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First occurrence of Arnold Chiari type II malformation and associated abnormalities in a Gir calf produced *in vitro* from Brazil – case report

[*Primeira ocorrência de malformação de Arnold Chiari tipo II e anormalidades associadas em uma bezerra Gir de produção* in vitro *no Brasil – relato de caso*]

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ABSTRACT

This study characterized the clinical, radiological, ultrasound, and necroscopic findings of a case of Arnold-Chiari type II malformation in a Gir breed calf from Brazil. The animal was hospitalized at sixty days of age, in permanent sternal recumbency, cutaneous appendix at the 4th lumbar vertebra and kyphoscoliosis of the caudal and lumbosacral thoracic spine. Radiographic examination of the spine and skull revealed spina bifida and suspected occipital hypoplasia. Upon examination of myelography with an injection of lumbar and atlantooccipital contrast, it was possible to visualize the meningocele at the 4th lumbar vertebra region and findings at the rhombencephalon level of increased regional pressure with failure to fill the contrast in the posterior fossa, in the presence of clear demarcation of the circumvolutions of the cerebral cortex and the subarachnoid space of the cervical spinal cord. Ultrasonographic examination of the cerebellum showed an insinuation of the cerebellar worm through the foramen magnum. The animal did not show changes in complete blood count, biochemical series, and cerebrospinal fluid and was negative for Pestivirus. There was a worsening of the clinical conditions and the animal died. This malformation of unknown etiology must be studied as a differential diagnosis of the nervous system disorders.

Keywords: neurological syndrome, reproductive biotechnologies, congenital diseases, neuropathology

RESUMO

Este estudo caracterizou os achados clínicos, radiológicos, ultrassonográficos e necroscópicos de um caso de malformação de Arnold-Chiari tipo II em uma bezerra Gir no Brasil. O animal foi hospilatizado aos 60 dias de idade, apresentando decúbito esternal permanente, apêndice cutâneo na altura da quarta vértebra lombar e cifoescoliose da coluna vertebral torácica caudal e lombossacra. Ao exame radiográfico da coluna e do crânio, foram observadas espinha bífida e suspeita de hipoplasia occipital. Ao exame de mielografia com injeção de contraste lombar e atlanto-occipital, foi possivel visualizar a meningocele na altura da quarta vértebra lombar e achados em nível rombencefálico de aumento da pressão regional com falha de preenchimento do contraste na fossa posterior, na presença de nítida demarcação das circunvoluções do córtex cerebral e do espaço subaracnoide da medula espinhal cervical. Ao exame ultrassonográfico do cerebelo, foi observada insinuação do verme cerebelar através

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do forame magno. O animal não apresentou alterações em hemograma completo, série bioquímica e fluido cérebro-espinhal e foi negativo para Pestivirus*. Houve uma piora do quadro clínico e o animal morreu. Essa malformação de etiologia desconhecida deve ser estudada como um diagnóstico diferencial.*

Palavras-chave: síndrome neurológica, biotecnologias reprodutivas, doenças congênitas, neuropatologia

INTRODUCTION

Arnold-Chiari malformation is a very rare anomaly characterized by occipital bone hypoplasia and rhombencephalon complications such as herniation of the cerebellar vermis and caudal brain stem through the foramen magnum (Cho and Leipold, 1977). In calves, it is bilateral, symmetrical, and may occur simultaneously to spina bifida, meningomyelocele, hydrocephalus, syringomyelia (Loughin, 2016), arthrogryposis, lumbar kyphoscoliosis and cleft lip (Gülbahar *et al*., 2005). Even though it's occurrence and descriptions are exceptionally rare in animals, it's characterization is similar to the Arnold-Chiari malformation in humans (Lempe *et al*., 2012; Loughin, 2016).

