applied to them at room temperature for 30 min. After washing in PBS (3-5 min), all sections were incubated with peroxidase-bound Streptavidin (Genemed Biotechnologies REF 54-0003) for 30 min at room temperature. A solution of 3.3-diaminobenzidine tetra hydrochloride (DAB) (Genemed Biotechnologies REF 10-0048) was used as a chromogen for 15 min. The sections were treated with Mayer's Hematoxylin for 30 second and washed in running water for 5 min, dehydrated in graded alcohols, cleared in xylene and coated with entellan. Primary antibodies were omitted from the negative control sections and were treated with diluted normal serum. The slides prepared after the covering were examined under a light microscope and photographed via the Cell^P program. Analyzes of the images were done with Image J Program (Beytut, 2017).

RESULTS

Table I shows the data and results of calves with anomalies. Histopathological examination of the A1 number anasarca (Fig. 1A) was determined interstitial pneumonia characterized by edema and excessive thickening of the table in the lung alveolar walls in the lungs of patients. In addition, severe hydropic hepatocyte degeneration was detected in the liver (Fig. 1B, C). Anatomic examination revealed excessive edema in skin and subcutaneous tissue, especially in the cranium region.

In A2 anasarca case, there was excessive edema throughout the body. Histopathologically, severe bleeding, hyperemia and atelectasis were detected in the lung. In addition, the bronchi and bronchioles were full (Fig. 2A, B). Numerous gigantic cystic structures were observed in the kidney, while nonpurulent interstitial nephritis was dominant around these structures (Fig. 2C, D). Nonpurulent myocarditis was detected in the heart (Fig. 2E, F). Hydropic degeneration and multifocal neutrophilic inflammatory cell infiltration were observed in the liver (Fig. 2G, H). Histopathologic examination of case A3 revealed fibrinopurulent meningitis, diffuse gliosis, perivascular cell infiltration, and severe neuronal degeneration in the brain. The most important histopathological result was the inversion of white and gray matter in the brain. Hyperemia and bleeding areas in the lung, intense bacterial foci in

the liver, and cystic areas were determined. Anatomical examination revealed that there was another small, undeveloped nose (2nd nose) on the frontal bone above the concha nasalis dorsalis (Fig. 3A, B). Cranium had a 13 cm calvarium at the junction of the parietal bone and the frontal bone (Fig. 3C).

Table I.- Data and results of anomaly calves.

No.	PN	Breed	Age	Anomaly	Results
A1	01-05-17	Brown Swiss	2 months before birth (abort)	Anasarca	Lung, thickening of the alveolar wall Liver, degeneration of hepatocytes
A2	02-25-17	Simental	1.5 months before birth (abort)	Anasarca	Lung, Atelectasis Lung, Severe bleeding and hyperemia, content in bronchioles Kidney, cystic structures, nonpurulent interstitial nephritis Heart, nonpurulent myocarditis Liver, inflammatory cell infiltration
A3	3-89-18	Simental	2-3 days	In the upper part of the frontal, nasal concha, small nose, calvarium, bone in the head	Brain, purulent meningitis, vascular hyperemia and inflammatory cell infiltration, calcification, gliosis, neuronal degeneration Synaptophysin positive areas in the region where the substantia alba in the area where substansia grizea is located in the inner brain region Lung, hyperemia and bleeding Liver, cystic structures, Bacterial foci in cystic structures Lung, interstitial pneumonia, Heart, bleeding, Liver, sinusoidal hyperemia, mononuclear cell infiltration, fat degeneration
A4	4-123-18	Brown Swiss	1 day	Anencephaly	Lung, interstitial pneumonia, Heart, bleeding Liver, sinusoidal hyperemia, mononuclear cell infiltration, fat degeneration Brain, purulent meningitis, Kidney, bleeding, atrophy and necrosis in the glomeruli
A5	5-65-19	Simental	1 day (breech birth)	Diprosopus monauchenos Diencephaly	Lung, edema, bronchiole content, hyperemia, Liver, sinusoidal hyperemia, fat degeneration in hepatocytes, inflammatory cell infiltration, Kidney, hyperemia, bleeding, atrophy and necrosis of glomeruli
A6	6-49-19	Simental	1-2 days	Schistosoma reflexum	Openness of the abdominal and chest cavity Limbs sideways

A, case order; PN, protocol number registered in the registration book.

