Optic pathway degeneration in Japanese black cattle

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ABSTRACT. Degeneration of the optic pathway has been reported in various animal species including cattle. We experienced a case of bilateral optic tract degeneration characterized by severe gliosis in a Japanese black cattle without any obvious visual defects. To evaluate the significance, pathological nature and pathogenesis of the lesions, we examined the optic pathway in 60 cattle (41 Japanese black, 13 Holstein and 6 crossbreed) with or without ocular abnormalities. None of these animals had optic canal stenosis. Degenerative changes with severe gliosis in the optic pathway, which includes the optic nerve, optic chiasm and optic tract, were only observed in 8 Japanese black cattle with or without ocular abnormalities. Furthermore, strong immunoreactivity of glial fibrillary acidic protein was observed in the retinal stratum opticum and ganglion cell layer in all 5 cattle in which the optic pathway lesions could be examined. As etiological research, we also examined whether the concentrations of vitamin A and vitamin B12 or bovine viral diarrhea virus (BVDV) infection was associated with optic pathway degeneration. However, our results suggested that the observed optic pathway degeneration was probably not caused by these factors. These facts indicate the presence of optic pathway degeneration characterized by severe gliosis that has never been reported in cattle without bilateral compressive lesions in the optic pathway or bilateral severe retinal atrophy.
KEY WORDS: axonal degeneration, gliosis, Japanese black cattle, optic pathway degeneration

Degeneration of the optic pathway, such as the retina, optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiation and cortex of the occipital lobe, has been reported in various animal species and humans [3, 7, 11–19, 22–25, 27, 29–32]. Vitamin A deficiency [14, 23, 24, 37], vitamin B12 deficiency [3, 15, 16, 18, 29, 30], injury to the retina and/or optic nerve [13, 27], trauma [22], optic neuritis [24, 37] and compression of the optic nerve by intraorbital or intracranial neoplasia [24, 37] are all potential causes of optic pathway degeneration.

In calves, vitamin A deficiency causes optic canal stenosis by including developmental bone anomalies and eventually leads to degeneration of the optic chiasm due to compression [5, 33, 34, 37]. In addition, reducing the vitamin A consumption of beef cattle, such as Japanese black (JB) cattle, in order to improve the quality of their meat has been demonstrated to cause visual impairment [2, 6, 33].

In cattle, congenital bovine viral diarrhea virus (BVDV) infection causes ocular lesions, including retinal dysplasia and hypoplasia of the optic nerve [8, 37].

We experienced a case of bilateral optic tract degeneration characterized by gliosis in a JB cattle without any obvious visual deficits or ocular lesions. However, we could not clarify the animal's pathological condition. Thus, in this study, we examined the optic pathway of cattle with or without ocular abnormalities to evaluate the significance, pathological nature and pathogenesis of the lesions. In addition, we also examined whether vitamin A or vitamin B12 deficiency or congenital BVDV infection is associated with such conditions.

MATERIALS AND METHODS

Animals: A total of 60 cattle with or without ocular abnormalities were used in this study (Tables 1 and 2). All of the cattle were euthanized and necropsied. Then, their tissues were subjected to histopathological examinations. The examined animals included 41 JB (20 males, 20 females and 1 castrated male), 13 Holstein (Hol; 2 males and 11 females) and 6 crossbreed cattle (Japanese black x Holstein: F1; 3 males and 3 females). None of the animals developed optic canal stenosis. The animals' ages and sexes are listed in Tables 1 and 2. The sample collection methods and necropsy procedure were approved by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine.

Pathological examination: In addition to the major organs including the liver, spleen, kidneys, heart and lungs, we examined the bilateral retina, optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiation and cortex of the occipital lobe as much as possible.

