FULL PAPER *Pathology*

A Pathologic Study on Ocular Disorders in Calves in Southern Kyushu, Japan

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ABSTRACT. Of 822 calves, ranging in age between one day and six months necropsied between 1996 and 1998 at Miyazaki University, histological examination showed that 25 (3.0 %) had ocular lesions. These ocular lesions consisted of suppurative inflammation (13 cases), cataract (seven cases), and retinal atrophy (five cases). Inflammatory changes were classified as suppurative keratitis (one case), keratitis and uveitis (ten cases), and uveitis and retinitis (two cases). Cataract was subclassified into three categories; cortical (three cases), nuclear (one case), and mature (three cases). These lesions were characterized by degenerative changes in the lens fibers and the appearance of eosinophilic globules known as Morganian globules. In the most severely affected case, there was capsular rupture of the lens, resulting in severe infiltration by eosinophils and histiocytes of the whole anterior chamber. Almost all the calves with retinal atrophy had been suffering from severe hydranencephaly and three had significantly raised levels of neutralization antibodies for the Akabane and/or Aino viruses. This study indicates that congenital arbovirus infections may predispose calves to ocular diseases, especially retinal atrophy.—KEY WORDS: bovine, cataract, keratitis, retinal atrophy.

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It is known that various ocular disorders may occur in young calves. These can be caused by any of the following: congenital viral infections including Akabane or bovine viral diarrhea-mucosal disease (BVD-MD), nutritional defects such as hypovitaminosis A, hereditary factors, bacterial infections, trauma and several environmental factors [1, 5, 6, 12, 16, 22]. Most ocular diseases appear sporadically and cause relatively minor economic losses. However, an outbreak of bovine cataract caused by unknown factors has been reported [8]. Although there are many cases of bovine ocular disease, in only a few has the epidemiology and clinical pathology been studied and described.

In southern Kyushu, Japan, severe outbreaks of viral abortion and congenital abnormalities caused by the Akabane or Chuzan (Kasba) viruses among new-born calves have occurred during the past few years [11, 14, 17–19, 24]. Bistner *et al.* [3] attempted to develop experimental congenital BVD-MD infections by injecting the viruses into pregnant cows and reported that their calves had ocular abnormalities, including cataract and retinal atrophy, as well as cerebellar hypoplasia. Similar observations have been confirmed in several previous reports of BVD-MD infections [1, 22]. Even in experimental Akabane disease, one case of keratitis has been recorded [17], but the pathologic features were unclear. Despite numerous outbreaks of congenital arbovirus infections among calves in Japan, there is little information about ocular abnormalities in these animals.

In this study, 822 calves were examined to determine the incidence of various ocular diseases, and histopathologic changes were detected in 25 cases. This report describes the pathologic features of ocular diseases in these southern Kyushu calves and discusses their pathogeneses.

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MATERIALS AND METHODS

Calves: Gross examination was performed on a total of 822 calves, ranging in age between one day and 6 months. These calves were affected by various disorders and were necropsied at the Department of Veterinary Anatomy, Miyazaki University between September 1996 and November 1998. Forty-eight of the calves were examined histopathologically. These 48 calves comprised the following breeds: Japanese Black (JB; 35), Holstein-Friesian (HF; 11), and a cross between JB/ HF cross (two).

Histopathology: Eyes with the optic nerves attached were collected at necropsy and fixed in Methanol-Carnoy's solution for 1 hr. Connective tissues around the eyeball including the eyelid and glandular tissues, were then removed. The fixed eyeballs were sectioned laterally and refixed over night in Carnoy's solution. The eyes were dehydrated in 100% methanol for 12 hr, 100% methanol for 2 hr, and chloroform for 2 hr. The dehydrated eyes were then immersed in toluene and embedded in paraffin. The other organs except for the eyes were fixed in 10% formalin solution. Sections were stained with hematoxylin and eosin (HE).

Neutralization antibody assay: Precolostral sera and/or pleural effusion fluid were collected at necropsy. After centrifugation at 3,000 rpm, the supernatant was collected and stored at −20°C until use. A viral neutralization test was performed using the Aino virus (strain JaNar 28) and the Akabane virus (strain JaGar 39) at Miyazaki Livestock Hygiene Service Center.

RESULTS

Ocular lesions were demonstrated by histopathologic examination in 25 calves. The ocular lesions of these 25 cases were briefly classified into three categories; suppurative inflammation (13 cases), cataract (seven cases), and retinal atrophy (five cases). The pathologic lesions of each disorder were described as follows.