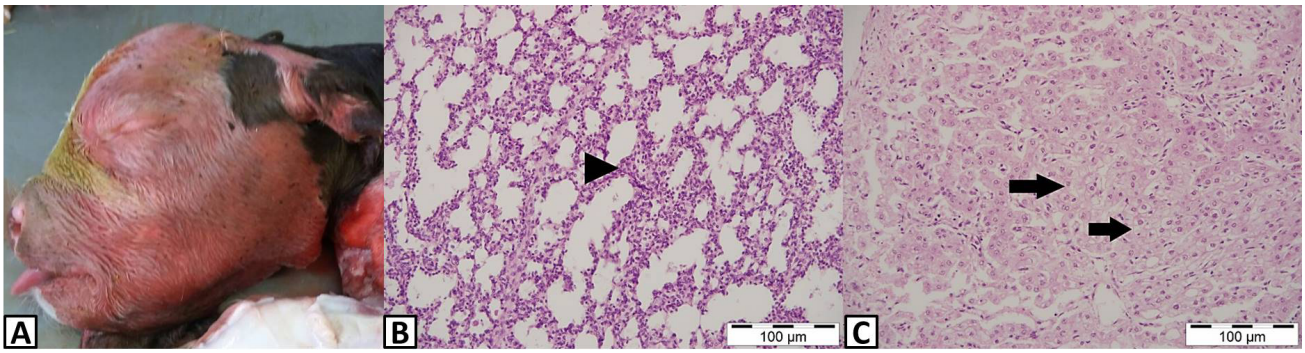

Fig. 1. **A**, Anasarca. **B**, Lung, thickening of the alveolar wall (arrowhead), H&E, 100 µm. **C**, Liver, hydropic degeneration of hepatocytes (arrows), H&E, 100 µm.

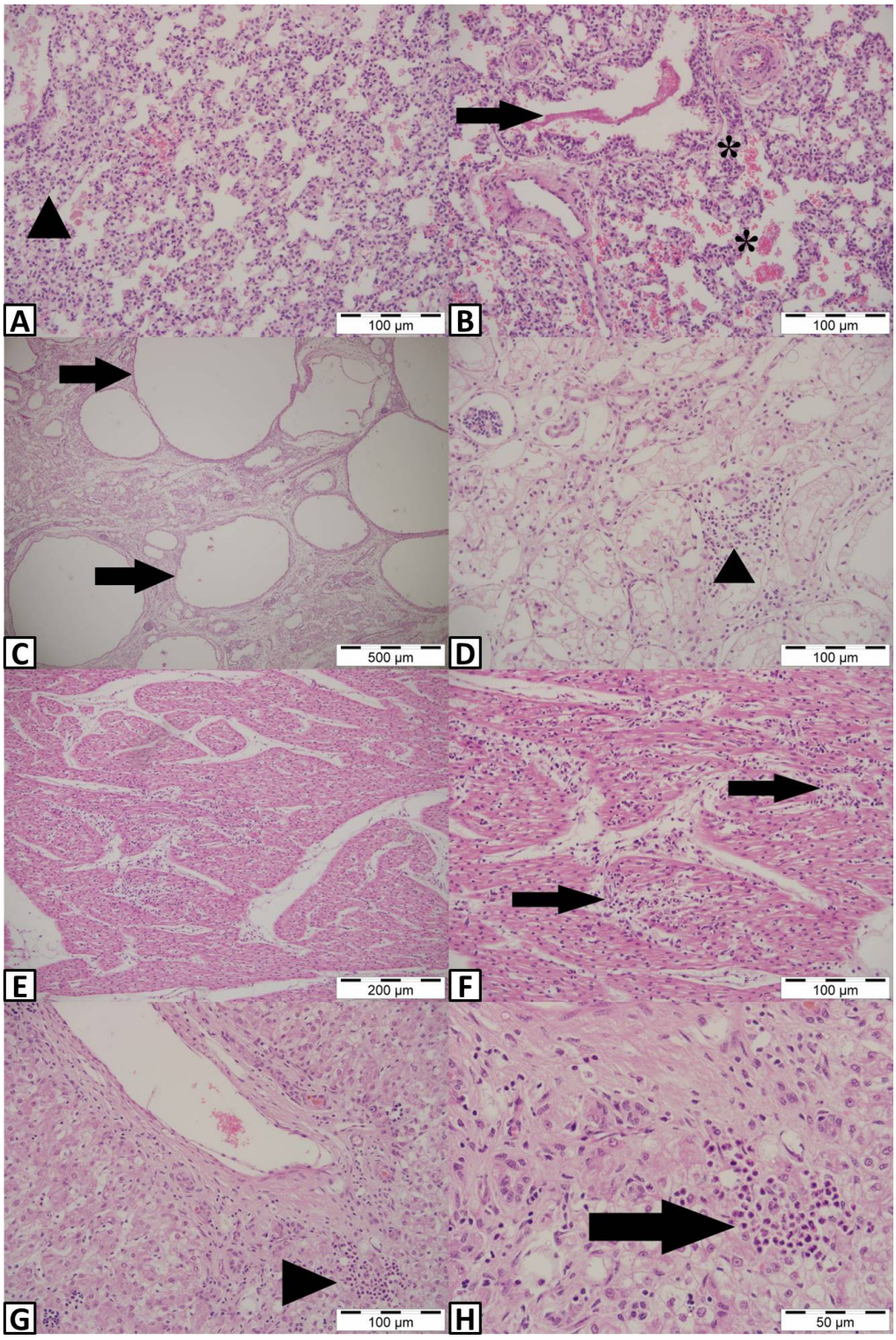

Fig. 2. **A**, Lung, Atelectasis (arrowhead), H&E, 100 µm. **B**, Lung, Severe bleeding and hyperemia (stars), content in bronchioles (arrow), H&E, 100 µm. **C**, Kidney, cystic structures (arrows), H&E, 500 µm. **D**, Kidney, nonpurulent interstitial nephritis (arrowhead), H&E, 100 µm. **E**, Heart, nonpurulent myocarditis (arrows), H&E, 200 µm. **F**, Higher magnification, H&E, 100 µm. **G**, Liver, neutrophilic inflammatory cell infiltration (arrowhead and arrow), H&E, 100 µm. **H**, Higher magnification, H&E, 50 µm.