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Table 1. Summary of the Japanese black cattle cases

Case No.	Age*	Sex**	Major clinical diagnosis (symptoms)	Ocular abnormalities	Optic pathway lesions
No. 1	1 d	F	Ectopia cordis	–	–
No. 2	1 d	F	Opisthotonos	–	–
No. 3	2 d	F	Skeletal malformation	–	–
No. 4	4 d	M	Weak calf syndrome	–	–
No. 5	10 d	M	Brain abscess	–	–
No. 6	12 d	F	Skeletal malformation	–	–
No. 7	15 d	M	Cardiac malformation	–	–
No. 8	18 d	M	Bleeding tendency	–	–
No. 9	19 d	M	Diarrhea	–	–
No. 10	20 d	M	Skeletal malformation	–	–
No. 11	44 d	F	Funisitis	–	–
No. 12	1 m	M	Claudin-16 deficient	–	–
No. 13	1 m	M	Convulsions	–	–
No. 14	1 m	M	Enteritis	–	–
No. 15	1 m	F	Astasia, keratitis	Keratitis (L)	+
No. 16	2 m	M	Astasia, intraocular abscess (R)	Intraocular abscess (R)	+
No. 17	2 m	M	Diarrhea	–	–
No. 18	2 m	M	Weak calf syndrome	–	–
No. 19	2 m	F	Hydrocephalus	–	–
No. 20	3 m	M	Peritonitis	–	–
No. 21	3 m	F	Pneumonia	–	–
No. 22	5 m	F	Pneumonia	–	–
No. 23	5 m	F	Claudin-16 deficient	–	–
No. 24	6 m	F	Bloat	–	–
No. 25	6 m	F	Brain abscess	–	+
No. 26	8 m	M	Pneumonia	–	–
No. 27	8 m	F	Encephalitis	–	–
No. 28	9 m	M	Claudin-16 deficient	–	+
No. 29	9 m	M	Growth retardation	–	–
No. 30	9 m	c-M	Bloat	–	–
No. 31	11 m	M	Urethral calculi	–	–
No. 32	1 y 1 m	M	Claudin-16 deficient	–	–
No. 33	1 y 3 m	F	Myositis	–	–
No. 34	1 y 9 m	M	Astasia	–	+
No. 35	1 y 10 m	M	Urethral calculi	–	–
No. 36	2 y 8 m	F	Bloat	–	–
No. 37	2 y 8 m	F	Bloat	–	–
No. 38	5 y	F	Fat necrosis	–	–
No. 39	8 y	F	Keratitis	Nonsuppurative endophthalmitis	+
No. 40	14 y	F	Dilated pupil, keratoconjunctivitis	Keratoconjunctivitis	+
No. 41	20 y	F	No significant lesions	–	+

Absent, –; present, +. *d, day (s); m, month (s); y, year (s). **M, male; c-M, castrated male; F, female. L, left side; R, right side.

For the histopathological examinations, brain tissue and other organs, such as the eye and optic nerves, were fixed in 15% neutral buffered formalin and processed by routine procedures for paraffin embedding. Five-micrometer-thick paraffin sections were stained with hematoxylin and eosin (HE). In addition, sections of the optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiation and cortex of the occipital lobe were also stained with Luxol fast blue-hematoxylin eosin (LFB-HE).

Sections of the abovementioned structures were also subjected to immunostaining using a streptavidin-biotin peroxidase complex method (Histofine SAB-PO kit; Nichirei, Tokyo, Japan). The following primary antibodies were used:

mouse monoclonal anti-neurofilament antibodies (Dianova-Immunotech, Hamburg, Germany) and rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) antibodies (Dako Cytomation, Kyoto, Japan). The reactions were visualized using 3′3-diaminobenzidine (Nichirei), and the sections were counterstained with Mayer's hematoxylin.

Sera were collected at autopsy and then frozen at −30°C until use.

