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NOTE

Internal Medicine

Ophthalmic findings in a septic calf with the concurrent exhibition of meningitis and endophthalmitis

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ABSTRACT. The aim of this study was to evaluate the impacts of ophthalmic findings obtained from both macroscopic examination and ocular ultrasonography when diagnosing bovine endophthalmitis. A newborn crossbreed (Japanese black and Holstein breeds) calf was suspected of visual impairment and central nervous system (CNS) symptoms, such as decreased activity and weak drinking performance. This calf was found to display macroscopic signs, such as clouded lens, convergent strabismus, and horizontal nystagmus, in both eyes. On ocular ultrasonography of both eyes, a V-shaped, thickened, hyperechoic structure was present in the anechoic vitreous humors, indicating retinal detachment. The animal died 4 days after the examination. Sepsis was evident in this case, as *Escherichia coli* was isolated from multiple organs. The autopsy and histological examination revealed meningitis, encephalitis, and secondary hydrocephalus in the CNS, and endophthalmitis and retinal detachment in both eyes. In this case, the ophthalmic findings did not provide definitive evidence for a diagnosis of endophthalmitis. However, this study indicated that retinal detachment might be an ultrasonographic finding that is suggestive of bovine endophthalmitis.

KEY WORDS: calf, *Escherichia coli*, endophthalmitis, meningitis, ultrasonography

Newborn calves are susceptible to systemic bacterial infection from various portals of entry, particularly calves with immature immune systems and insufficient colostrum intake [3]. In bovines, sepsis can frequently accompany infectious in various organs, including the meninges and the brain (referred to as septic meningitis and encephalitis, respectively) [13]. The eye, especially the uvea, is a susceptible organ, and infection in the eye can cause uveitis and hypopyon, a focal lesion within the anterior chamber of the eye [3, 24, 30]. Endophthalmitis is a septic ophthalmic disease, which may occur at the terminal stage, and which is characterized by the formation of multiple intraocular lesions (a condition also referred to as panophthalmitis) [6, 14]. Thus, ophthalmic findings both within and outside the eye may typically be indicative of bovine sepsis [14].

Macroscopically, strabismus and nystagmus are sepsis-induced signs associated with encephalitis and secondary hydrocephalus [9, 13, 30]; however, congenital nystagmus may occur in Holstein-Friesian and Guernsey breeds [19], and congenital multiple ocular defects (MODs) have been reported in Japanese black breed [1, 29]. Clouded lenses associated with sepsis-induced infection of the anterior chamber sometimes resemble lenses affected by bovine congenital cataract [13, 18].

Ocular ultrasonography enables effective evaluation of intraocular abnormalities and is the first choice among available ophthalmological procedures for differentiating between focal and systemic types of infectious ophthalmic diseases in human medicine [5, 7, 8]. In veterinary medicine, ultrasonography has also been used to diagnose various ophthalmic diseases in cats, horses, goats, sheep, and camels [4, 11, 12, 26]. Previous studies involving bovines have described the use of ocular ultrasonography to observe multiple congenital abnormalities [17, 29]. However, endophthalmitis in bovine cases has not

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previously been evaluated using ocular ultrasonography.

This study aimed to (1) describe ophthalmic findings in the bilateral eyes of a calf diagnosed with endophthalmitis when evaluated both macroscopically and by ocular ultrasonography and (2) discuss ophthalmic findings in comparison with observations from autopsy and pathological examination.

