The Journal of Veterinary Medical Science

NOTE

Theriogenology

Prenatal diagnosis of foetal hydrocephalus and suspected X-linked recessive inheritance of cleft lip in a Chihuahua

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J. Vet. Med. Sci.
82(2): 212–216, 2020
doi: 10.1292/jvms.18-0516

Received: 28 August 2018
Accepted: 15 December 2019
Advanced Epub:
27 December 2019

ABSTRACT. A 3.5-year-old, 2.9 kg, multiparous Chihuahua presented with abdominal distension; pregnancy was diagnosed. On Day 7 before parturition, prenatal sonograms showed anechoic bilateral dilated cerebral lateral ventricles, suggesting fluid-filled regions (ventriculomegaly) in one foetus. A Caesarean section was performed and the male newborn had an abnormally enlarged dome-shaped head and a cleft lip, and died 6 days after birth. According to the family pedigree, the X-linked recessive inheritance of an orofacial cleft from the unaffected mother was suggested. This report clearly demonstrates that canine foetal ventriculomegaly (hydrocephalus) can be diagnosed *in utero*. For dog breeds predisposed to congenital ventriculomegaly, early detection is important for the prediction of perinatal survival and adequate supportive care can be applied at delivery.

KEY WORDS: cleft lip, dog, prenatal, ultrasonography, ventriculomegaly

The diagnosis of hydrocephalus is primarily based on clinical features and brain imaging [27]. Brain imaging is commonly used to confirm the disorder by evaluating the ventricle size of the brain [5]. Clinical signs are present postnatally within the first few months, with varying degrees of disease progression. Some animals may not develop signs of encephalopathy until adulthood, thus resulting in misdiagnosis due to the normal awkwardness of the affected puppies. Most hydrocephalus cases are considered congenital and are predominantly found in small rather than large dog breeds, such as the Chihuahua, Maltese, Pomeranian, Yorkshire Terrier, English Bulldog, Boston Terrier, Pug, Pekingese and Toy Poodle [5, 19, 27].

An orofacial cleft−a cleft lip and/or cleft palate (CL/P)−is one of the most common congenital defects in dogs, especially in brachycephalic breeds [1]. CL/P is a failure of the closure of embryonic structures forming the primary and secondary palate (palatogenesis), which includes the lip, alveolus, and the hard and soft palate [12]. The etiology of orofacial clefts is linked to several factors, including the environment, genetics and genetic-environment interactions [3, 4]. In general, a CL/P with no family history is likely to be related to environmental risks, whereas the presence of one or more defected relatives in the same family strongly suggests the contribution of genetic factors.

Ultrasonography is a useful tool for early pregnancy diagnosis and monitoring foetal development in the dog and cat [15]. Furthermore, the technique is effective for the evaluation of foetal viability, gestational age and the prediction of the parturition date [6, 9]. In veterinary obstetrics, prenatal foetal congenital abnormalities are rarely reported by an ultrasonographic technique, most likely due to the technique's limited sensitivity [18]. The objective of this report was to describe a foetal sonographic congenital ventriculomegaly (hydrocephalus) and suspected X-linked recessive inheritance of cleft lip in a dog.

A 3.5-year-old, 2.9 kg, multiparous female Chihuahua presented with abdominal distension; pregnancy was diagnosed upon a transabdominal ultrasonographic examination. Serial prenatal sonograms were performed to evaluate foetal development and to predict the parturition date using foetal biparietal diameter (BPD) measurements [9] on Days -14, -7, -4 and -2 before parturition (Table 1). BPD was measured from the outer edge of the proximal calvarial wall to the outer edge of the distal calvarial wall, at the widest part of the skull [15]. At the first prenatal visit (14 days before parturition), three foetuses showed normal ultrasonographic

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Table 1. The measurement of foetal biparietal diameter (BPD) of each foetal head in relation to the days before parturition

Days before parturition	BPD in each foetus (mm)		
	1	2	3[a)]
14	12.7	13.5	14.2
7	20.8	21.6	22.4
4	21.4	22.2	23.5
2	22.9	23.2	27.7
0	23.1	23.3	27.8

a) Illustrated the data of hydrocephalic foetus.