Assessment of astrogliosis in the optic pathway using GFAP immunostaining: For evaluation of the optic pathway (optic nerve, optic chiasm, optic tract, lateral geniculate body, optic radiation and cortex of the occipital lobe) for astrogliosis, we counted the number of GFAP-positive cells

Table 2. Summary of the Holstein and F1 cattle cases

Holstein cattle	Age*	Sex**	Major clinical diagnosis (symptoms)	Ocular abnormalities	Optic pathway lesions
No. 1	4 d	F	Astasia	–	–
No. 2	5 d	F	Cardiac malformation, keratitis (B)	Keratitis (B)	–
No. 3	5 d	F	Cerebellar disorder	–	–
No. 4	17 d	F	Cerebral disorder	–	–
No. 5	22 d	M	Keratitis (L)	Keratitis (L)	–
No. 6	22 d	F	Torticollis, strabismus (L)	–	–
No. 7	1 m	M	Enteritis	–	–
No. 8	1 m	F	Diarrhea	–	–
No. 9	1 m	F	Opisthotonos, keratitis (R)	Keratitis (R)	–
No. 10	2 m	F	Pneumonia	–	–
No. 11	3 m	F	Renal failure	–	–
No. 12	5 m	F	Pneumonia	–	–
No. 13	1 y 3 m	F	Keratitis (L)	Keratitis (L)	–
F1 cattle					
No. 1	1 d	F	Skeletal malformation	–	–
No. 2	1 m	F	Pneumonia	–	–
No. 3	1 m 15 d	M	Renal failure	–	–
No. 4	3 m	F	Otitis media	–	–
No. 5	3 m	F	Otitis media	–	–
No. 6	9 m	M	Bloat	–	–

Absent, –; present, +. *d, day (s); m, month (s); y, year (s). **M, male; F, female. L, left side; R, right side; B, both sides.

in 3 randomly selected areas (using a x40 objective lens) and scored the samples according to the mean number of GFAP-positive cells detected: 0 to 0.2 =–, 0.3 to 1.0 =±, 1.1 to 10.0 =+, 10.1 to 20.0 =++, 20.1 ≥+++.

For evaluation of gliosis in the retina of 5 affected JB cattle (Nos. 15, 16, 25, 39 and 40) that could be examined, we evaluated the intensity of GFAP immunoreactivity at the nerve fiber layer, astrocytes and Müller cells. For comparison, 2 unaffected JB cattle (Nos. 12 and 23) were used as controls.

Measurement of vitamin A and vitamin B12 concentrations: The serum vitamin A (IU/dl) and vitamin B12 (pg/ml) concentrations of the affected and unaffected JB cattle were examined by Daiichi Kishimoto at their clinical examination center (Obihiro, Japan). The serum vitamin A concentrations of 8 affected JB cattle (Nos. 15, 16, 25, 28, 34, 39, 40 and 41) and 11 unaffected JB cattle (Nos. 2, 3, 7, 17, 18, 20, 31–33, 36 and 37) were measured, as were the serum vitamin B12 concentrations of 7 affected JB cattle (Nos. 15, 16, 25, 28, 39, 40 and 41) and 11 unaffected JB cattle (Nos. 2, 3, 7, 17, 18, 20, 31–33, 36 and 37).

The results are expressed as mean ± standard deviation values. The significance of any difference was determined using the two-sided Student's t test. P-values of <0.05 were considered to be significant.

Detection of the BVDV gene: We attempted to detect the BVDV gene in order to determine whether BVDV infection is involved in optic pathway degeneration.

To detect BVDV, sample RNA was extracted from the serum samples collected from the affected JB cattle with optic pathway degeneration (Nos. 15, 16, 25, 28, 39 and 40) using a QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Each sample was processed using the single-tube

RT-PCR method to detect the BVDV gene using a Gene-Amp® EZ rTth RNA PCR Kit (Applied Biosystems, Foster City, CA, U.S.A.), according to the method described in a previous report [36]. As a positive control, a 2 years old Holstein cow with mucosal disease was used.

