

# Ultrasonographic approach and findings in calves with hydranencephaly

Morteza Gorjidoz<sup>1</sup> | Peyman Dehghan Rahimabadi<sup>2</sup>  | Afshin Raoofi<sup>2</sup> |  
Majid Masoudifard<sup>3</sup> | Seyed Hossein Mardjanmehr<sup>4</sup>

<sup>1</sup> Department of Clinical Sciences, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar, Iran

<sup>2</sup> Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

<sup>3</sup> Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

<sup>4</sup> Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

## Correspondence

Afshin Raoofi, Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.  
Email: [raoofi@ut.ac.ir](mailto:raoofi@ut.ac.ir)

**Background:** Teratogenic viral infections may proceed to hydranencephaly in cattle. Post-mortem and antemortem diagnosis can be achieved by necropsy or ultrasonography, CT-scan and MRI techniques.

**Objectives:** The aim of this study was to determine how effective ultrasonography approach is in detecting hydranencephaly in calves.

**Methods:** In this study, ultrasonography images were obtained from brains of nine Holstein calves, of the same age, with neurological signs (due to Akabane virus infection), approaching from the caudal part of the temporal bone. To confirm the obtained images, the same approach was used to obtain images from a normal calf of the same age. The thickness of the temporal bone was measured and compared in seven affected and the healthy calves, using CT-scan images.

**Results:** In ultrasonographic images, temporal bone (as a hyperechoic structure) and temporal cortical mantle (as an echogenic structure) were noted in the right and left side of the skull. The medial part of the image showed presence of fluid in an anechoic region, instead of brain parenchyma. Falx cerebri was also seen as a floating hyperechoic line in the middle part in all patients. There was no statistically significant difference between the thickness of temporal bone in normal and affected calves ( $p = 0.502$ ). All findings were confirmed by necropsy.

**Conclusions:** Transtemporal approach is a novel and easy approach to study the brain in calves. This is the first study on the hydranencephalic brains of calves, using ultrasonography by transtemporal approach.

## KEYWORDS

calf, hydranencephaly, transtemporal approach, ultrasonography

## 1 | INTRODUCTION

Hydranencephaly may occur in cattle as a result of infection with Akabane virus, Aino virus, Bovine Viral Diarrhea virus, Bluetongue virus, Schmallenberg virus, Chuzan virus, Cache Valley virus, Rift

Valley fever virus and Wesselsbron virus (Baiker et al., 2010; Kirkland, 2015). This condition is recognizable soon after birth by characteristic clinical signs such as blindness, dullness and inability to suck the udder (Kirkland, 2015). Evaluation of frequency of central nervous system (CNS) defects is difficult in animals. Many of

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these defects also are identified after necropsy. Occasionally, where necropsy is not performed, CNS defects are missed, although sometimes they can be diagnosed before death (Kirkland, 2015; Washburn & Streeter, 2004).

The first report on clinical use of echoencephalography was published in 1956, increasing the interest of using this method for intracranial pathologies (Chambers et al., 1985). Neurologic ultrasonography allows diagnosis and confirms known or suspected brain abnormalities (Di Salvo, 2001). Ultrasound is widely used in humans to detect cerebral, periventricular and intraventricular hemorrhages, periventricular leukomalacia, hydrocephalus, brain atrophy and other brain disorders (Hudson et al., 1989; Hudson et al., 1998). Pathological changes of the brain alter the normal echogenicity of the brain tissue depending on their size and location which is detectable with ultrasonography (Lorigados & Pinto, 2013). Accurate diagnostic procedures are necessary for clinical diagnosis of hydranencephaly in cattle (Hudson et al., 1990). Before the development of ultrasonography, computed tomography scan (CT-scan) and air ventriculography were used to diagnose intracranial disorders in humans (Chambers et al., 2013). Ultrasonography is an appropriate method for examination of the brain in human, and it is often compared to CT-scan (Hudson et al., 1991; Vasiljevic et al., 2012). Despite the development of CT-scan and magnetic resonance imaging (MRI), to date, ultrasound is still the most common method for examination of the brain in human neonates. Unlike CT and MRI, there is no need for sedation, anaesthesia and ionising radiation (Chambers et al., 2013; Fox, 2009). In veterinary medicine, evaluation of ventriculomegaly by ultrasonography through open fontanel is possible in kittens and puppies, as it can be performed in human neonates (Chambers et al., 2013; Lorigados & Pinto, 2013).

Skull bones limit brain evaluation. These bones are insurmountable barriers for ultrasound image formation and cause refraction as a result of poor image quality obtained from the brain (Lorigados & Pinto, 2013; Spaulding & Sharp, 1990; White et al., 1978;). Hyperostosis of the skull is a major obstacle for brain imaging using ultrasound (Postert et al., 1997). Studies on human skulls have shown that acoustic energy transmission is variable and depends on bone structure and thickness. The transmitted energy is always equal to or less than 35% of the emitted energy (Lorigados & Pinto, 2013). According to literature, in adult animals 100% of sound waves are reflected by the bone, making it impossible to observe the brain (Lorigados & Pinto, 2013; Spaulding & Sharp, 1990). It should be noted that some skull bones (such as the frontal and dorsal portion of parietal bones) have a layer of spongy bone with several bone spicules in various shapes and directions. This spicule structure leads to scattering of ultrasound beams (Lorigados & Pinto, 2013). The compact bone (such as temporal bone) can cause refraction of the ultrasound beams (White et al., 1978). According to the acoustic behaviours of the skull bones and small thickness of the temporal bone (especially in young animals), it can be used as an acoustic window (Berland et al., 1988; Chambers et al., 2013; Hudson et al. 1998; Lorigados & Pinto, 2013). This bone is the only suitable available window in most people (Berland et al., 1988), but its size and the degree of penetrability are variable in humans. Thus, only lesions in the center of the

brain or portions of the temporo-parietal lobes were seen clearly, and frontal, occipital and cerebellar lesions were less clear (Berland et al., 1988) on the opposite side.