Soon after a normal birth, a newborn crossbreed (Japanese black and Holstein breeds) calf presented with abnormal behaviors, including decreased vitality, depression, and reduction of suckling reflex. The animal had a fever (40.0°C), and the umbilical cord could be palpated and appeared to be slightly swollen when diagnosed on postpartum day 6. The animal presented with severe strabismus in both eyes, and macroscopically, white-colored areas of the cornea were observed. The strabismus was the convergent type. On ophthalmic examination, visual impairment on both eyes was suspected on the basis of reduced dazzle and pupillary reflexes. Additionally, both eyes presented with horizontal nystagmus. Ocular ultrasonography was conducted using a portable ultrasound machine (CTS-800 KS, Kyoritsu Seiyaku Ltd., Tokyo, Japan). A 7.5 MHz linear transducer was applied to the surface of the cornea soon after the topical application of an ultrasound gel, and the animal was maintained in a standing position without sedation. On the ultrasonograms of both eyes, a common intraocular abnormal characteristic was the presence of a V-shaped, membranous structure within the vitreous body. The tip of the V-shaped structure was located near the area of the optic disk, and the two proximal edges ended in the scleroretinal rim near the ciliary body, although the entire structure could not be visualized in the same image (Fig. 1A, 1B). These V-shaped structures within both eyeballs were heterogeneously echogenic and irregular. The thickness of the structures was greater at the tip of the V shape than at the two proximal segments and measured 5–7-mm. The vitreous humor appeared anechoic and did not include any echoic deposits. On the other ocular ultrasonograms visualizing the whole structures of the lens, a normal appearance of the lenses in both eyes was evident; the anechoic lens structure was surrounded by intermittent, hyperechoic lines of the anterior and posterior lens capsules. The small structures of the anterior chambers were ultrasonographically unclear in both eyes. Hematological examination revealed a high white blood cell count (14,750/µl) than the value (mean ± standard deviation) measured in 16 heads of 1-week-old healthy Japanese black calves (10,081.3 ± 4,136.6/µl; Table 1). Compared with the mean level in age-matched animals (481.1 ± 318.3 U/l), an abnormally low level of γ-glutamyltranspeptidase (23 U/l) was identified, indicating insufficient intake of colostrum [2]. The levels of blood urea nitrogen (40.1 mg/dl) and aspartate transaminase (95 U/l) were higher than those in age-matched animals (12.0 ± 5.9 mg/dl and 37.4 ± 4.3 U/l, respectively). The animal was treated by 4 day intravenous injection of oxytetracycline (Engemycin 10% DD solution for injection, MSD Animal Health, Tokyo, Japan). However, the condition of the animal gradually deteriorated, resulting in the development of ataxia, with the legs flailing, followed by signs of coma. The animal died 4 days after the examination.

At autopsy, congestion was evident in the subcutaneous tissues of the umbilical cord. Pale-yellow synovial effusions accumulated together with white deposits within the right hip, the left stifle, and the atlantooccipital joints. Yellowish-white purulent material adhered to the round ligament of the liver, the greater omentum, and the serosal surface of the mesentery. The structure of the lungs was characterized by congestion but not pneumonic changes. In both eyeballs, fibrous deposits were present within the anterior chamber. The lens structures were not macroscopically abnormal within both eyeballs. Within the vitreous body, the retinal structure appeared to be detached between the area of the optic disc and the scleroretinal rim near the ciliary body. Macroscopically, thickening of the meninges of the brain and the dura mater of the spinal cord was evident. The lateral ventricle was markedly extended. *Escherichia coli* (*E. coli*) was isolated from swabs obtained from the heart, liver, spleen, kidney, cerebrum, cerebrospinal fluid, and the aqueous humors of both eyes.

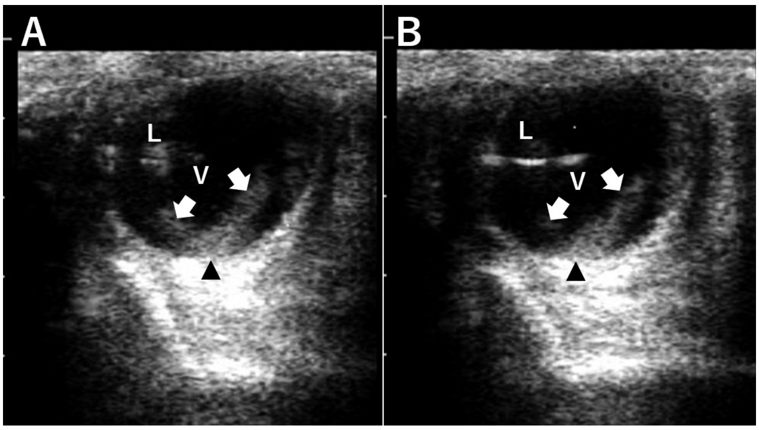

Fig. 1. Ocular ultrasonograms of the left and right eyeballs (**A** and **B**). The lens structure (L) is represented by the anechoic lens and the intermittent, hyperechoic lines of the anterior and posterior lens capsules. The vitreous humor (V) appears anechoic. A V-shaped membranous structure is evident within the left and right eyeballs (arrows). The tip of the V-shaped structure is located near the area of the optic disc (arrowheads). Scale bar=10 mm.