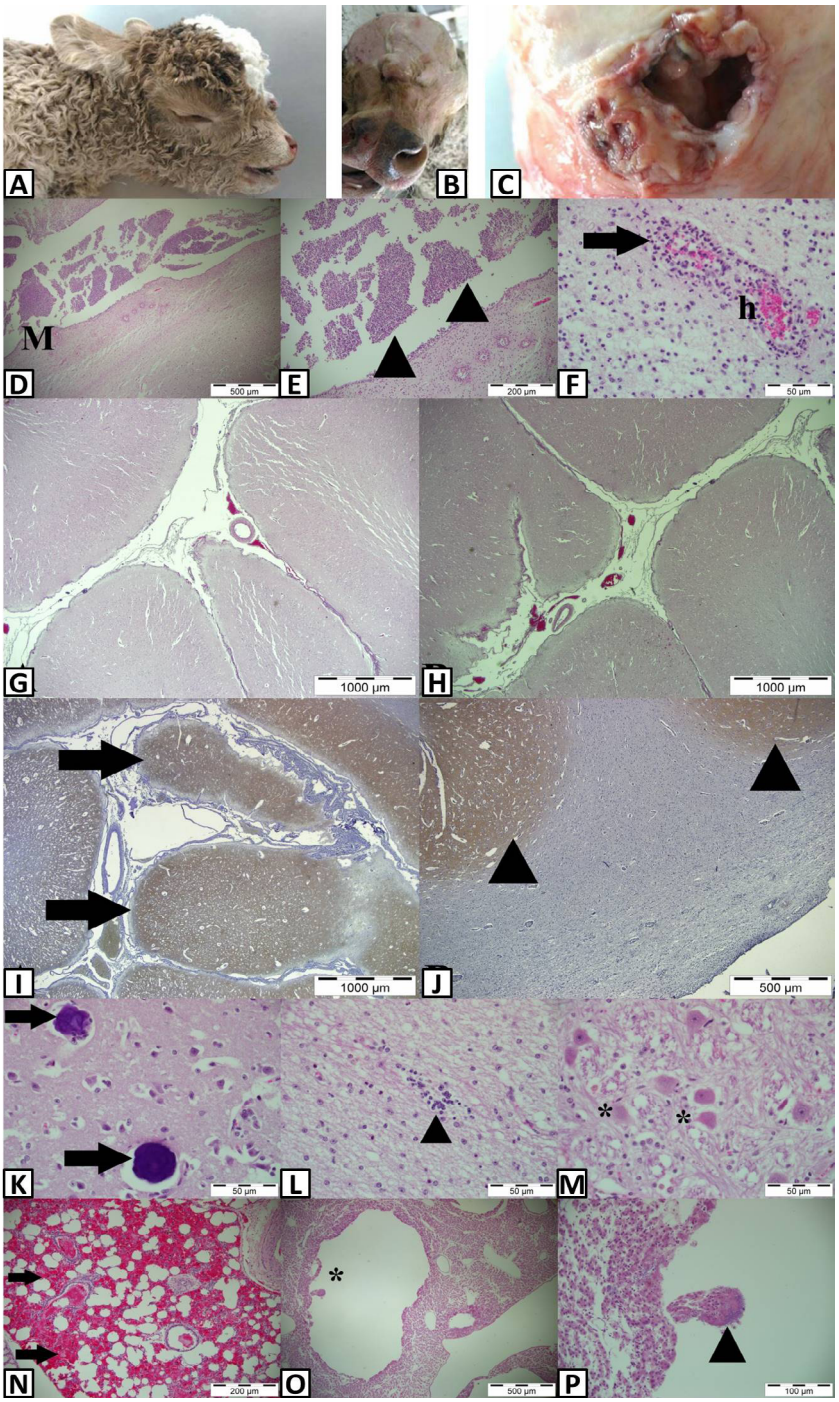

Fig. 3. **A and B**, Two-nosed calf. **C**, Opening in the cranium. **D**, Brain, purulent meningitis (M), H&E, 500 µm. **E**, Higher magnification, purulent meningitis (arrowheads), H&E, 200 µm. **F**, Brain, vascular hyperemia (h) and inflammatory cell infiltration (arrow), H&E, 50 µm. **G and H**, Substansia grizea with meninges in the brain. H&E, 1000 µm. **I**, Synaptophysin positive areas in the region where the substantia alba in the area where substansia grizea is located in the inner brain region (arrows), IHC, 1000 µm. **J**, Synaptopysin positive areas in substansia grizea (arrowheads) and Synaptopysin negative areas in substansia alba, IHC, 500 µm. **K**, Brain, calcification (arrows), H&E, 50 µm. **L**, Brain, gliosis (arrowhead), H&E, 50 µm. **M**, Brain, neuronal degeneration (stars), H&E, 50 µm. **N**, Lung, hyperemia and bleeding (arrows), H&E, 200 µm. **O**, Liver, cystic structures (star), H&E, 500 µm. **P**, foci that resemble bacteria in terms of morphological structures (arrowhead) in cystic structures, H&E, 100 µm.