RESULTS

Cases with optic pathway degeneration: Among the 60 cattle examined, bilateral degenerative changes in the optic pathway were only observed in 8 JB cattle (Nos. 15, 16, 25, 28, 34, 39, 40 and 41). The affected animals ranged in age from 1 month old to 20 years old and included 3 males and 5 females. Each of the affected animals was from a different farm. Of the 8 JB cattle with optic pathway degeneration, 4 JB cattle (2 cases with keratitis, Nos. 15 and 39; 1 case with intraocular abscess, No. 16; 1 case with keratoconjunctivitis, No. 40) had ocular lesions, and 4 JB cattle (Nos. 25, 28, 34 and 41) did not. Two of the 8 JB cattle with unilateral ocular lesions (No. 15, keratitis (L); No. 16, intraocular abscess (R)) also had bilateral optic pathway degeneration. Moreover, 33 of the 41 JB cattle without ocular lesions did not exhibit optic pathway degeneration.

Of the 13 Hol cattle, 4 Hol cattle (Nos. 2, 5, 9 and 13) had ocular lesions, and 9 Hol cattle did not. Optic pathway degeneration was not observed in any of the Hol cattle. In addition, optic pathway degeneration was not observed in 6 F1 cattle without ocular lesions.

Histopathological findings: The details of the optic pathway degeneration observed in the 8 JB cattle are as follows. Lesions similar to those observed in the optic pathway were not observed in other regions of the central nervous system. However, slight to mild perivascular inflammatory cell cuff-
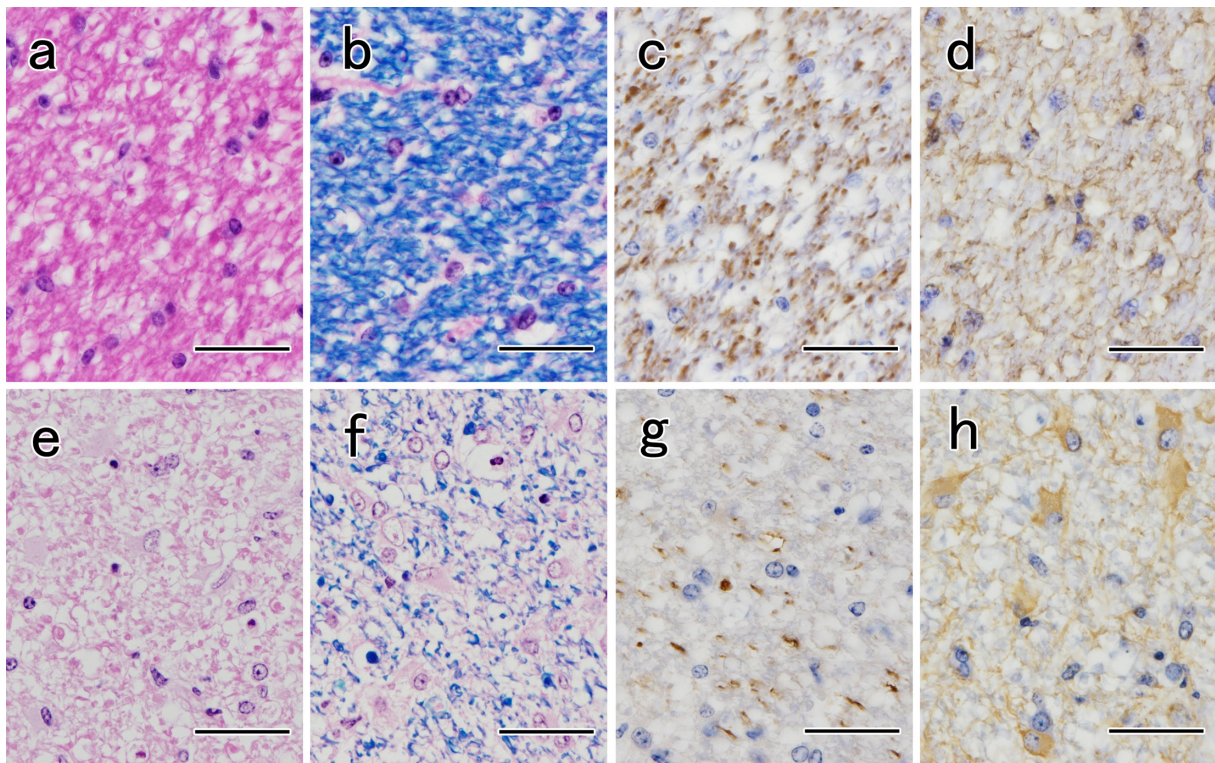
Fig. 1. (a, b, c, d) Histological and immunohistological findings of the optic tract in a Japanese black cow (No. 23) without optic pathway degeneration (control). (e, f, g, h) Histological and immunohistological findings of the optic tract in a Japanese black bull (No. 16) with optic pathway degeneration. (e) Astrogliosis is evident in the HE-stained sections. Bar=25 μm. (f) Decrease in myelin sheath density, formation of myelin ovoid and macrophage infiltration are observed. LFB-HE. Bar=25 μm. (g) Anti-neurofilament immunohistochemistry demonstrates a reduction in axon density. Bar=25 μm. (h) Anti-GFAP immunohistochemistry detected an increased number of GFAP-positive astrocytes. Bar=25 μm.