Ultrasonographic diagnosis of hydranencephaly and fluid replacement in the brain of both dog and human fetuses have been reported (Cruz et al., 2003), but there is no report of diagnosis of hydranencephaly by ultrasound in the calves. Tsuka, et al. (2002), used transorbital echoencephalography in normal cattle, younger than 1-year-old, to show that this imaging technique is beneficial for the diagnosis of bovine hydranencephaly.

The aim of this study was to determine how effective ultrasonography approach is in detecting hydranencephaly in calves.

## 2 | MATERIAL AND METHODS

### 2.1 | Animals

Twenty Holstein calves with neurological signs were born in a dairy farm in Varamin (a county in Tehran Province, Longitude 51.39° E, Latitude 35.19° N). Blindness and unintelligence (dumb calves that were unresponsive to stimuli) were seen in all calves, and seventeen calves were depressed. Deafness, kicking, head pressing, tongue paralysis and hyperexcitability were less common signs in these calves. All calves were born naturally, and average birth weight was  $38.45 \pm 5.12$  Kg. The owner had decided to keep and care for these calves for 3 months (Table 1).

### 2.2 | Ultrasonography protocol

Ultrasonographic images of the brains of nine calves (five male and four female, with the consent of the owner) with neurological signs were obtained by an ultrasound machine (SonoSite-MicroMaxx, WA 98021 USA) equipped with microconvex (5–8 MHz) and phased-array (1–5 MHz) transducers. During the imaging procedure the calves were restrained by hand. The hair on the skin was shaved at the transducer site, and ultrasound gel was used as an acoustic coupler between the transducer and skin. The transducers were positioned at 90° relative to left and right aspect of the temporal region (Figure 1). Caudal part of the temporal bone was chosen as the ultrasonic approach, and the temporal bone was used as an acoustic window. In each calf, B-mode images were also obtained in transverse and dorsal planes.

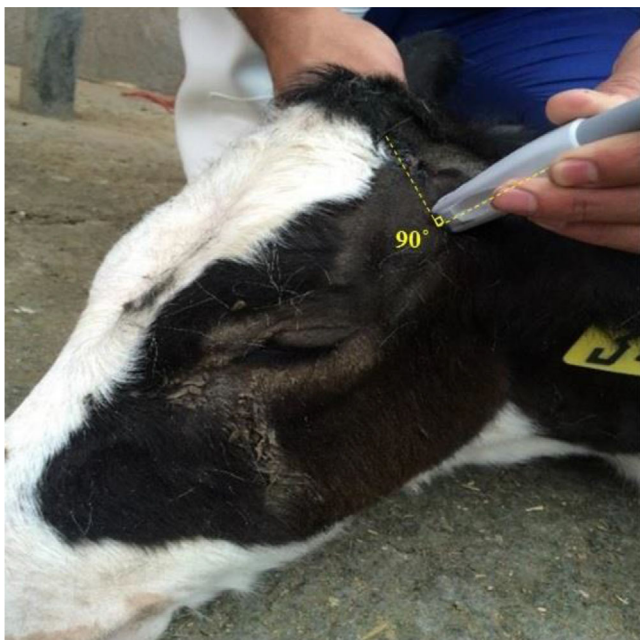
To confirm images obtained from the brains of patients, ultrasound images of the brain were also obtained from a normal calf (without neurological disorders) of the same age as the affected ones in the similar protocol.

Other parts of the skull were evaluated via transorbital approach for imaging the brain. Both transducers were placed separately over the eyelid and turned caudally and dorsally at an angle of about 21 and 20 degrees, respectively. The hair of the eyelid was not clipped, while an acoustic gel was applied between the transducers and the skin (Tsuka et al., 2002).

**TABLE 1** Age, breed, sex, body weight, CT-scan examination, clinical signs and necropsy findings of calves with hydranencephaly and normal calf at the time of ultrasonography

Information	Calf number	1	2	3	4	5	6	7	8	9	Normal calf
Age (months)		3	3	3	3	3	3	3	3	3	3
Breed		H	H	H	H	H	H	H	H	H	H
Sex		F	F	F	F	M	F	M	M	F	M
Body weight/kg		30	34	26	43	41	38	35	37	35	45
CT-scan performed		Y	Y	Y	Y	Y	Y	Y	N	N	Y
Clinical signs	Blindness	P	P	P	P	P	P	P	P	P	A
	Unintelligence	P	P	P	P	P	P	P	P	P	A
	Head pressing	P	A	A	A	A	A	A	A	A	A
	Tongue paralysis	P	A	A	A	P	A	A	A	A	A
	Hyperexcitability	A	A	A	P	A	A	A	A	A	A
	Deafness	A	P	A	A	A	A	A	A	A	A
	Kicking	A	P	A	A	P	P	A	A	A	A
Necropsy findings	Fluid-filled sacs	P	P	P	P	P	P	P	P	P	
	Frontal lobe	Pf	Nf	Nf	Nf	Pf	Nf	Nf	Nf	Nf	
	Occipital lobe	Nf	Pf	Pf	Nf	Nf	Nf	Nf	Nf	Nf	
	Temporal lobe	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	
	Parietal lobe	Pf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	
	Pyriform lobe	Cf	Pf	Cf	Pf	Pf	Pf	Pf	Pf	Cf	
	Falx cerebri	Cf	Cf	Cf	Cf	Cf	Cf	Cf	Cf	Cf	