Since the triple ligament band (extensor digiti III, extensor digitorum communis, extensor digitalis lateralis muscles tendon) on the corpus of the facies dorsalis of os metacarpale III-IV is not sufficiently developed, it is bent shortly on the margo lateralis. Initially it was found that it was completely lateral to articulationes carpometacarpeae and it was completely median line from corpus metacarpalis to caput metacarpalis. M. extensor digitorum communis was observed to terminate in phalanx distalis (proc. extensorius). As the extensor muscles did not develop sufficiently in the forefoot, it was determined that the leg remained in flexion and caused the animal to be unable to walk. Arthrogryposis and hydrocephalus were seen.

Histopathologically, fibrinopurulent meningoencephalitis was dominant in the brain. The vessels were highly hyperemic. Infiltration of neutrophils and mononuclear cells was detected around the vessels (Fig. 3D, E, F). In the mid-section of the sections taken from the existing structure other than the normal formation at the medulla oblangata and pons level in the brain, it was found that in the outer part of the meninges, an inversion of the substantia alba was formed in an outer layer, as if the substance was invaginated (Fig. 3G, H). To determine the presence of synaptic vesicles in these areas, synaptophysin staining was performed as IHK. As a result, the synaptophysin positive areas where synaptic vesicles were found in the mentioned region were revealed (Fig. 3I, J). In addition, calcifications, gliosis and neuronal degeneration were found in the brain (Fig. 3K, L, M). The lung was highly hyperemic and bleeding. In the liver, large cystic structures and foci that resemble bacteria in terms of morphological structures were found within these cystic structures (Fig. 3N, O, P).