ing was observed in regions of the central nervous system outside of the optic pathway in 4 of the affected JB cattle (Nos. 25, 34, 39 and 40). Of these, two affected JB cattle had central nervous system lesions (No. 25, a brain abscess limited to right ventricle; No. 39, nonsuppurative meningo-encephalitis).

Optic nerve, optic chiasm and optic tract: The degenerative changes involved diffuse axonal degeneration and astrogliosis affecting the bilateral optic nerve, optic chiasm and optic tract (Fig. 1e). The extent of astrogliosis was determined as moderate to severe based on immunohistochemical staining of GFAP (Fig. 1h and Table 3).

Moreover, macrophage infiltration was also observed in such lesions. In LFB-HE-stained sections, loss of myelin and myelin ovoid formation were noted in the same areas (Fig. 1f). In addition, the axon density, which was evaluated using anti-neurofilament immunohistochemistry, was also decreased in these regions (Fig. 1g). None of the affected animals had histological lesions of fibrosis at the optic nerve, optic chiasm and optic tract.

Lateral geniculate body, optic radiation and cortex of the occipital lobe: Among the 8 JB cattle that exhibited degenerative changes in the optic nerve, optic chiasm and optic tract, slight histological changes were detected in the
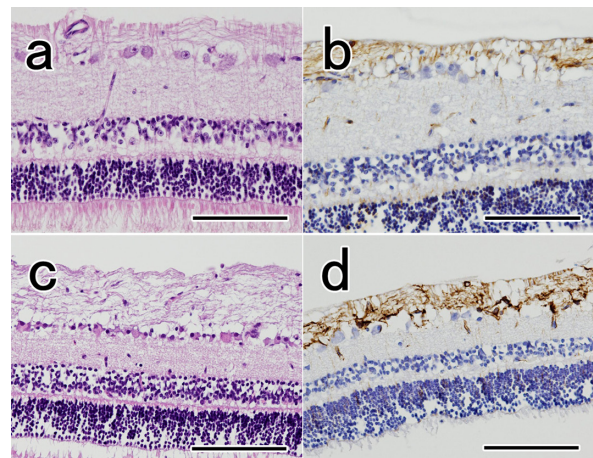

Fig. 2. (a, c) Histological findings of the retina in a Japanese black cow without optic pathway degeneration (No. 23) (a) and a Japanese black bull with optic pathway degeneration (No. 16) (c). No significant changes are observed. HE. Bar=50 μm. (b, d) Immunohistological evaluation of the retina in a Japanese black cow (No. 23) (b) and a Japanese black bull (No. 16) (d). Strong GFAP immunoreactivity is detected in the stratum opticum and ganglionic cell layer in No. 16 compared with No. 23. Anti-GFAP immunohistochemistry. Bar=50 μm.