Abbreviations: A, absent; Cf, completely formed; F, female; H, Holstein; M, male; N, no; Nf, not formed; P, present; Pf, partially formed; Y, yes.

**FIGURE 1** Brain ultrasonographic approach in the calf, microconvex transducer was positioned at 90° relative to the temporal bone

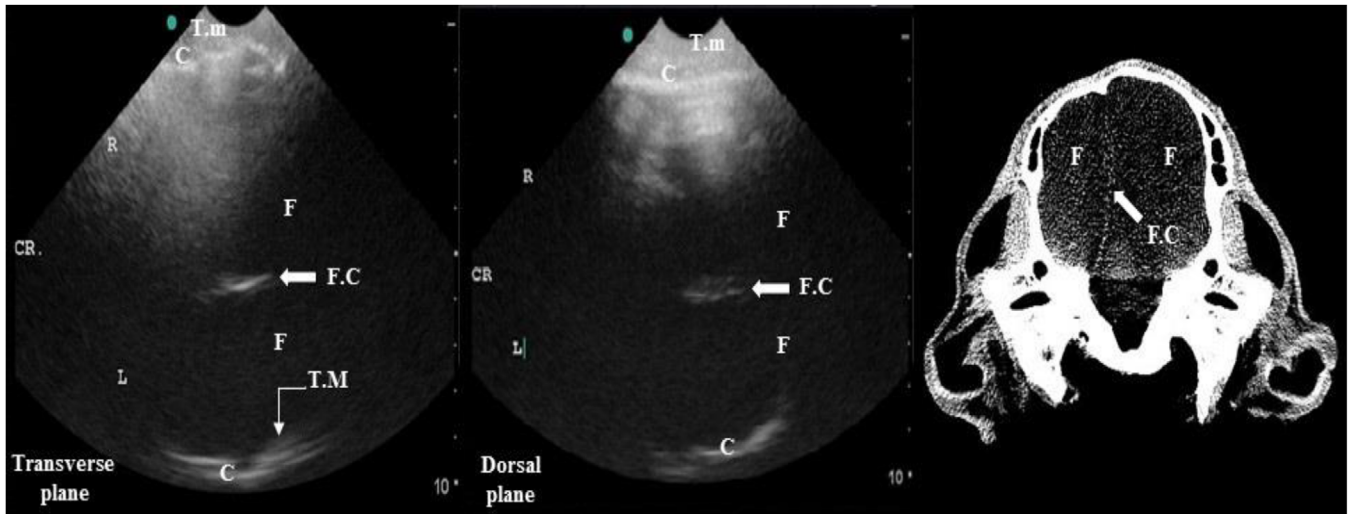
The calves were slaughtered due to difficulties in nursing. Immediately after slaughter, images were obtained from the brains through transtemporal approach to investigate probable changes.

### 2.3 | CT-scan protocol

To study temporal bone thickness in the calves, heads were obtained from necropsy. CT images were obtained in transverse plane from seven skulls of calves with neurological signs and one skull of normal calf using a multi detector CT scanner (Somatom spirit 2, Siemens, Germany). The CT-images were reconstructed in transverse planes using the program Image (Syngo image 5.5). After recording the temporal bone thickness in affected calves, the results were compared to temporal bone thickness of the normal calf by one-sample *t*-test method (SPSS-22 software, IBM Co., New York, USA), and differences were considered statistically significant when  $p < 0.05$ . Also, using the method introduced by Lee, et al (2010), the volume of the fluid-filled sacs in affected calves was calculated by multiplication of the total sac area measured on each transverse image by the total slice thickness. Maximum height of these sacs was determined by software in transverse plane of CT images in brain window.