Table 1. Hematology and blood biochemistry in this case

	This case	Age-mathed calves (n=16)
Red blood cell count (×10^4/µl)	1,013	839.1 ± 118.7
White blood cell count (/µl)	14,750	10,081.3 ± 4,136.6
Hemoglobin (g/dl)	12.4	10.6 ± 1.5
Hematocrit (%)	39.3	34.3 ± 6.3
Urea nitrogen (mg/dl)	40.1	12.0 ± 5.9
Creatinine (mg/dl)	1.46	0.90 ± 0.19
Aspartate transaminase (U/l)	95	37.4 ± 4.3
γ-glutamyltranspeptidase (U/l)	23	481.1 ± 318.3

Specimens of the eyeballs and various organs were fixed in 10% neutral phosphate-buffered formalin and embedded in paraffin wax. Paraffin wax-embedded tissues were sectioned at a 4 µm thickness for hematoxylin and eosin staining. Sections of the cerebral tissues were subject to immunostaining using rabbit antibodies against *E. coli* O119 (DENKA SEIKEN Co., Ltd., Tokyo, Japan) and immunoperoxidase polymer (Simple stain; Nichirei Biosciences, Tokyo, Japan). Histological examination of the abdominal organs revealed the accumulation of the inflammatory cells, predominantly comprising neutrophils and macrophages, within the umbilical cord, liver, spleen, and kidney. The accumulation of inflammatory cells was partially accompanied by congestive, degenerative, and necrotic changes. Bacterial aggregates were distributed throughout the lumina of the capillary vessels within the myocardium, spleen, and hepatic sinusoid.

Histological examination of the eyeballs revealed degenerative, necrotic, edematous changes to the inner plexiform layer of the retina, sclera, and cornea, and swollen pigment epithelial cells were observed in the tapetum. The retina was completely detached from the sclera (Fig. 2A). Inflammatory cells, predominantly neutrophils, infiltrated into the iris, ciliary body, sclera, and cornea (Fig. 2B). The infiltration of inflammatory cells was observed in combination with necrotic changes along the perineurium of the optic nerve (Fig. 2C). These pathological changes were less pronounced in the right eyeball than in the left eyeball. No histopathological abnormality in the lens structures of both eyeballs was evident.

The structures of the meninges were histologically hemorrhagic, edematous, and infiltrated by inflammatory cells. In the cerebral parenchyma, perivascular cuffing of neutrophils and the formation of glial nodules were predominant (Fig. 3). Bacterial aggregates were commonly scattered within the cerebral parenchyma and the meninges. Immunohistochemistry with anti-*E. coli*-specific antibodies revealed positive reactions in large areas of the cerebrum. Based on the histological findings, this case was suspected to involve sepsis, resulting in endophthalmitis (panophthalmitis), comprising purulent scleritis, uveitis, and keratitis, and perineuritis of the optic nerve in both eyeballs. In the central nervous system (CNS), diffuse purulent meningitis, mild encephalitis, and secondary hydrocephalus were evident.

Genomic DNA was extracted from paraffin-embedded tissue samples of the affected calf. Polymerase chain reaction (PCR) was conducted to amplify the targeted area, which was the location of a single-nucleotide insertion mutation (*WFDC1*:c.198_199insC) previously identified in MOD [1]. Amplifications were performed using forward (5′-TAGGCGGAGGAGGTAGGC-3′) and reverse (5′-CAGCCGTTGTAGCAGCAGC-3′) primers, and the amplified band was sequenced using Sanger sequencing. The sequence of the affected calf was shown to be homozygous wild-type and did not include the previously identified mutation (Fig. 4).