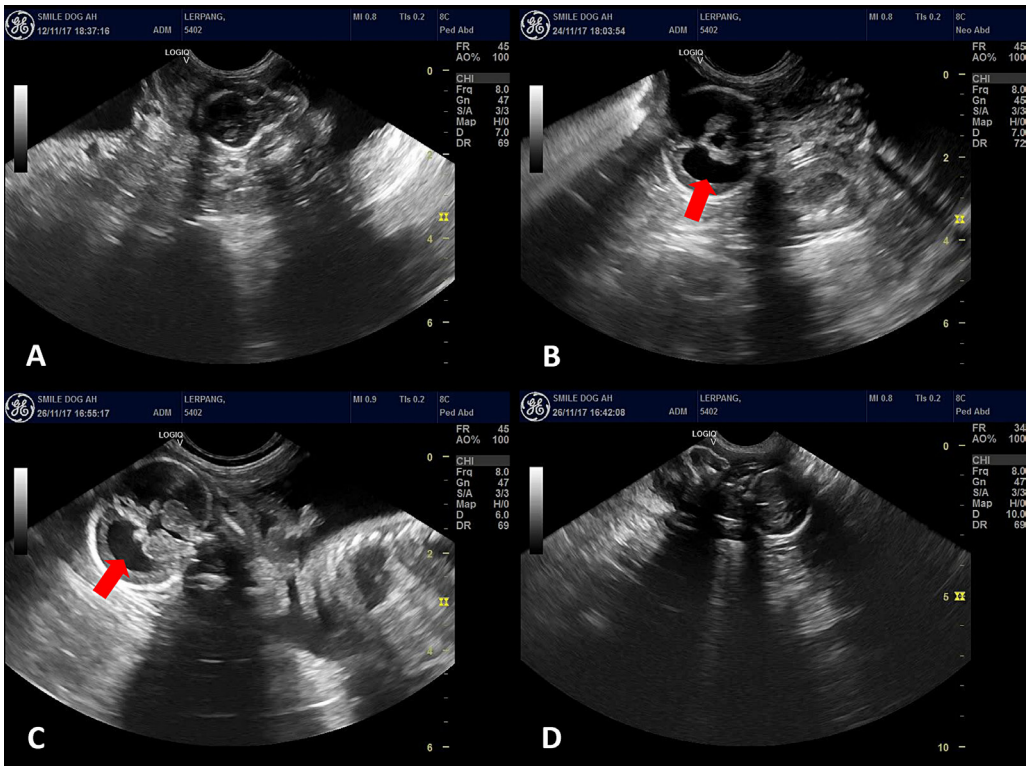

Fig. 1. Ultrasonographic appearance of the foetal skull of a normal foetus (A: 14 days before parturition; D: day of parturition) and the affected abnormal male foetus (B: 2 days before parturition; C: day of parturition). Symmetrical dilated cerebral lateral ventricles that appear anechoic surrounding the choroid plexus (foetal ventriculomegaly) are clearly observed (arrow; B and C).

features and the foetal heart rates (200–240 beats per min; bpm) were within a normal range. An example of an ultrasonographic scan of a foetal skull is shown in Fig. 1A. On Day-7, symmetrically bilateral dilated cerebral lateral ventricles that appeared anechoic (fluid-filled regions surrounding the choroid plexus) were apparent in one foetus, indicating foetal ventriculomegaly, whereas the brain tissues of the other two littermates appeared sonographically normal. On Day-2, the abnormal appearance of the anechoic dilated ventricles became more obvious (Fig. 1B), and BPD measurements revealed that the affected foetus had a larger BPD (27.7 mm) compared to the other two littermates (22.9 and 23.2 mm) and the mean BPD value (25.1 mm) of small-sized dogs on the day of parturition [9].