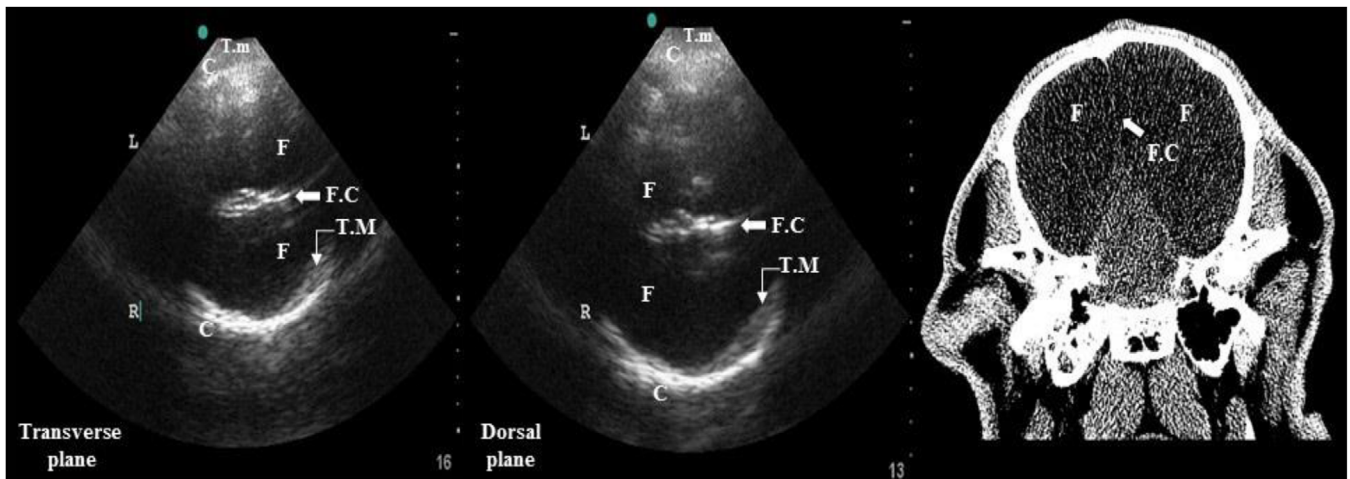
### 2.4 | Necropsy

As the final stage, six skulls of calves with neurological signs were necropsied (three skulls were not necropsied due to the owner's disapproval) to confirm the imaging findings, and brain tissue was examined macroscopically to compare to the results obtained from ultrasonographic examination.



**FIGURE 2** Ultrasonography of the brain in the calf with hydranencephaly, presence of fluid in the anechoic region on both sides of the falx cerebri in the transverse (left) and dorsal (middle) planes in the transtemporal approach (microconvex transducer: 5–8 MHz); transverse CT-scan image of the same calf (right)

Abbreviations: C, calvarium; CR, cranial; F, fluid; F.C, falx cerebri; L, left hemisphere; T.M, temporal cortical mantle; T.m, temporal musculatures; R, right hemisphere.



**FIGURE 3** Ultrasonography of the brain in the calf with hydranencephaly, presence of fluid in the anechoic region on both sides of the falx cerebri in the transverse (left) and dorsal (middle) planes in the transtemporal approach (phased-array transducer: 1–5 MHz); transverse CT-scan image of the same calf (right)

Abbreviations: C, calvarium; F, fluid; F.C, falx cerebri; L, left hemisphere; R, right hemisphere; T.M, temporal cortical mantle; T.m, temporal musculatures.

Six samples of the remaining brain tissue were collected (approximately 10 grams) to detect Akabane virus mRNA. RNA was extracted using the MBST kit (Molecular Biological System Transfer, Iran) from 0.05 g of the remaining brain tissue according to the manufacturer's instructions. Then the residual Akabane virus acid nucleic was identified, using Reverse Transcription-Polymerase Chain Reaction (Qiagen, Hilden, Germany) with forward and reverse primers for the Akabane genome (F1: TAACTACGCATTGCAATGGC; R1: TAAGCTTAGATCTG-GATACC).

### 3 | RESULTS

#### 3.1 | Ultrasonographic findings

Using both transducers (microconvex: 5–8 MHz and phased-array: 1–5 MHz) in the transtemporal approach, images were obtained from brain structure of alive affected calves (Figures 2 and 3). In transverse and dorsal planes, temporal bone and temporal cortical mantle were seen (as hyperechoic and echogenic structures, respectively) in the

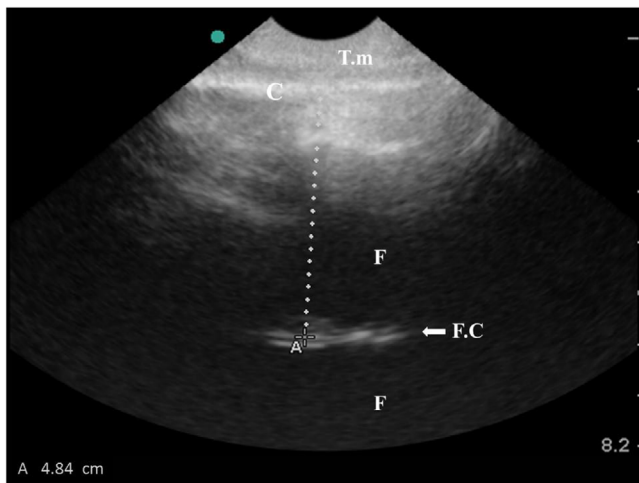
**TABLE 2** Ultrasonographic findings of two different transducers in dorsal and transverse planes of calves with hydranencephaly and normal calf

Calf number	Side of skull	1		2		3		4		5		6		7		8		9		Normal calf				
		D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T			
Microconvex (5-8 MHz)	Calvarium (Temporal bone)	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V		
		L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
		R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Nv	
	Anechoic region (fluid)	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Nv	Nv
		R	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		L	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
	Cerebral paranchyma	R	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		L	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		R	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V
	Temporal cortical mantle	L	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V
		R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
		L	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V	Nv	V
Temporal masclatures	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
Falx cerebri	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
Phased-array (1-5 MHz)	Calvarium (Temporal bone)	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
		L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
		R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Nv	
	Anechoic region (fluid)	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	Nv	Nv
		R	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		L	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
	Cerebral paranchyma	R	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		L	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	Nv	V
		R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
	Temporal cortical mantle	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
		R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
		L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
Temporal masclatures	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
Falx cerebri	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	R	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
	L	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	

Abbreviations: D, dorsal plane; L, left; Nv, non visible; R, right; T, transverse plane; V, visible.