In human medicine, John Cleland made the first description of a congenital malformation of the brain and cerebellum with herniation through the foramen magnum in 1833, and only in 1891, Hans Von Chiari characterized the malformation, which was finally named by Júlio Arnold in 1894 as Arnold-Chiari malformation (Aranda *et al*., 2011).

In cattle, the Arnold-Chiari malformation is the result of abnormal embryonic development of neural tissue and bone structures (Cho and Leipold, 1977; Madarame *et al*., 1993; Gülbahar *et al*., 2005), the malformation of the skull base, and the enlargement of the foramen magnum are common (LeClerc *et al*., 1997).

There is no description of this rare congenital anomaly occurrence in cattle from Brazil, but has already been reported in North America, Europe and Oceania, in the breeds Japanese Shorthorn (Madarame *et al*., 1991), Japanese Black (Madarame *et al*., 1993), Simmental (LeClerc *et al*., 1997) e Holstein (Gülbahar *et al*., 2005). Thus, the objective of this work is to report the first occurrence of Arnold-Chiari type II malformation in a Gir calf produced *in vitro* from Brazil, and its clinical and diagnostic aspects.

MATERIAL AND METHODS

A Gir breed calf of *in vitro* production, at sixty days of age, with spinal deformity and permanent recumbency since birth was seen at the University of Cuiabá Veterinary Hospital. In the anamnesis, the owner reported that, at birth, the calf was unable to stand and remained in sternal recumbency with the pelvic limbs always turned to the left, only being able to feed on her own mother with support. The animal had, since its birth, a cutaneous appendix (meningocele) in the lumbar region, inability to stand and move around, in addition to episodes of depression and ataxia. Upon admission of the animal in the veterinary hospital, exams such as complete blood count, serum aspartate aminotransferase activity, alkaline phosphatase, creatine phosphokinase, gamma glutamyl transferase, and serum concentrations of albumin, bilirubin, total calcium, cholesterol, creatinine, total protein, sodium, triglycerides, and urea were performed but didn´t evidence any alteration.

This work was approved by the Ethics Committee for the Use of Animals and internationally recognized high standards of veterinary clinical care for the individual patient were followed. This work involved the use of non-experimental animals (owned) and an informed consent (either verbal or written) was obtained from the owner or legal custodian. The veterinary hospital's medical record has a term where the owner authorizes the use of clinical and laboratory data for use in scientific studies and works, a term evaluated by the Ethics Committee for the Use of Animals.

CASUISTRY

The animal was normothermic, normocardic, normopneic, normovolemic and conscious. On clinical examination, a cutaneous appendix was found at the 4th lumbar vertebra region measuring 8.3 cm wide and 14.8cm long (Figure1A), alteration on the spinous processes of the vertebrae from L1 to L6 on palpation, musculature pelvic limbs atrophy, spine kyphoscoliosis in the caudal thoracic and lumbar region (Figure 1B).

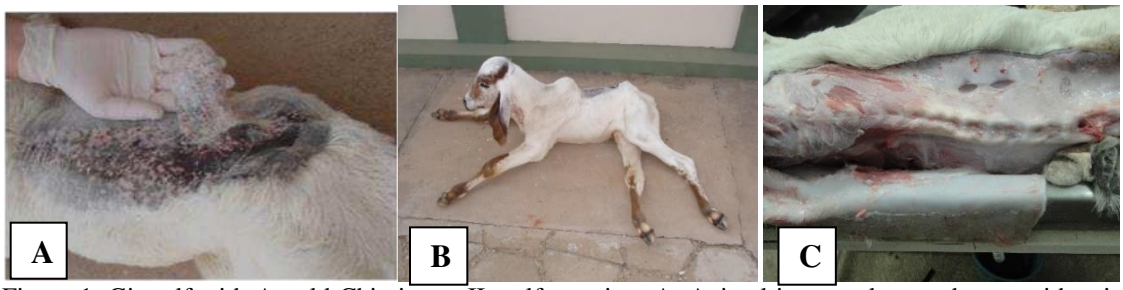

Figure 1. Gir calf with Arnold Chiari type II malformation. A: Animal in sternal recumbency with spine kyphoscoliosis in the caudal thoracic and lumbar region; B: Presence of cutaneous appendix at the 4th lumbar vertebra region indicating meningocele; C: Presence of communicating fistula from meningocele to spinal cord in the 4th lumbar vertebra and spina bifida.