Due to the high risk of dystocia in small-sized dogs, a planned Caesarean section was requested by the dog's owner. An elective Caesarean section was performed when the bitch showed a decreased appetite and her body temperature dropped below 37.7°C. Before surgery, the BPD of the affected foetus was 27.8 mm; ultrasonographic findings of the brain tissues with dilated cerebral lateral ventricles are shown in Fig. 1C. Foetal heart beat monitoring revealed that one foetus without ventriculomegaly had a low heart rate of 181 bpm, indicating the presence of foetal distress [8]. Three puppies were delivered (two females and one male). The heart rates of all the newborns were over 200 bpm, with a respiratory rate of 20–35 breaths per minute. All pups showed normal behaviors (nipple searching, suckling behavior, righting and rooting responses) and locomotion during the early postnatal period. The male newborn had an enlarged dome-shaped head (Fig. 2), which corresponded to bilateral ventriculomegaly (hydrocephalus) diagnosed *in utero* and a cleft lip (Fig. 3). There were no signs of neurological disorders. The abnormal newborn with hydrocephalus and a cleft lip died 6 days after parturition due to progressive weakness.

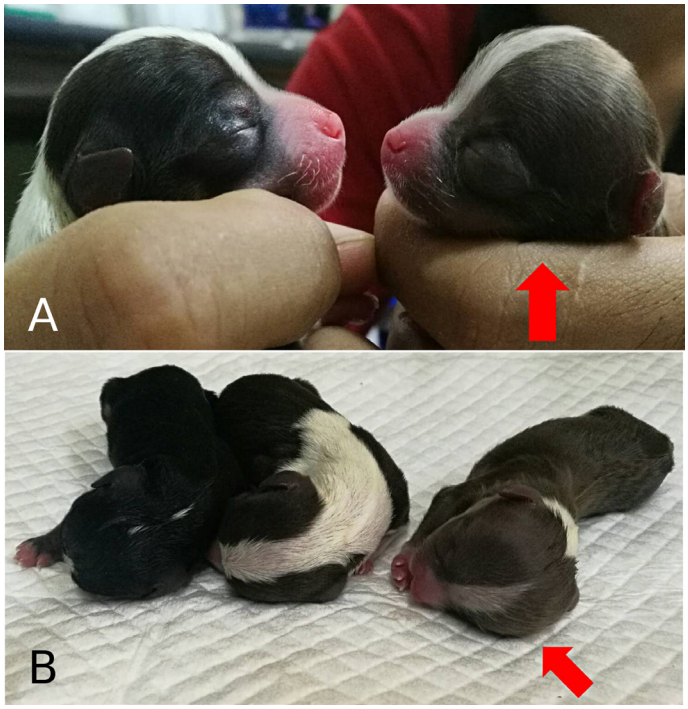

Fig. 2. An enlarged, dome-shaped head of an abnormal male puppy (1-day-old; arrow; A: lateral view; B: dorsal view) diagnosed as having congenital hydrocephalus, compared to the other littermates.

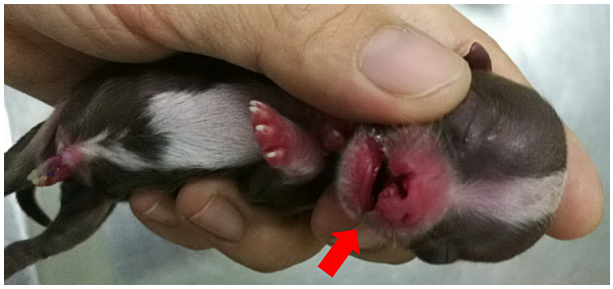

Fig. 3. A cleft lip (arrow) was present in the affected newborn with an enlarged dome-shaped head.