**TABLE 3** Size of fluid-filled sacs on the transverse plane in the ultrasonograms

Calf NO.	Side of skull	Size (cm)
1	Righ	5.67
	Leftt	5.92
2	Righ	4.85
	Leftt	4.25
3	Righ	4.75
	Leftt	4.42
4	Righ	5.74
	Leftt	5.32
5	Righ	5.89
	Leftt	5.28
6	Righ	5.14
	Leftt	5.96
7	Righ	4.84
	Leftt	4.12

**FIGURE 4** Size of fluid-filled sac in the ultrasonogram (microconvex transducer: 5–8 MHz)

Abbreviations: C, calvarium; F, fluid; F.C, falx cerebri; T.m, temporal musculatures.

left and right sides of skull, and a wide middle portion of the images appeared as anechoic region, indicating the presence of fluid instead of brain tissue. Falx cerebri as a hyperechoic and floating line was clearly visible in the center of the anechoic region (Table 2). Additionally, the largest fluid-filled sac noted in the ultrasonogram was in calf NO. 6 (5.96 cm, left sac), and the smallest sac was in calf NO. 7 (4.12 cm, left sac) (Table 3, Figure 4).

In the normal calf, temporal bone appeared as hyperechoic structure in the left and right sides of skull. Hemispherical echogenic structures were seen in the middle part of the images. Falx cerebri was also characterized as a hyperechoic line among the hemispheres (Figure 5).

There was no difference between the images obtained from the structures of the brain in alive and slaughtered affected calves. The

images obtained from calves' brains using the microconvex transducer (5–8 MHz) were clearer than the phased-array (1–5 MHz) transducer.

It should be noted that no image was formed when transorbital approach was used as an acoustic window in the calves.

### 3.2 | CT-scan findings

In transverse plane of the skulls, temporal bone thickness was determined after reconstruction of images (Figure 6). Maximum and minimum thicknesses of the temporal bone were recorded in calf NO. 7 (4.12 mm) and calf NO. 4 (3.65 mm), respectively (Table 4). The difference between the thickness of the temporal bone in the affected calves and the normal calf (3.83 mm) was not significant ( $p = 0.502$ ). All the ultrasound images were of high resolution at the mentioned bones thicknesses.

CT images also showed asymmetrical fluid-filled sacs separated by falx cerebri in affected calves. Mean height of the left sacs and the right sacs were  $6.01 \pm 0.5$  cm and  $6.01 \pm 0.3$  cm, respectively. Maximum and minimum heights of the fluid-filled sacs were also determined. Maximum height was recorded in calf NO. 7 (left sac, 6.84 cm), and minimum height was seen in calf NO. 3 (left sac, 5.29 cm) (Figure 6). Mean volume of the left sacs and the right sacs were  $80531.43 \pm 28602.42$  mm<sup>3</sup> and  $85987.14 \pm 22302.42$  mm<sup>3</sup>, respectively. The left sac in calf NO. 7 was the most voluminous one, measuring 124640 mm<sup>3</sup>, and the least fluid volume measured 46750 mm<sup>3</sup>, in the left sac in calf NO. 3 (Table 4).

### 3.3 | Necropsy findings

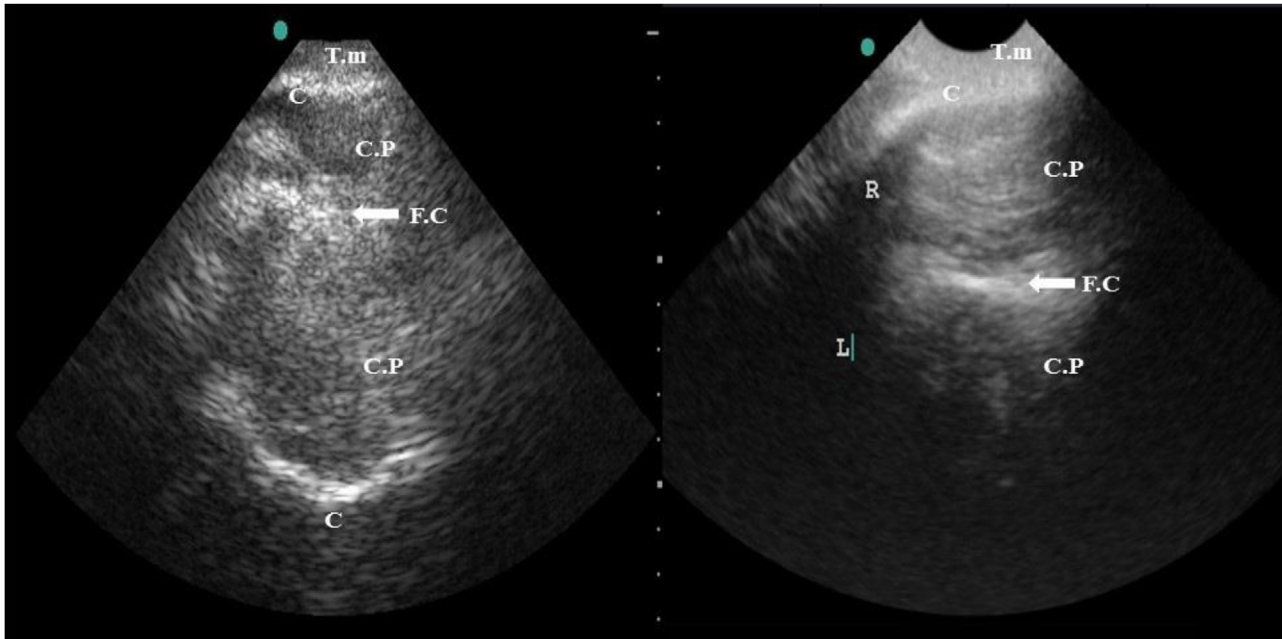
Ultrasonographic results have correlated well with pathologic lesions, and hydranencephaly was confirmed in affected calves.