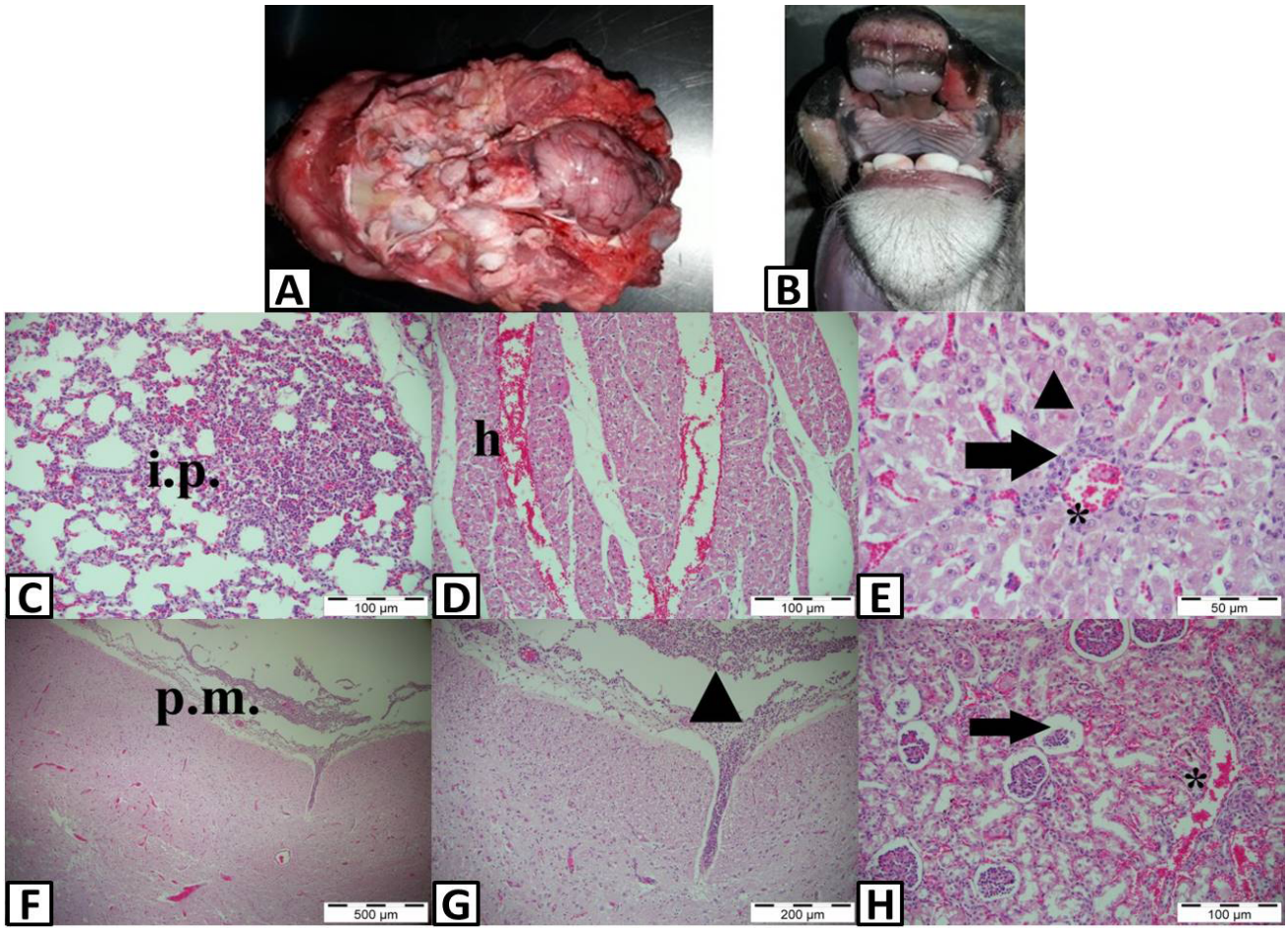

Fig. 4. **A**, Anencephaly. **B**, Palatoschysis. **C**, Lung, interstitial pneumonia (i.p.), H&E, 100 µm. **D**, Heart, hemorrhage (h), H&E, 100 µm. **E**, Liver, sinusoidal hyperemia (star), mononuclear cell infiltration (arrow), fat degeneration (arrowhead), H&E, 50 µm. **F**, Brain, purulent meningitis (p.m and arrowhead), H&E, 500 µm. **G**, Higher magnification, H&E, 200 µm. **H**, Kidney, hemorrhage (star), atrophy and necrosis in the glomeruli (arrow), H&E, 100 µm.

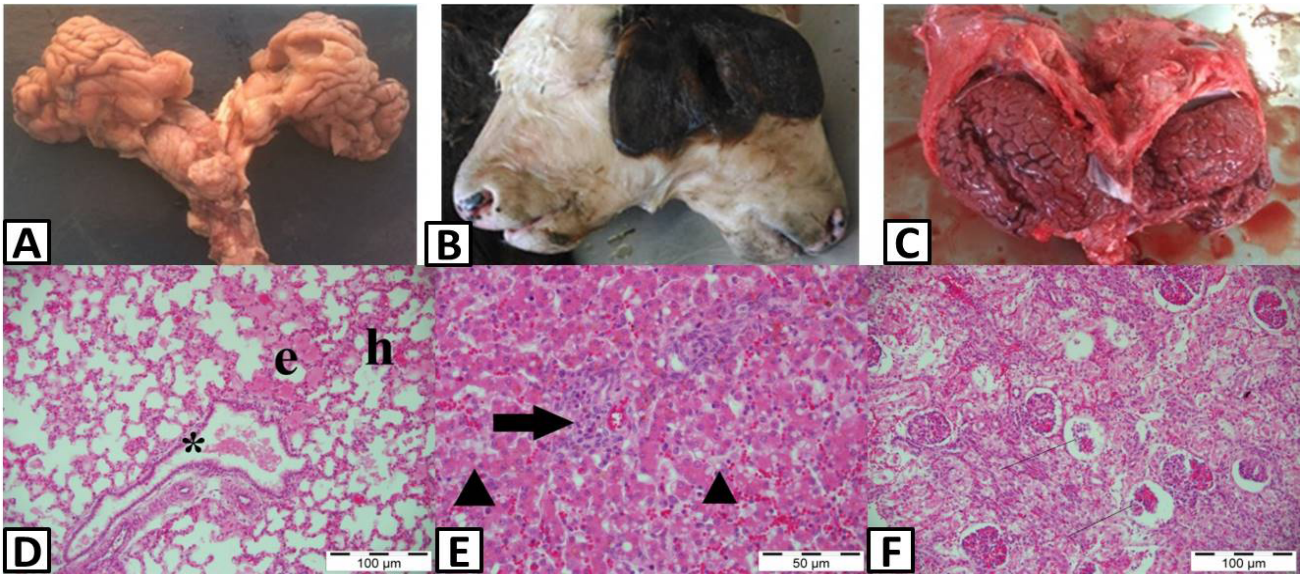

Fig. 5. A, Diencephali. B, Diprosopus monauchenos. C, Diencephali. D, Lung, edema (e), bronchiole content (star), hyperemia (h), H&E, 100 µm. E, Liver, sinusoidal hyperemia, fat degeneration in hepatocytes (arrowheads), inflammatory cell infiltration (arrow), H&E, 50 µm. F, Kidney, hyperemia, hemorrhage, atrophy and necrosis of glomeruli (lines), H&E, 100 µm.