Neurological examination revealed normal responses in the thoracic limbs, but the pelvic limbs presented reduced proprioception, increased muscle tone and hyperreflexia of the spinal stimulus to the patellar, cranial tibial and gastrocnemius reflexes.

Simple and contrasted radiographic examinations (myelography) of the craniocervical and thoracolumbar regions were performed. In the craniocervical region, images suggestive of occipital hypoplasia with decreased posterior fossa volume were observed when compared to calves of the same breed and age. There was also a marked attenuation of the iodinated contrast filling in the sulcus of the cerebellar folia in the presence of marked iodinated contrast filling the sulcus of the cerebral cortex and subarachnoid space at the cervical spine suggesting increased pressure in the caudal fossa and compression of the rombencephalic tissues (Figure 2). Examination of the spine in the thoracolumbar and lumbosacral regions revealed the non-fusion / opening of the spinous process from L1 to L6 (Figure 3B), confirming the diagnosis of spina bifida with the presence of meningocele at the 4th vertebra lumbar region.

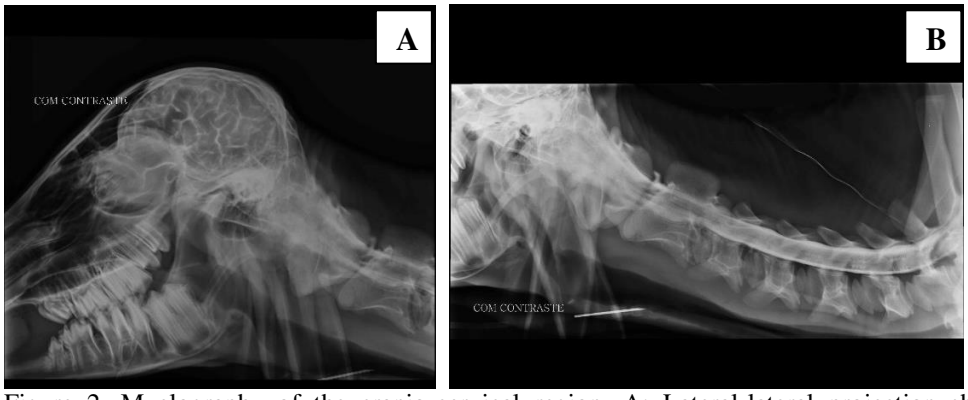

Figure 2. Myelography of the cranio-cervical region. A: Lateral-lateral projection showing contrast progression by retrograde pathway to the brain with marked attenuation of the iodinated contrast filling in the sulcus of the cerebellar folia in the presence of marked iodinated contrast filling the sulcus of the cerebral cortex; B: Lateral-lateral projection showing marked iodinated contrast filling the subarachnoid space at the cervical spine.