After necropsy and removing the calvarium and dura matter, fluid-filled sacs, surrounded by a thin membrane, were observed in the cranial cavity. Examination of telencephalon showed that frontal and occipital lobes were partially formed in two cases, and in other cases these parts were not formed. Parietal lobe was found to be incomplete only in one case and was not seen in other cases. Temporal lobe was not formed in any of the cases. Pyriform lobes were complete in three cases and in other three cases were partially formed. In all cases, thalamus, cerebral peduncles, rostral and caudal colliculi, cerebellum, pons and medulla oblongata were completely formed without any major changes. Falx cerebri was completely formed, and it was floating in the fluid (Table 1, Figure 7).

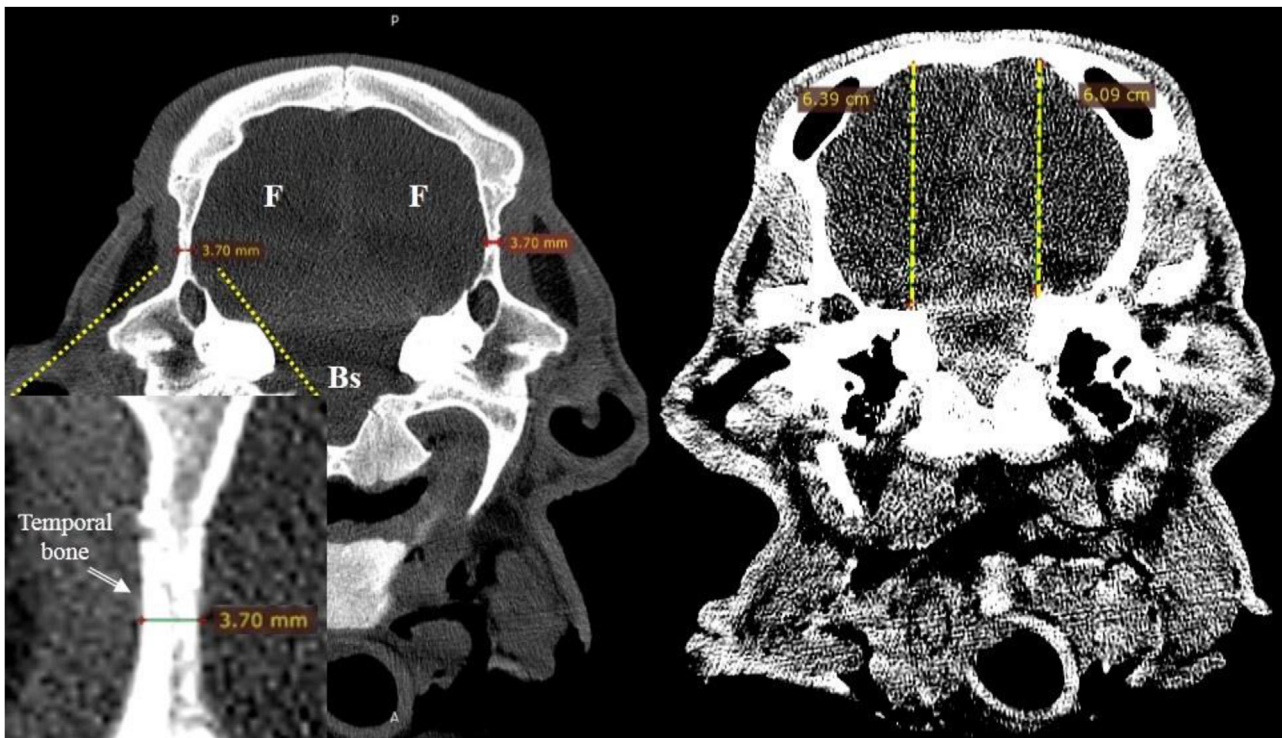
It must be mentioned that Akabane virus was the causative agent of hydranencephaly in the calves and was detected by RT-PCR in the remaining brain tissue.

## 4 | DISCUSSION

Optimal imaging of the brain requires a good acoustic window which in humans and animals are the unfused cranial sutures and open fontanel



**FIGURE 5** Ultrasonography of the normal brain in a calf, presence of cerebral parenchyma as echogenic structures in both sides of the falx cerebri in the transtemporal approach (phased-array transducer: 1–5 MHz [left] and microconvex transducer: 5–8 MHz [right])  
Abbreviations: C, calvarium; C.P, cerebral parenchyma; F.C, falx cerebri; L, left hemisphere; R, right hemisphere; T.m, temporal musculatures.