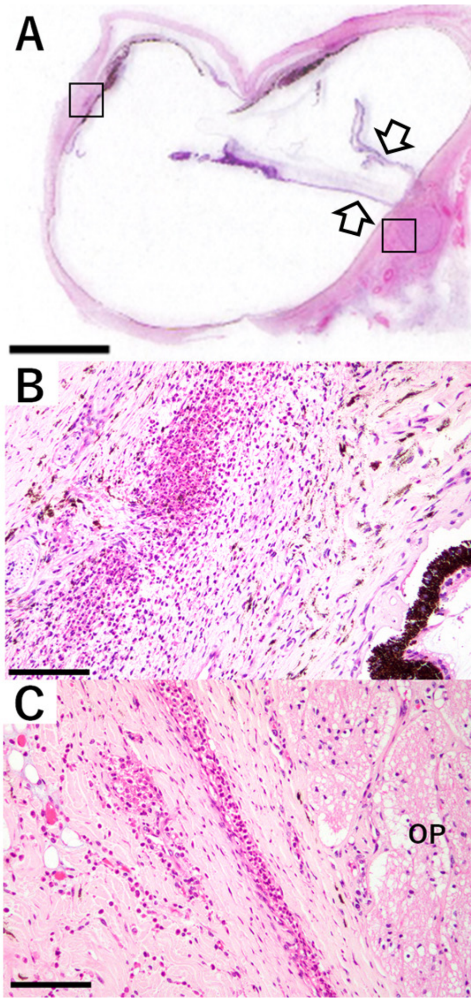

Fig. 2. Histopathology of the left eyeball. **(A)** Suppurative inflammation is evident in the entire ocular structure, and the detachment of the retina appears as a V-shaped structure (arrows). **(B)** A magnified view of the left upper square in image A shows uveitis accompanied by the infiltration of neutrophils. **(C)** A magnified view of the right bottom square in image A shows predominant inflammation in the perineurium of the optic nerve (OP). Hematoxylin and eosin (HE) staining; scale bars=500 µm (A), and 100 µm (B and C).

This animal exhibited bilateral ophthalmic signs, which were characterized macroscopically by strabismus and ultrasonographically by retinal detachment. The concurrent pattern was suggestive of MOD, even though this case was a crossbreed animal presenting with nystagmus, whose observations are inconsistent with MOD [1, 29].

In this case, critical CNS involvement was a possible cause for the observed bilateral strabismus, which was classified as the convergent type and referred to as esotropia [9]. Strabismus, esotropia, particularly, and nystagmus can be induced by hydrocephalus, which is associated with the decreased absorption of cerebrospinal fluid because of inflammation of the choroid plexus [9, 13, 30]. This case might have involved the terminal stage of sepsis, as indicated by the presence of encephalitis and secondary hydrocephalus, which are typically the most deeply diffused lesions associated with sepsis [6, 14]. However, distinguishing between strabismus and nystagmus caused by septic CNS involvements and those caused by congenital ocular diseases can be challenging, as the presentations of these symptoms are similar [1, 9, 13, 19, 29, 30]. Thus, the presence of CNS symptoms should prompt careful observation of any abnormal behaviors (such as decreased vitality and reduction of suckling reflex, as were observed in this case) and the evaluation of concurrent intraocular abnormalities [19].

Ocular ultrasonography is an essential ophthalmic examination, which can be performed to differentiate between various ophthalmic diseases in human medicine, including endophthalmitis [5, 7]. Based on ultrasonographic evidence, endophthalmitis can be classified into five types: anterior and posterior focal infections, anterior and posterior diffuse infections, and panophthalmitis

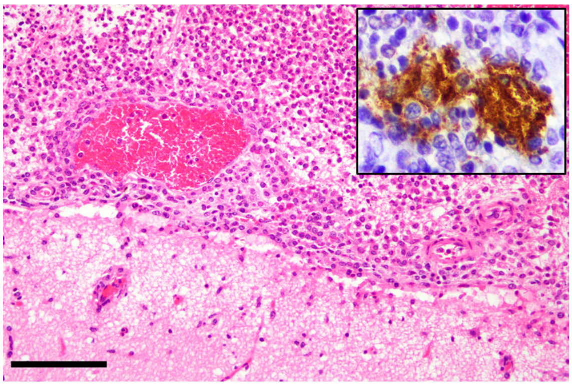

Fig. 3. Histopathology of the cerebrum. A marked inflammatory reaction is evident on the surface of the cerebrum. Hematoxylin and eosin (HE) staining; scale bar=100 µm. Insertion shows a positive reaction by immunohistochemistry with anti-*Escherichia coli*-specific antibodies.

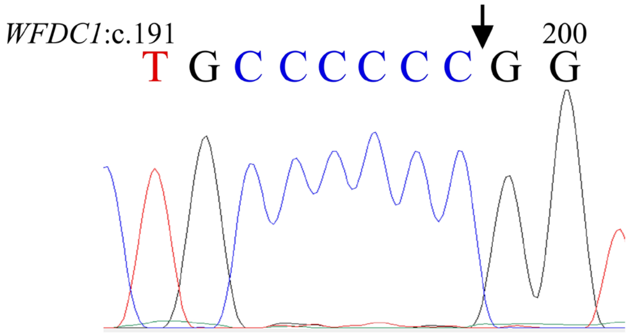

Fig. 4. Partial sequence (c.191_200) of the bovine *WFDC1* gene in the affected animal. The arrow shows the location of the c.198_199insC mutation, which causes bovine multiple ocular defects. This sequence appears to be wild-type.