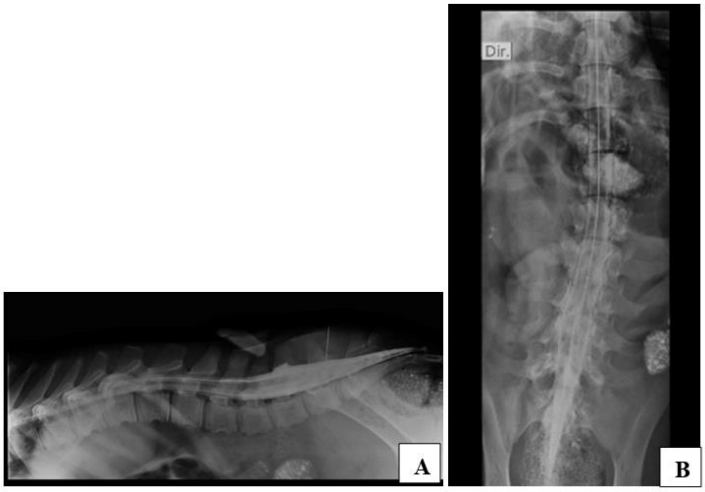

Figure 3. Myelography of the spine. A: Lateral-lateral projection, showing contrast progression through the medullary canal; B: Ventrodorsal projection showing kyphoscoliosis and spina bifida throughout the lumbar region.

The ultrasound examination of the atlantooccipital region was performed, flexing the head 90º, and the images taken with the linear transducer positioned in the transverse and longitudinal orientations, using the frequency of 7.5 MHz. In the exam, it was observed insinuation of the cerebellar worm through the foramen magnum and its location next to the spinal cord in the cranial aspect of the vertebral canal of the atlas. In the sagittal view, the caudal part of the cerebellar worm in tapered shape was verified, exceeding the limits of the occipital bone (Figure 4), and in the transversal view, the spinal cord was observed ventral with hyperechoic lines typical of the folia of the cerebellar worm dorsally in the cranial aspect of the vertebral canal of the atlas.

RT-PCR was performed from the sequence of the untranslated region (5'UTR) of the Bovine Viral Diarrhea Virus (BVDV) genome with serum samples from the calf and the mother (Vilček *et al*., 1994) and virus neutralization was performed according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Aspartate..., 2018) and the results were negative for *Pestivírus*.

There were no changes in complete blood count, serum aspartate aminotransferase activity, alkaline phosphatase, creatine phosphokinase, gamma glutamyl transferase and serum concentrations of albumin, bilirubin, total calcium, cholesterol, creatinine, total protein, sodium, triglycerides, urea dosed weekly since the animal´s admission and in the cerebrospinal fluid (CSF) analyses that was dosed at the time of myelography.

The animal was kept hospitalized for two months, receiving daily care, such as feeding and intensive assistances, including physiotherapy, however, there was a worsening of the clinical conditions, and died at 130 days of life, when an autopsy was performed.

On necroscopic examination, external inspection revealed the presence of pressure ulcers in the regions of the most prominent bony tuberosities of the pelvic region on the right side, due to the permanent position on this side, caused by kyphoscoliosis, and the internal examination, presence of pulmonary congestion and onset of pneumonia. In addition, when examining the spine, after bouncing the skin in the 4th lumbar vertebra over the meningocele, the presence of a fistula communicating with the spinal cord and the non-fusion of the spinous process (spina bifida) was noted throughout the lumbar spine (Figure 1C).