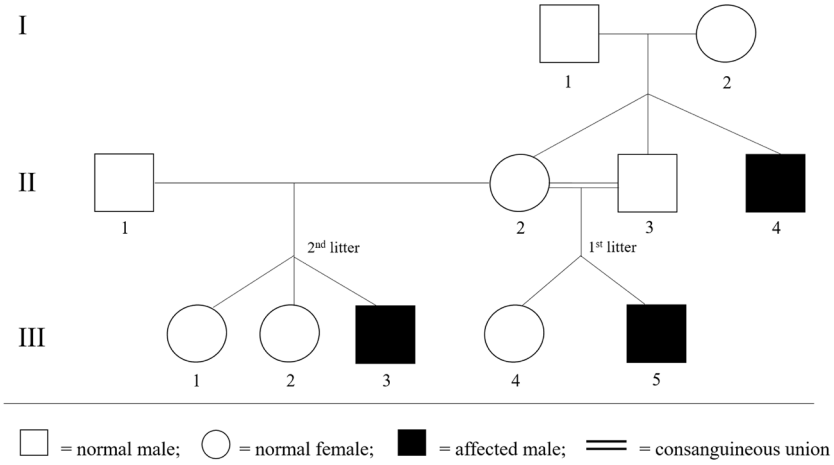

Fig. 4. A family pedigree illustrating the segregation of the phenotype with an isolated clefting defect, suggesting the X-linked recessive inheritance of a cleft lip and/or palate (CL/P).

According to the family pedigree (Fig. 4), the dam had two male siblings, one of which had an orofacial cleft. For the first pregnancy, the dam was inbred with her normal sibling (1st litter, Fig. 4) and delivered two newborns (one male and one female); the male pup had a cleft lip and showed clinical signs of epilepsy. In the present report, the bitch was bred with a normal healthy male with no history of hydrocephalus in the family pedigree. The second pregnancy resulted in two normal females and a hydrocephalic male newborn with a cleft lip (2nd litter, Fig. 4). The dam had no history of teratogenic substance exposure during either pregnancy and had been fed with premium commercial dog food.

In veterinary obstetrics, the *in utero* diagnosis of canine foetal abnormalities is rarely reported. The prenatal diagnosis of foetal anasarca or hydrops fatalis has been recently reported [2, 6]. To our knowledge, descriptions of foetal ventriculomegaly/hydrocephalus diagnosed prenatally in the dog are scarce, possibly due to its low prevalence or overlooked diagnoses. The early detection of foetal abnormalities can be useful for perinatal survival and monitoring parturition to minimise foetal loss at delivery [2].

In the present study, hydrocephalus was diagnosed in a Chihuahua, which is in agreement with previous reports demonstrating a higher incidence of this particular abnormality in small and toy breeds [5, 19, 27]. In a study by Freitas and colleagues, foetal hydrocephalus, as indicated by an increased amount of cerebrospinal fluid accumulation inside the ventricles, was sonographically diagnosed in dogs from the 7th week of pregnancy, and there was only a moderate accumulation of fluid in the cerebral ventricles, with no significant change in the BPD measurement [6]. In our study, anechoic lesions in the foetal cerebral ventricles were apparent at the 8th week of pregnancy (Day-7) and the affected foetus had a larger BPD compared to the other littermates and the average BPD of full-term foetuses of small-sized dogs [9]. Moreover, the common physical characteristic of a large, dome-shaped head, indicating a hydrocephalus condition [5], was observed in this puppy. Our findings suggest that conventional transabdominal ultrasonographic examination is a useful tool to clearly diagnose congenital hydrocephalus during late pregnancy (approximately 7 days before parturition; Fig. 1B, 1C). In clinical practice, when the pregnancy monitoring of foetal development or the prediction of the parturition date using BPD measurements is performed in dog breeds that are prone to congenital hydrocephalus, the foetal brain and cerebral ventricles must be scanned carefully. Various degrees of ventricle dilatation/fluid accumulation and clinical outcomes of hydrocephalic newborns should be further investigated.