In case A4, the brain did not develop (Fig. 4A), and palatoshysis was seen (Fig. 4B). The cerebellum showed purulent meningitis and perivascular cell infiltration. Interstitial pneumonia in the lung, hyperemia in the kidneys, hemorrhagic areas, atrophy and necrosis in the glomerulus, sinusoidal hyperemia in the liver, mononuclear cell infiltration, fat degeneration, hemorrhage in the heart were detected (Fig. 4C-H). Macroanatomic most important results anencephaly and was palatoschysis.

In case A5, diprosopus monauchenos (double-headed calf), dicephaly and chondrodysplasia were observed. Pathological and anatomical investigations revealed that the two cerebrum hemispheres showed independent and complete development in the cavum cranii, but that the cerebellums were hanging out of the foramen magnum (Fig. 5A, B, C). Basal formations of the brain were found to be pairs up to the pons and were single when they were moved backwards from the pons. Two rima oris, 2 nose, 4 nares, 4 orbita, 4 bulbus oculi (tetraophtalmus), 2 tongue, 4 pinna and 4 mandible were found. It was found that the hard palate was slit along the median line (palatoschisis) in both heads. The right and left eyes were in normal position and structure and the middle eye was surrounded by lamina orbitalis of two ethmoidal bones and frontal bone. Histopathologically, edema and hyperemia were observed in the lung (Fig. 5D). Hyperemia in liver sinusoids and fatty degeneration in hepatocides has been determined (Fig. 5E). Hyperemia, glomerular atrophy and necrosis were seen (Fig. 5F).

In the case of schistosoma reflexum number A6, hind legs were seen at cranium level (Fig. 6). Kidneys, lungs, hearts were visible from the outside. The diaphragm had not developed. The liver, stomach, most of the intestines were absent.

Fig. 6. *Schistosoma reflexum*.

Congenital anomalies are structural disorders seen with birth. Most of these disorders are caused by genetic factors and environmental factors (infection, toxins, fertilization techniques, nutrition) or a mixture of these. Usually the main cause cannot be diagnosed. Multiple organ deformations can be seen in most congenital anomalies (Pothiappan *et al*., 2012). In a study, it was

observed that anomalies were mostly located in the skeletal system in a calf (Demiraslan *et al*., 2014).

DISCUSSION

In the last period of pregnancy, in the anasarca cases where fluid accumulation and subcutaneous edema are observed in the soft tissues of the calf, the calf has the appearance of a hippopotamus. It has been reported in various studies that anasarca cases may occur in single (Švara *et al*., 2016) or multiple (Kaya *et al*., 2015) pregnancies. In our study (A1-A2), the anasarca in the singleton newborn calves. Anasarca and pulmonary hypoplasia were detected together in chica cattle. In the same case, edematous of the interlobular connective tissue and bronchial walls. Also pulmonary parenchyma were found to be mostly atelectatic. In addition, severe inflammation with multifocal and severe dilated lymph vessels was detected in the subcutis and skeletal muscles (Švara *et al*., 2016). In another reported case of anasarca were found to be, severe subcutaneous edema, palatoschisis in the upper palate, lungs small and atelectatic (Yüksel *et al*., 2019). Histopathologically, in addition to edema in soft tissue, there was thickening of the lung wall, severe bleeding, hyperemia and atelectasis. In our study, both clinical and histopathological symptoms detected in anazarka cases are similar. Many factors play a role in the etiology of anasarca cases. Therefore, it is thought that it may be beneficial to remove the cows that produce repetitive anomalies or calves with calves in the farms and change the sperm used.

Congenital anomalies in the nasal region in animals can often be seen with craniofacial defects and palatoshysis (Milli and Hazıroğlu, 2000). In the case numbered A3, there was a second small nose above the frontal bone. In the same case, a 13 cm long calvarium opening was observed between frontal and parietal bone. This result confirms the information that congenital anomalies in the nasal area are mostly accompanied by craniofacial defects expressed by Milli and Hazıroğlu (2000). In addition, there were cases of calves born with two noses (Anonymous, 2020). One of the most important results of this case in our study was that the substantia grisea and substantia alba in the brain were reversed. In the literature searches, such a case reported in animals was not encountered. It is important as it will be the first in this sense.

In four calves with anencephaly, cranioschisis, absence of diencephalon with cerebral hemisphere and rostral midbrain, various eye defects and relatively normal development of caudal brain stem, cerebellum and spinal cord were determined in pathological examination. In addition, it was determined that there was no cerebellum in one case. It has been reported that anencephaly in calves has a limited localized defect in the brain, eye and skull (Cho and Leipold, 1978). In another case, many defects such as anencephaly, anophthalmos, permanent truncus arteriosus, duodenal atresia were observed in a stillborn female calf (Hiraga and Abe, 1986). Multiple organ malformations are frequently encountered in cases of anomalies shaped in calves, and in some cases, palatosysis is also observed (Hobbenaghi *et al*., 2015). In the A4 case in our study, there were no brains, interstitial pneumonia in the lung, atrophy and necrosis in the glomerulus, sinusoidal hyperemia in the liver and bleeding in the heart. Macroanatomically, we found that anencephaly and palatoschisis were together in this case. This finding supports that multiple organ defects can be seen in anencephaly cases.