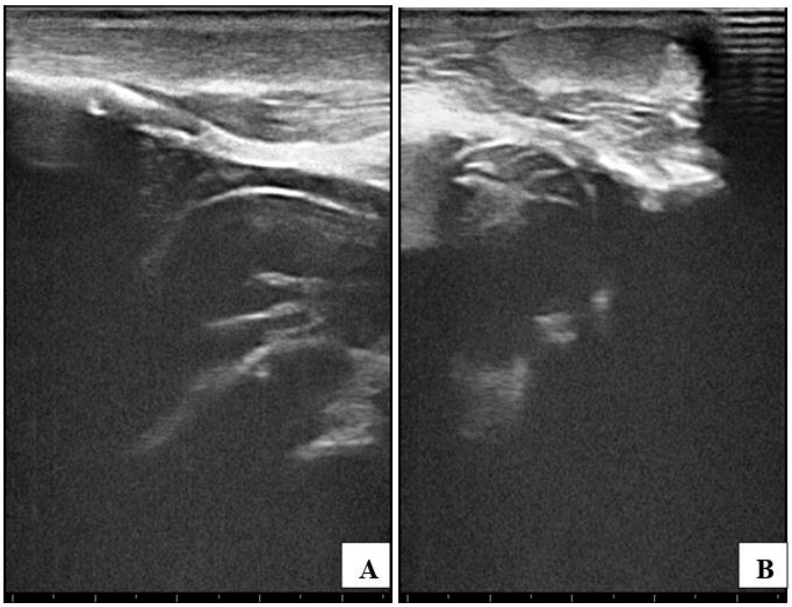

Figure 4. Ultrasonography of the atlantooccipital region. A: Longitudinal section (sagittal view) of the affected calf - caudal part of the cerebellar worm in tapered shape was verified, exceeding the limits of the occipital bone; B: Longitudinal section (sagittal view) of a control calf of the same age and breed - caudal part of the cerebellar worm in rounded shape was verified, not exceeding the limits of the occipital bone.

DISCUSSION

In the present case, herniation of the cerebellum through the foramen magnum was observed in ultrasound of the atlantooccipital joint. This finding, together with other clinical, radiographic and necroscopic findings, resulted in the present diagnosis of Arnold-Chiari malformation and other abnormalities in the development of the neural tube, such as spina bifida, meningocele and kyphoscoliosis in the caudal thoracic and lumbar spine. It is important to emphasize that this report of Arnold-Chiari malformation in a Gir breed calf produced in vitro from Brazil is unprecedented, whereas, until now the description of this rare congenital anomaly in cattle were reported in North America, Europe and Oceania, in the breeds Japanese Shorthorn (Madarame *et al*., 1991), Japanese Black (Madarame *et al*., 1993), Simmental (LeClerc *et al*., 1997) e Holstein (Gülbahar *et al*., 2005).

In humans, the Arnold-Chiari malformation is classified into four types: type I presents a variable inclination of the cerebellar tonsils and the lower part of the cerebellum to the vertebral canal, without displacement of the IV ventricle to the spinal canal; type II is associated with myelomeningocele, so that the hernial sac may contain some parts of the spinal cord, spinal membranes and cerebrospinal fluid; type III as immersion of the bulb of the IV ventricle and the whole cerebellum in an occipitocervical meningocele; type IV presents cerebellar hypoplasia (Aranda *et al*., 2011; Lempe *et al*., 2012). Thus, the present case is classified as Arnold-Chiari type II malformation in a calf.

Animals with Arnold-Chiari malformation have a variable expression of clinical signs that depends on the position, compression degree, extent of cerebellar tonsils cells degeneration and the presence or absence of syringomyelia / hydromyelia (Loughin, 2016). Malformation usually occurs with spina bifida and meningocele in the lumbosacral region (LeClerc *et al*., 1997), as seen in this clinical case.

The CSF physical-chemical examination was unchanged in the present case report, which corroborates to the observations of Loughin (2016) who demonstrated the CSF without alterations in animals with Arnold-Chiari malformation.

Spina bifida is described as a failure in the fusion of the vertebral arches, varying in degrees, from the hidden spina bifida, in which the defect is only in the dorsal bony arch, to the open spina bifida, associated with the meningocele (protrusion of the meninges) or myelomeningocele (protrusion of neural elements in addition to meninges) and kyphoscoliosis (Lempe *et al*., 2012).

Other changes such as unilateral renal agenesis, unicorn uterus, anal atresia, kyphoscoliosis and cleft palate may be associated with spina bifida in cattle (Nicácio *et al*., 2013), and in the present case only kyphoscoliosis was present. The unusual condition of meningomyelocele associated with cerebellar hernia through the foramen magnum, as in the present case, has already been described in a foal (Lempe *et al*., 2012).