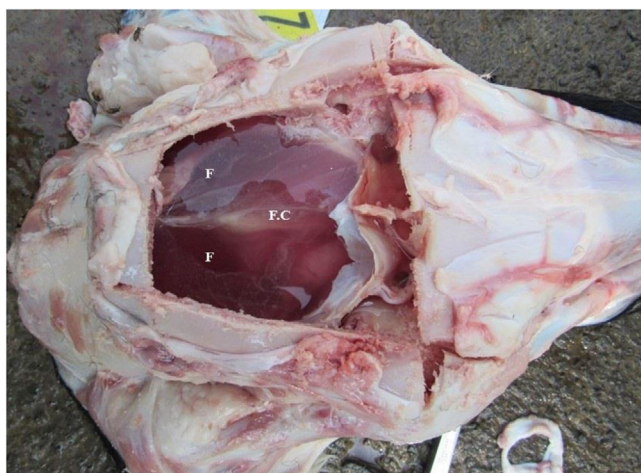


**FIGURE 6** Measurement of temporal bone thickness in the calf with hydranencephaly by CT-scan in bone window. It shows that the slight thickness of the caudal part of the temporal bone provides an acoustic window for brain ultrasonography in the calves (left); measuring the height of the fluid-filled sac in brain window of the same calf (right)  
Abbreviations: Bs, brainstem; F, fluid.

**TABLE 4** Thickness of the temporal bone, height and volume of the fluid filled sacs in the studied calves using CT-scan

Calf No.	Thickness of temporal bone (mm)	Fluid-filled sacs			
		Height (cm)		Volume (mm <sup>3</sup> )	
		Left	Right	Left	Right
1	3.70	6.39	6.09	115600	95030
2	3.85	5.93	6.20	64680	85320
3	3.74	5.29	5.84	46750	63250
4	3.65	6.16	5.45	72700	52300
5	4.03	5.69	6.23	74350	105650
6	4.09	5.77	5.98	65000	85000
7	4.12	6.84	6.32	124640	115360
Mean ± SD	3.89 ± 0.19	6.01 ± 0.5	6.01 ± 0.3	80531.43 ± 28602.42	85987.14 ± 22302.42

Thickness of temporal bone in the normal calf measured 3.83 mm.



**FIGURE 7** Necropsy in a calf with hydranencephaly. Presence of fluid-filled sacs in both sides of the falx cerebri  
Abbreviations: F, fluid; F.C, falx cerebri.

(Di Salvo, 2001). Lorigados and Pinto (2013), used parietal and temporal bones as an acoustic window to examine the brain in dogs, and perfectly observed the frontal, temporal, and occipital lobes through the left and right temporoparietal window, but visualization of the lateral ventricles was difficult in this approach. In a more detailed study, Fukushima et al. (2000), successfully examined the middle cerebral artery in dogs through the transtemporal approach; hence, the present study used a similar approach for brain imaging in affected calves. Hydranencephaly was successfully observed in the images. Images obtained from the normal calf demonstrated normal brain structure that was compared to affected ones. The results also correlated well with CT-scan and necropsy findings. Results of this study indicate that this approach is appropriate for brain imaging in calves, which can help the diagnosis of hydranencephaly, as transtemporal approach had been previously used in ultrasound imaging of the human brain, and approved to be efficient (De Souza-Daw et al., 2012; Postert et al.,

1997). Meanwhile Testoni et al. (2010) used craniocervical junction as an acoustic window for detection of caudal structures of brain, including occipital lobe, cerebellum and caudal aspect of brain stem in a calf, being followed by Lorigados and Pinto (2013) who successfully examined the cerebellum with the same approach. Transorbital echoencephalography was also described by Tsuka et al. (2002) for detection of cerebral parenchyma and lateral ventricles in cattle by positioning the transducer at specific angles, but in this study, no images were obtained from the brain tissue, using this method.

Thickness of the temporal bone may be the most important factor affecting ultrasound beam penetration (Kwon et al., 2006), and as it has been proven in the same animal species, that the thickness of this bone tends to be quite variable (Lorigados & Pinto, 2013); however the thickness can be estimated using a skull CT-scan (Ammi et al., 2008; Kwon et al., 2006). Results showed that there was no significant difference in temporal bone thickness between affected and normal Holstein calves. This bone was identified in the ultrasonography as a hyperechoic structure on both sides of the image. Kwon et al. (2006) stated that attenuation of the ultrasound beam significantly increases in humans, if the temporal bone thickness is more than 2.7 mm and has a positive correlation with age, while it seems that in Holstein calves, even up to the age of 3 months (despite temporal bone thickness of 4.12 mm measured via CT-scan), this bone is not an insurmountable barrier. Therefore, brain can be observed in the calves of the same age, using ultrasound waves via transtemporal approach. But it seems that as calves age, acoustic shadowing caused by the skull bones, leads to decreased image quality in this approach. The authors believe that temporal approach in immature cases was more practical than other approaches. Because in this region the bone thickness is reduced, an appropriate acoustic window for evaluation of the brain was prepared especially in the hydranencephalic cases.