Cranial duplications can be formed more frequently as a congenital anomaly in cows and sheep. In such cases, dystocia is unavoidable and usually the calves are born dead. Although cranial duplication is not seen in cases, it can occur in defects in various parts of the body (Rani *et al*., 2013; Roberts, 1986). In the physical examination of the reported cases, it was observed that two heads, four ears, four eyes and skulls fused in the occipital region (Gadre *et al*., 2018; Salami *et al*., 2011; Rani *et al*., 2013). When we examined our duplication case, it was found that there were two rima oris, two noses (four nostrils), two orbits, four bulbus oculi, two tongues and four mandibles similar to the studies. In some reported cases, no defect was detected in the palate (Karthik *et al*., 2013; Salami *et al*., 2011), in our case, palatoschisis was detected in both palate similar to Kerr (2007) and Atasever and Ekebaş (2016). It was also stated that there were two ears in diprosopus cases (Karthik *et al*., 2013; Salami *et al*., 2011; Sharma *et al*., 2010) but in our study, four ears were identified. Careful rectal examination should be performed during delivery, as these types of cases cause particularly difficult deliveries. After the diagnosis of diprosopus is made, the mode of intervention should be determined accordingly. In addition, defects may occur in the palate in diprosopus cases.

Schistosoma reflexum, the cause of which is not fully understood, is more common in cows. It is an anomaly in which the chest and abdominal cavities are not closed in general, and the sacrum, coxae and posterior extremities are in different directions (Aydın *et al*., 2006; Semacan *et al*., 2012). In our study, defects in cases with schistosoma reflexum reported (Aydın *et al*., 2006; Semacan *et al*., 2012) were found. İt was reported that since diaphragma did not occur in a case with schistosoma reflexum, cavum abdominis and cavum thoracis were communicating. İn addition lumbal, sacral and caudal vertebrae were unseen (Aydın *et al*., 2006). Similarly, in the presented case,

it was seen that the diapragma did not develop, and the majority of the liver, stomach and intestines did not exist. Schistosoma reflexum is a congenital anomaly and causes economic losses due to the birth of dead calves in farms. Since the reason is not known exactly, it will be beneficial for the future of the farm to make good analysis of farm pedigree and not to use the breeding cows with defect calves like schistosoma reflexum in the past. Also, since some schistosoma reflexum case may be confused with uterine rupture during rectal examination during delivery, it should not be forgotten in the differential diagnosis.

CONCLUSION

As a result, congenital anomalies are structural disorders and most genetic factors are affected by environmental factors (infection, toxins, nutrition). It was determined that there were multiple defects pathologically and anatomically in calves born in the cases examined. In terms of farm management, after the pedigree analysis, it may be useful to remove the cows that are estimated to give birth to congenital defect calves due to genetic factors. It should not be forgotten that healthy calves are the future of the farm.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Aksoy, Ö., Kılıç, E., Öztürk, S., Özaydın, İ., Kurt, B. and Baran, V., 2006. Congenital anomalies encountered in calves, lambs and kids 1996-2005 (262 cases). *Kafkas Üniv. Vet. Fakul. Derg.*, **12**: 147-154.

Anonymous, 2020. *Two-nosed calf born in Wis.; smaller, functioning nose on top of first*. https://www.postindependent.com/news/two-nosed-calf-born-in-wis-smaller-functioning-nose-on-top-of-first/ (accessed on 15 February, 2020).

Atasever, A. and Ekebaş, G., 2016. A case of diprosopus in brown swiss calf. *Ankara Üniv. Vet. Fakul. Derg,* **63**: 415-419. https://doi.org/10.1501/Vetfak_0000002763

Aydın, M., Karan, M. and Yüksel, M., 2006. The anatomical comparison of different *Schistosoma reflexum* cases in calves. *Fırat Üniv. Sağ. Bil. Derg.,* **20**: 249-252.

Bekyürek, T., Canooğlu, E., Demiral, Ö.O. and Abay, M., 2004. Fissura abdominalis in a calf. *Erciyes Üniv. Vet. Fakul. Derg.*, **1**: 161-163.

Beytut, E., 2017. Pathological and immunohistochemical evaluation of skin and teat papillomas in cattle. *Turk. J. Vet. Anim. Sci.,* **41**: 204-212. https://doi.org/10.3906/vet-1609-65

Buck, B.C., Ulrich, R., Wohlsein, P. and Distl, O., 2009. A case of schistosoma reflexum in a black and white German Holstein calf. *Deut. Tierärztl. Wochensch.,* **116**: 363-368.

Cho, D.Y. and Leipold, H.W., 1978. Anencephaly in calves. *Cornell Vet.,* **68**: 60-69.

Demiraslan, Y., Aslan, K., Gürbüz, İ. and Özen, H., 2014. Multiple congenital anomalies in a Simmental calf. *Kafkas Univ. Vet. Fakul. Derg.*, **20**: 629-632.