The clinical signs observed in this case were more severely associated with spina bifida than related to the cerebellar changes, since the animal showed signs of hyperreflexia and non-ambulatory spastic paraparesis only of the pelvic limbs, while the thoracic limbs were normal. The lack of protection of the spinal cord caused by spina bifida can result in neurological deficiencies, with sensory, motor and orthopedic disorders in the pelvic limbs (Nicácio *et al*., 2013).

Calves with Arnold-Chiari malformation that do not have spina bifida or arthrogryposes, may not show clinical signs of neurological deficiencies (Cho and Leipold, 1977) and have a favorable prognosis (Lempe *et al*., 2012). It was difficult to predict the real contribution of the cerebellar repercussions on the clinical manifestation, since due to severe non-outpatient paraparesis, it was not possible to assess locomotion, which would provide sufficient information regarding cerebellar involvement, although there was compression of the cerebellum in the caudal fossa by occipital hypoplasia, as evidenced by the radiographic examination and ultrasonographic signs.

The clinical rhombencephalic repercussions probably were discreet in this case, because if there were intense changes, this animal would present cervical paresthesia, thoracic and pelvic limbs weakness, abnormal vocalizations, cranial nerves deficits (mainly VI, VII, VIII, IX, X and XI) and neck ventral flexion (Loughin, 2016).

Changes in the skull during embryonic development are mainly responsible for the Arnold-Chiari malformation, which also has a small caudal and/or rostral cerebral fossa. These abnormalities in the bone growth of the skull result in an unusually small brain space, leading to a caudal displacement of the brain / cerebellar tissue (LeClerc *et al*., 1997), as seen in the x-ray and ultrasound of this region for the animal studied, confirming that the smaller space for the brain (rhombencephalon), favored cerebellar herniation and the characterization of the malformation.

The spina bifida of the case presented was observed by the appearance of spinous processes not fused throughout the lumbar spine together with the presence of the cutaneous appendix and meningocele in the 4th lumbar vertebra. Ventrodorsal or dorsoventral radiographic positioning is preferable in young calves, compared to laterolateral projections that result in overlapping of the unfused parts of the spinous process (Lempe *et al*., 2012; Nicácio *et al*., 2013).

Ultrasonography was useful to assess soft tissue involvement, in addition, it served to exclude other causes of neurological changes at the cervical cranial junction such as hematoma, neoplasia or vertebral fractures. Cerebellar herniation was diagnosed using the acoustic window that forms at the cervical cranial junction, being conducive to ultrasound examination and detection of abnormalities in the caudal brain, cerebellum and cranial spinal cord in calves (Testoni *et al*., 2010).

The general prevalence of Chiari II in humans described in the United States is 0.44 in 1,000 live births, however it has been reduced (70%) due to prophylaxis with daily folic acid at a dose of 4mg. In Brazil, studies carried out by Unicamp in the Perinatal Genetics Program from 1982 to 2001 indicated an even greater result, with 2.28 for every 1000 births (Sbragia *et al*., 2004). This may be a hypothesis for this malformation occurrence in cattle, although there are no studies around the folic acid supplementation during cattle gestation with this propose, so studies are valid and justifiable, as

also this case report highlighting this research line.

Although congenital malformations are recognized by the morphological and functional changes produced, their causes are not easily detected (Nicácio *et al*. 2013) and the high differentiation of the nervous system increases susceptibility to abnormalities, which may be associated with toxic, environmental and infectious factors (Aranda *et al*., 2011). The use of in vitro production for breeding cattle is also related to congenital anomalies in calves (Branco *et al.,* 2017; Torres *et al*., 2013). The calf was negative for *Pestivirus* that may induce congenital anomalies in the central nervous (Gülbahar *et al*., 2005). Assisted reproduction techniques, such as *in vitro* embryo production, have been frequently related to the birth of calves with congenital anomalies (Branco *et al*., 2017), which could suggest the etiology of the present description.

CONCLUSION

The present report represents the first description of the occurrence of Arnold-Chiari type II malformation in a calf produced *in vitro* from Brazil.

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