The abnormal newborn showed no signs of behavioral or gait abnormalities that are commonly observable in hydrocephalic dogs [19]. The affected newborn could move and demonstrated nipple searching and suckling behavior like the other siblings from the same litter. The hydrocephalic newborn, however, died 6 days after birth due to progressive weakness. The cause of death probably involved increased brain impairment. This is agreeable with the early *in utero* onset evidence, implying the worst prognosis outcome likely due to extensive neuronal cell death from prolonged hydrocephalus [10]. Prolonged ventriculomegaly during the foetal phase may cause extensive damage and the death of neurons responsible for circulatory function and metabolism.

The etiology of congenital hydrocephalus could be related to the environment, genetics or an environment-genetic interaction, including teratogenic substances, infectious agents and gene mutations [3–5]. Considering the breed and the occurrence individually, concomitantly with no history of teratogenic exposure, the presumptive cause of hydrocephalus in this case was genetic defects of the foetus.

The affected newborn also suffered from a cleft lip. Congenital CL/P defects are considered a common birth defect in several mammalian species [11, 14, 20, 21, 24, 25]. A previous study indicated that neonatal CL/P incidences were most frequent in brachycephalic breeds [1]. According to our review, the prevalence of CL/P in Beagles and Pyrenean Shepherds is 0.11% [16] and 2.2% [7], respectively. Because an animal with a CL/P is likely to have difficultly feeding and hearing due to their craniomaxillofacial abnormalities, which may bring about nasal infection and/or aspirated pneumonia [17], this might have contributed to the cause of death in the affected newborn in the present report. In humans, orofacial clefts can occur in conjunction with other defects, including brain abnormalities [23]. There are reports of brain abnormalities associated with a CL/P, including intracranial volume and specific deficits of tissue volume within the cortical and subcortical structures, and irregularities in cerebellum tissue [23]. A further investigation of the association between orofacial clefts and brain abnormalities in the dog is warranted.

The presence of a CL/P in the case's relatives (Fig. 4) strongly suggested genetic factors as the major contributors to the defect of this newborn. The recessive mode of inheritance, monogenic autosomal recessive or X-linked recessive transmission, plays an important role in CL/P [7, 11, 12, 21, 22, 28]. Monogenic autosomal recessive disorders normally occur when two of the defective genes are inherited from both of the parents as carriers of the mutated gene. In contrast, X-linked recessive disorders occur when a mother carrying the affected gene on the X chromosome passes it to its female newborn as a carrier, while there is a 50% chance that her male newborn with suffer from the disorder. With no history of teratogenic exposure and the presence of a large proportion of male siblings with congenital CL/P defects, the family pedigree of this case presentation indicates the X-linked recessive transmission of aberrant genes associated with cleft palates from the unaffected mother to the male newborn. Since X-linked recessive inheritance of CL/P was highly suspected, any further breeding of the parents (especially the bitch) was strongly discouraged [13].

In the case of neonatal canine CL/P, only somatic gene mutations–*ADAMTS20* and *DLX6* genes–were reported in the Nova Scotia Duck Tolling Retriever [28, 29]. Interestingly, X-linked mutations, *DMD*, *FGF13* and *EGFL6* genes, and additional Loci at Xp22.2. on the X chromosome, were recently reported to be associated with CL/P development in humans [26]. Our study, hereby, encourages further investigation into the X-linked recessive inheritance of CL/P in dogs.

In conclusion, sonographic examination allows for the prenatal diagnosis of hydrocephalus approximately 1–2 weeks before parturition. This early detection of foetal anomalies in dog breeds predisposed to congenital ventriculomegaly is thus important for the prediction of perinatal survival and close monitoring/assistance around the time of parturition.

ACKNOWLEDGMENT. We would like to thank all our colleagues and the staff of the Smile Dog Small Animal Hospital for their participation and technical support.

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