Table 3.   Astrogliosis in the optic pathway as evaluated by GFAP immunohistochemistry

Case No.	Optic nerve		Optic chiasm	Optic tract		Retina	
	L	R		L	R	L	R
No. 15	++*	++	++	+++	+++	+[a]	+[a]
No. 16	++	++	NA	++	++	+[a]	NA
No. 25	+++	NA	+++	+++	+++	+[a]	NA
No. 28	NA	NA	NA	NA	+++	NA	NA
No. 34	+++	NA	+++	+++	+++	NA	NA
No. 39	+++	+++	+++	+++	+++	+[b]	+[b]
No. 40	+++	+++	+++	+++	+++	+[b]	+[b]
No. 41	NA	NA	NA	+++	NA	NA	NA

*Moderate, ++; severe, +++. +[a], Strong GFAP immunoreactivity was detected in the stratum opticum and ganglionic cell layer., +[b], Strong GFAP immunoreactivity was detected from the stratum opticum to the outer granular layer. To assess the presence or absence of astrogliosis in the optic nerve, optic chiasm and optic tract, we counted the numbers of GFAP-positive cells (x40 objective lens) and scored each sample according to the mean number of GFAP-positive cells observed: 0 to 0.2 =-, 0.3 to 1.0 =±, 1.1 to 10.0 =+, 10.1 to 20.0 =++, 20.1 ≥ +++. L, left side; R, right side; NA, not available.

optic radiation. Although gemistocytic astrocytes were not detected in the bilateral optic radiation in the JB cattle with optic pathway degeneration, a mild increase in the number of GFAP-positive cells was observed. However, we did not detect any significant changes in the lateral geniculate body and cortex of the occipital lobe in the JB cattle with optic pathway degeneration in the applied methodology.

Retina: We could examine the retina in 5 of the 8 cases (Nos. 15, 16, 25, 39 and 40). No significant changes in retinal structure were detected in 3 of the 5 cases in which HE staining-based histological evaluations could be carried out (Nos. 15, 16 and 25). However, in one of these cases (case No. 16), the retinal structures of the animal's right side had disappeared due to intraocular abscess formation. Bilateral plasma cell infiltration around the retinal capillaries was noted in the remaining 2 cases (Nos. 39 and 40). None of the affected animals exhibited histopathological changes, such as loss of the photoreceptor cell, degeneration of the photoreceptor cell and retinal pigment epithelial cells, retinal atrophy, and retinal dysplasia. Furthermore, none of the affected animals had histopathological changes of the optic disk cupping.

In addition, immunohistological evaluations detected strong immunoreactivity of GFAP in the stratum opticum and ganglionic cell layer in all of the abovementioned 5 JB cattle (Fig. 2d). Moreover, the strong immunoreactivity of GFAP was also detected in the inner and outer granular layers in 2 cases (Nos. 39 and 40) in which plasma cell infiltration was observed around the retinal capillaries. The GFAP immunoreactivity was weak in the retina of two JB cattle (Nos. 12 and 23) without optic pathway degeneration that were used as controls.

Vitamin A and vitamin B12 concentrations of Japanese black cattle with optic pathway degeneration: There were no significant differences in the concentrations of vitamin A or B12 between the JB cattle with and without optic pathway degeneration (Table 4).

Detection of the BVDV gene in Japanese black cattle with optic pathway degeneration: We attempted to detect the BVDV gene in 6 of the 8 JB cattle (Nos. 15, 16, 25, 28, 39 and 40) with optic pathway degeneration. However, the BVDV gene was not detected in the sera of any of these animals.