[7]. Applying Christensen's typing to veterinary medicine, the anterior infection type (local or diffuse) and panophthalmitis type have been observed commonly in the feline and equine cases diagnosed as endophthalmitis by ocular ultrasonography, respectively [4, 26]. Additionally, retinal detachment was reported to be concurrently evident on ultrasonograms, indicating severe endophthalmitis in both the feline and equine cases [4, 26]. Retinal detachment, referred to as a "seagull sign", could be detected in 18% of the eyes affected by endophthalmitis in human patients [8]. These reports from human and veterinary medicine indicate that ocular ultrasonography would be useful for detecting retinal detachments in this case.

In ruminants, hypopyon, which is an anterior chamber infection type, has previously been diagnosed based on the ultrasonographic detection of heterogeneously increased echogenicity within the anterior chamber in 16% of ovine and caprine cases diagnosed with ophthalmic diseases [12]. An anterior infection type associated with a penetrating corneal wound has also been ultrasonographically observed in 23% of camel cases [11]. However, panophthalmitis may occur in most newborn calves with ophthalmic diseases because this type is commonly caused by sepsis [6, 14]. This case also involved the panophthalmitis type, in which the infectious lesions were spread over all intraocular structures, as observed at autopsy.

On the ocular ultrasonograms obtained from trans-corneal scanning using a 7.5 MHz linear transducer, the typical pattern associated with the panophthalmitis type could not be identified, as represented by a diffuse hyperechogenicity in the posterior segment, although the "seagull sign" of the detached retina could be identified in both eyes of this case [7, 8, 15, 20, 22, 23]. This could make it difficult to diagnose bilateral endophthalmitis, and could result in confusion in terms of distinguishing between congenital and acquired ocular diseases. The frequency of ultrasound wave used in this case was at the recommended level (>7.0 MHz) for examining intraocular structures of large animals [26] but might be too low to enable visualization of minute hyperechoic deposits within the vitreous humor [7, 8]. The use of ultrasound transducers employing wave frequencies of ≥10 MHz is recommended because enable high-quality image resolution, leading to the identification of <1-mm-sized structures [26]. Such minute intraocular lesions may include vitreous hemorrhage, ultrasonographically resembling vitreous inflammatory deposits, and vitreous membranes and posterior vitreous detachments, represented by membranous structures floating inside the vitreous cavity [15, 20, 21, 23]. Ultrasonographic findings of the persistent hyaloid artery may rarely resemble those of the retinal detachment [22]. Ocular ultrasonography can be helpful for the diagnosis of bovine endophthalmitis, if accurate discrimination of these intraocular lesions is possible.

In our case, topical corneal anesthesia was not used for the trans-corneal scanning although ultrasound gel was applied; however, care was taken to operation the ultrasound transducer gently. Trans-corneal scanning without topical anesthesia is similar to previously reported methods conducted for echobiometric assessments of bovine eyes [25, 28]. Also, the use of an ultrasound gel can prevent accidental trauma within the surface of the cornea, to which the ultrasound transducer is applied [28]. Regardless of the use of an ultrasound gel, application of a topical anesthetic drug to the cornea is recommended, which may prevent corneal damage [10, 11, 16], and which may control the movements of the eyeball triggered by the transducer. Compared with the trans-corneal technique, application of the transducer to the surface of the eyelid, as an alternative scanning method [10, 16] does not entail a risk of corneal injury. However, based on our experience, this trans-palpebral technique may make it difficult to perform the scanning accurately because of the movements of the eyelid during examination [23]. General anesthesia and sedation have not been commonly used for the ruminants when examined by ocular ultrasonography [10–12, 16, 17, 25, 28]. Examination with uses of general anesthesia and sedation may make it difficult to provide the diagnosable image including the optimal axis (the lens-vitreous body-optic disc) [16]. Additionally, this method is not suitable for the kinetic evaluation, helpful for differentiation of the vitreous membranous lesions in human medicine [21].

The prognosis is commonly poor in calves diagnosed with endophthalmitis, as this typically represents the terminal stage of systemic bacterial spread [6, 14]. Thus, the diagnosis should be conducted as early as possible [14]. The isolation of pathogens from the aqueous and vitreous humors, cerebrospinal fluid, and blood specimens is strongly indicative of bacterial endophthalmitis and encephalitis in human cases [5, 7] and in bovine cases [14]. The abnormality of the ophthalmic findings, both macroscopically and ultrasonographically, appeared to represent supplemental support for the diagnosis of such diseases based on laboratory examinations. Additionally, ocular ultrasonography can provide guidance for aqueous and vitreous paracentesis [27].

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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