Dittmer, K.E. and Thompson, K.G., 2015. Approach to investigating congenital skeletal abnormalities in livestock. *Vet. Pathol.,* **52**: 851-858. https://doi.org/10.1177/0300985815579999

Gadre, K.M., Hemanth Gowda, K., Mohamed Faraman, F. And Mahesh, S.A., 2018. Dicephalus Dipus Iniodymus-A case report. *Indian J. Vet. Anat.,* **30**: 141-142.

Hiraga, T. and Abe, M., 1986. Anencephaly and other congenital defects in a calf. *Jpn. J. Vet. Sci.,* **48**: 595-598. https://doi.org/10.1292/jvms1939.48.595

Hobbenaghi, R., Dalir-Naghadeh, B. and Nazarizadeh, A., 2015. Congenital multi-organ malformations in a Holstein calf. *Vet. Res. Forum,* **26**: 257-260.

Jones, C.J., 1999. *Perosomus elumbis* (vertebral agenesis and arthrogryposis) in a Stillborn Holstein calf. *Vet. Pathol.,* **36**: 64–70. https://doi.org/10.1354/vp.36-1-64

Karthik, K., Muneeswaran, S.N., Shinde, S., Kesavan, M., Tamilmahan, P. and Prakash, D., 2013. Diprosopus monauchenos in a Jersey cross calf. *Int. J. Livest. Res.,* **3**: 74-76. https://doi.org/10.5455/ijlr.20130109094059

Kaya, S., Somalı, M., Kuru, M., Kaya, D. and Kaçar, C., 2015. A case of anasarca in a calf. *Harran Üniv. Vet. Fakul. Derg.,* **4**: 33-35.

Kerr, N.J., 2007. Diprosopus with multiple craniofacial, musculoskeletal, and cardiac defects in a purebred Suffolk lamb. *Can. Vet. J.*, **48**: 1074-1076.

Mahabady, M.K. and Barati, F., 2012. Multiple congenital cephalic defects in a calf. *Asian J. Anim. Vet. Adv.,* **7**: 767-770. https://doi.org/10.3923/ajava.2012.767.770

Milli, Ü.H. and Hazıroğlu, R., 2000. *Veterinary Pathology-I and II*. Medisan, Ankara, Turkey, pp. 353-500.

Özfiliz, **N.**, 1993. İnsan ve evcil hayvanlarda malformasyonların nedenleri. *Uludağ Üniv. Vet. Fakul. Derg.,* **12**: 77-83.

Pothiappan, P., Selvi, D., Kantharaj, K. and Thangapandiyan, M., 2013. Multiple congenital

anomalies in a newborn calf – a case report. *Tamilnadu J. Vet. Anim. Sci.,* **9**: 61-63.

Rani, R.U., Kalirajan, R., Vairavasamy, K. and Muruganandan, B., 2013. Dystocia due to Diprosopus monauchenos calf in a Crossbred cow. *Indian Vet. J.*, **90**: 90-91.

Roberts, S.J., 1986. *Veterinary obstetrics and genital disease (Theriogenelogy),* 3rd edn. Edwards Brothers, Woodstock.

Robert, S.J., 2004. *Veterinary obstetrics and genital diseases*. Indian Reprints, CBS, Publishers and Distributors, Delhi-110032, pp. 73-74.

Salami, O.S., Okaiyeto, S.O., Danbirni, S., Ibe, C., Allam, L. and Kudi, A.C., 2011. A case of diprosopus monauchenos in a day old calf (White Fulani × Friesian cross) in an integrated dairy farm. *Int. J. Livest. Prod.,* **2**: 55-58.

Semacan, A., Kaymaz, M., Fındık, M., Rişvanlı, A. and Köker, A., 2012. *Obstetrics and gynecology in farm animals*. Medipres Printing, Malatya, Turkey, pp. 163-170.

Sharma, A., Sharma, S. and Vasishta, N.K., 2010. A Diprosopus buffalo neonate: A case report. *Buffalo Bull.*, **29**: 72-75.

Švara, T., Cociancich, V., Šest, K., Gombač, M., Paller, T., Starič, J. and Drögemüller, C., 2016. Pulmonary hypoplasia and anasarca syndrome in Cika cattle. *Acta Vet. Scand.,* **58**: 36. https://doi.org/10.1186/s13028-016-0220-9

Taşal, İ. and Aytekin, Ö., 2015. A case of brachygnathia inferior (Micrognathia) in a calf. *Can. Vet. J.*, **26**: 157-159.

Yüksel, B.F., Ercan, K., Karabulut, B., Akdeniz İncili, C. and Kalkan, C., 2019. A case of pulmonary hypoplasia and anasarca syndrome with palatoschisis in a calf fetus. *Fırat Univ. Vet. J. Hlth. Sci.,* **33:** 207-209.

Zachary, J.F. and McGavin, M.D., 2013. *Pathologic basis of veterinary disease*, 5th edn. Elsevier, eBook ISBN: 9780323075343.