DISCUSSION

In the present study, we detected bilateral optic pathway degeneration in 8 of 41 Japanese black cattle. However, optic pathway degeneration was not observed in any of the examined Hol or F1 cattle. It is known that severe intraocular lesions can cause degenerative changes in the optic pathway [11, 12, 17, 25, 27, 28]. Among the cattle examined in this study, 4 of the 8 JB cattle that exhibited optic pathway degeneration also had ocular lesions, including 2 with keratitis, 1 with intraocular abscess and 1 with keratoconjunctivitis. Of these, the lesions were unilaterally observed in two animals. However, these animals also exhibited bilateral degenerative changes in the optic pathway. Thus, we considered that the optic pathway degeneration observed in these 4 JB cattle did not represent secondary changes due to such ocular lesions. In the present study, the remaining 4 cattle did not have any ocular problems, and the optic pathway degeneration observed in these cases (2 of 2 could be examined) was also bilateral. These facts may indicate the presence of optic pathway degeneration in JB cattle with little clinical significance.

As a central nervous system lesion outside the optic pathway, No. 25 had a brain abscess localized in the right frontal cerebral hemisphere. However, in this case, optic pathway degeneration characterized by severe gliosis was observed bilaterally in the same degree. Therefore, we considered that the optic pathway degeneration was not related to the brain abscess. No. 39 had a nonsuppurative meningoencephalitis. However, the degenerative change characterized by severe gliosis was observed as localized lesions in the optic pathway. Thus, we considered that the meningoencephalitis was not related to the optic pathway degeneration.

Optic pathway degeneration has been caused by numerous causes [1, 11, 22, 28, 37]. In various animal species and humans, inflammation of the optic pathway (optic neuritis);

Table 4. Vitamin A and vitamin B12 concentrations of Japanese black cattle

	Japanese black cattle with optic pathway lesions (n=8)*	Japanese black cattle without optic pathway lesions (n=11)**	*P*-value
Vitamin A (IU/d*l*)	51.8 ± 59.0	45.9 ± 44.3	NS
Vitamin B12 (*pg*/m*l*)	279.2 ± 114.9***	231.1 ± 188.0	NS

*Japanese black cattle with optic pathway lesions (Nos. 15, 16, 25, 28, 34, 39, 40 and 41). **Japanese black cattle without optic pathway lesions (Nos. 2, 3, 7, 17, 18, 20, 31-33, 36 and 37). ***n=7 (Nos. 15, 16, 25, 28, 39, 40 and 41). Values are shown as mean ± standard deviation (SD) values. NS, not significant.

glaucoma, in which retinal ganglion cells are affected by increased intraocular pressure; optic nerve trauma; and compression of the optic nerve have been listed as major causes of optic pathway degeneration [1, 11, 12, 17, 22–24, 28, 37]. Among the JB cattle examined in the present study, none of the animals exhibited histopathological changes that were suggestive of glaucoma, such as cupping of the optic disk. Similarly, the 8 JB cattle with optic pathway degeneration either did not display inflammation localized only in the optic pathway or traumatic or compressive lesions. Therefore, we considered that the optic pathway degeneration observed in the present study was probably not caused by such conditions.

In beef cattle, it has been known that the feeding method of limiting the amount of vitamin A supply improves the meat quality [6]. As a result of excessive limitation, vitamin A deficiency may be observed in herds [2, 33, 34, 37]. In vitamin A-deficient cattle, narrowing of the optic canal occurred due to bone hypoplasia and eventually caused compressive optic neuropathy [5, 11, 14, 34, 37]. However, this pathological condition was limited to cattle that were 2 years old or less [14]. Vitamin A deficiency also causes retinal degeneration in adult animals [2], and squamous metaplasia of the urinary, respiratory and/or gastrointestinal epithelia has been known to develop in such animals [2, 10, 14, 26, 33]. In the present JB cattle affected with optic pathway degeneration, these pathological findings including optic canal stenosis, retinal degeneration and retinal atrophy were not detected. In calves associated with maternal vitamin A deficiency, macroscopical examination of affected eyes revealed congenital ocular abnormalities, such as microphthalmos, ocular dermoids covering the external surfaces of the eyes and aphakia [4, 23]. Histopathological examination of these affected eyes revealed severe retinal dysplasia [4, 23]. These pathological changes were not observed in the present JB cattle affected with optic pathway degeneration. Furthermore, the mean serum vitamin A concentration of these animals was not significantly reduced. These results strongly suggest that vitamin A deficiency which has been reported was not involved in the optic pathway degeneration seen in the JB cattle in the present study.