Generally, temporal bone has three different layers: the inner and outer tables (compact bone) and middle spongy layer. The last layer is most responsible in aberration of beam in temporal bone (Ammi et al., 2008). The variation of sound velocity has been shown in different layers of the human skull. Sound velocity ranges from 2570 to 3030 ms in



the temporal bone (Ammi et al., 2008). Although this bone is present as a compact bone in the skull, its thin nature, especially in the caudal part, makes it possible to be considered as an acoustic window in brain ultrasonography (Sekhar & Estonillo, 1986). Berland et al. (1988) obtained good images, similar to our results, using temporal bone as an acoustic window to examine the brain in people. In adult people due to poor skull penetration, higher frequency real-time transducer is not applicable, while Hudson et al. (1998) stated that low frequency transducers (5 MHz) in dogs provided a good image through the transtemporal approach, but resolution was reduced. In most dogs, frequencies between 7 and 12 MHz are usually useful. Also, in some cases, a lower frequency transducer may be needed to penetrate deeper brain structures (Hudson et al., 1998; Tucker & Gavin, 1996). Although lower frequency of ultrasound wave is commonly used to evaluate brain structures through echoencephalography, smaller size of neonatal brains had led to the use of higher frequency transducers for brain imaging (Hudson et al., 1989). Generally, the higher frequency transducers (5–10 MHz) are used for better resolution (Tucker & Gavin, 1996). Desirable image resolution can be achieved by pulsed beams (Aldrich, 2007). High frequency transducers are composed of thin piezoelectric elements that emit fewer cycles in each pulse, compared to low frequency transducers. So, spatial pulse length is shorter in high frequency transducers where the wavelength is also shorter. Axial resolution is equal to half the spatial pulse length. Hence, the image quality is higher when the spatial pulse length is shorter (Alexander & Swanevelter, 2011). Therefore, in this study, microconvex transducer (5–8 MHz) with a B-mode ultrasound was used to obtain high quality images of the brain. Cruz et al. (2003) detected hydranencephaly in the fetuses of a 2.5-year-old female dog in their study using an almost similar microconvex transducer (5–7.5 MHz). Lorigados and Pinto (2013) have noted another advantage of this transducer; they believed that microconvex transducer is appropriate for brain ultrasonography due to the small surface contact area. Moreover, Berland et al. (1988) stated that the more skin contact with the transducer, the more difficult it is to manipulate the position of the transducer. According to the derived results, it can be stated that utilizing this transducer with the mentioned frequency, proper images can be obtained from brains of Holstein calves up to 3 months of age. In the current study, phased-array (1–5 MHz) transducer was also used, revealing less image quality compared to microconvex (5–8 MHz) transducer. Previously, Berland et al. (1988) stated that the low-frequency phased-array probe with convenient penetration of the skull provides a good image of the brain, unlike the current study. Di Salvo (2001), obtained high resolution images, using a phased-array (5–7 MHz) transducer, while examining human neonatal brain. However, artifacts may be seen in the images, which is due to the difference between acoustic impedance in bone and soft tissues.

It must be mentioned that transducer positioning on the temporal bone is important for penetration of the ultrasound through the skull and the best position for the transducer is  $90^\circ \pm 5^\circ$  to the temporal bone (Ammi et al., 2008). In the current study, the transducer position was  $90^\circ$  relative to the temporal region so that a proper image would be obtained from the brain.

In this study, falx cerebri, temporal cortical mantle, temporal bone and anechoic region (fluid-filled sacs) were clearly visible by ultrasonography, and these results have correlated well with pathologic findings. Also, falx cerebri was observed by Sepulveda et al. (2012) in human fetus with hydranencephaly that appeared as a floating hyperechoic line in the middle part of anechoic region (fluid-filled sacs) which was similar to our findings in the brains of affected calves. Khalid et al. (2012) believed that in most cases of hydranencephaly, lesions had developed after ventricular formation; thereby falx cerebri is intact in patients. Whitem (1957) similarly stated that the brain structure was first found to be normal in patients with hydranencephaly, and then the degenerative process began in this tissue. However, this may not be detectable in cases of severe hydranencephaly that have existed since the beginning of fetal development and may not be visible in ultrasonography (Cruz et al., 2003). Based on the data obtained from CT-scan, mean ventricular height is normally known to be about 5 mm in Holstein calves (Lee et al., 2010), while in the present study, fluid-filled sacs had replaced the cerebral hemispheres; hence they were more voluminous than that measured by Lee et al. (2010) and possessed a much higher height; as much as hemispheric heights.

The results of this study showed that there was no change in the quality of images in calves before and after slaughter. Therefore, calves' skull after slaughter (in shortest possible time) can be used for ultrasound imaging. According to the current study, ultrasonography can be used in caudal part of the temporal bone as a diagnostic technique for detection of hydranencephaly in suspected calves.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

#### AUTHOR CONTRIBUTIONS

Conceptualization: Morteza Gorjidoz and Majid Masoudifard. Formal analysis and investigation: Morteza Gorjidoz, Peyman Dehghan Rahimabadi, Afshin Raoofi, Majid Masoudifard and Seyed Hossein Mardjanmehr. Writing - original draft preparation: Peyman Dehghan Rahimabadi. Reviewer of literature: Peyman Dehghan Rahimabadi. Writing - review and editing: Peyman Dehghan Rahimabadi, Afshin Raoofi and Majid Masoudifard. Methodology: Majid Masoudifard and Seyed Hossein Mardjanmehr.

#### ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## PEER REVIEW

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## ORCID

Peyman Dehghan Rahimabadi  <https://orcid.org/0000-0002-2987-4479>

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