In humans and nonhuman primates, it has been reported that vitamin B12 deficiency can cause bilateral optic neuropathy [3, 15, 16, 18, 29, 30]. Vitamin B12 deficiency also causes lesions including demyelination and spongiosis to develop in the central nervous system white matter of such species [3, 15, 16, 18, 29, 30]. However, the JB cattle that were affected by optic pathway degeneration in the present study did not exhibit such changes in their central nervous system nor was their mean serum vitamin B12 concentration significantly reduced. These facts clearly indicate that vitamin B12 deficiency was not related to the optic pathway degeneration observed in the JB cattle.

In cattle, it is known that congenital BVDV infection can cause ocular lesions, including retinal dysplasia and hypoplasia of the optic nerve [8, 37]. Thus, we also examined whether persistent infection of BVDV was involved in the optic pathway degeneration observed in the present study. However, the BVDV gene was not detected in any of the JB cattle with the optic pathway degeneration. Moreover, these pathological findings, including retinal dysplasia and hypoplasia of the optic nerve, were not detected in the present JB cattle affected with the optic pathway degeneration. Therefore, we considered that there is no close relationship between optic pathway degeneration and persistent infection of BVDV in JB cattle.

In sheep and cattle, *Helichrysum argyrosphaerum* can cause visual defects like myelin vacuolation of the optic nerve fiber and retinal degeneration [4, 35]. In these animals, spongiosis of the white matter of the brain is also observed [4, 35]. The pathological findings observed in the present JB cattle affected with the optic pathway degeneration differed from those of *Helichrysum argyrosphaerum* poisoning. Furthermore, all the present JB cattle with optic pathway degeneration were being kept at different farms. Also, animals which have kept in the same farms did not show symptoms suggestive of the *Helichrysum argyrosphaerum* poisoning. Therefore, we considered that poisoning caused by this plant was not related to the optic pathway degeneration observed in the JB cattle.

Among the JB cattle affected with the optic pathway degeneration in which it was possible to perform histological evaluations of the retina, strong GFAP immunoreactivity was detected in the stratum opticum and ganglion cell layer. Moreover, strong GFAP immunoreactivity was also seen in the inner and outer granular layers in the 2 JB cattle that exhibited retinal perivascular inflammatory cell cuffing. It has been reported that glial cells distributed in the stratum opticum and ganglion cell layer also stained positive with GFAP immunohistochemistry in the normal retina [9, 20]. Also, it is known that the GFAP immunoreactivity is increased in retinal and optic nerve injury [9, 20, 21, 38]. In the present study, strong GFAP immunoreactivity was observed at the retina in the JB cattle with optic pathway degeneration.

Therefore, the strong GFAP immunoreactivity in the retina may indicate the presence of retinal damage. However, the retinal areas covered by the present examination were restricted to small areas (one to two sections for each grove). Therefore, we could not verify the precise time-dependent changes and significance of the retinal damage in the present optic pathway degeneration.

In the present study, we could not identify the cause of the optic pathway degeneration in each case. Also, the ages of the affected animals varied from 1 month old to 20 years old. However, optic pathway degeneration characterized by severe gliosis has never been reported in cattle without bilateral compressive lesions in the optic pathway or bilateral severe retinal atrophy. Therefore, the present study indicates the presence of sporadically detected optic pathway degeneration in JB cattle. For an exact understanding of the clinical significance and the disease pathogenesis, further examination is required.

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