Suppurative inflammations: Suppurative inflammations was detected in 13 calves (Table 1). The lesions were classified as suppurative keratitis (one case), suppurative keratitis and uveitis (ten cases), and suppurative uveitis and retinitis (two cases). In suppurative keratitis, neutrophils had diffusely infiltrated the ulcerated cornea. Fibroblast and capillaries had proliferated in the superficial area of the cornea in some cases to a mild and in others to a moderate extent (Fig. 1). In eye showing suppurative keratitis and uveitis, neutrophils and a few lymphocytes had infiltrated the perivascular area of the uvea mildly to moderately with diffuse edema (Fig. 2). In four of the 11 calves with corneal lesions (cases 12, 27, 30, 47), neutrophils had also infiltrated both the anterior and posterior chambers. In three calves with severe suppurative fibrinous keratitis and uveitis (cases 22, 28, 37), corneas were markedly ulcerated and edematous. Neutrophils, a few lymphocytes, and plasma cells had infiltrated the perivascular area of the uvea. A large amount of fibrinous exudate was also present in the anterior chamber of these eyes. Of the 11 calves with corneal lesions, four showed bilateral and seven hemilateral suppurative (Table 1). In addition to ocular lesions, there was mild to moderate dilation of the lateral ventricles (five cases), cerebellar hypoplasia (two cases) and suppurative meningitis (one case) (Table 1).

Suppurative inflammation in the uvea and retina without corneal lesions was detected in two calves (cases 8 and 48). Histopathologic examination showed that there was mild to

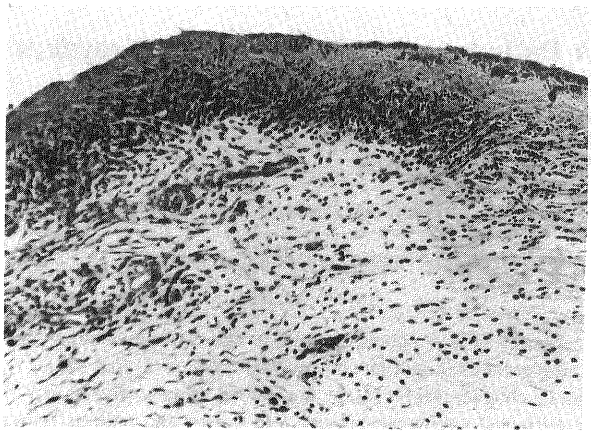

Fig. 1. Suppurative keratitis. Corneal ulceration with diffuse infiltration of neutrophils, edema, and proliferation of blood vessels. Case 11. HE stain. × 50.

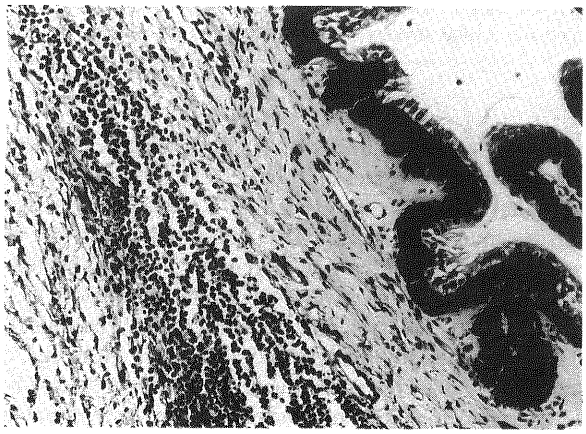

Fig. 2. Suppurative uveitis. Severe diffuse infiltration of neutrophils with fibrinous exudate in the ciliary body. Case 37. HE stain. × 50.

Table 1. Summary of calves with inflammatory ocular changes (n=13)

Histopathologic diagnosis	Case No.	Age (days)	*Breed	Localization	Major lesions beside eye	**Antibody titer
Suppurative keratitis *(n=1)*	11	14	HF	Right	–	N.E.
Suppurative keratitis and uveitis *(n=10)*	12	150	JB	Bilateral	Moderate hydrocephalus	N.E.
	16	2	JB	Right	Cerebellar hypoplasia	–
	22	7	HF	Bilateral	Mild hydrocephalus	N.E.
	27	12	JB	Bilateral	Suppurative meningitis	N.E.
	28	3	JB	Right	Moderate hydrocephalus	–
	30	1	JB	Left	Mild hydrocephalus	–
	37	3	JB	Bilateral	Cerebellar hypoplasia	–
	43	7	JB	Right	–	–
	45	33	HF	Right	Mild hydrocephalus	–
	47	4	HF	Left	–	AK>256
Suppurative uveitis and retinitis (n=2)	8	15	JB	Bilateral	Suppurative arthritis	–
	48	63	JB	Bilateral	Systemic mucor infection, suppurative arthoritis	N.E.

*Breed: HF; Holstein-Friesian, JB; Japanese Black.
**Antibody titer: N.E.; not examined, –; negative; AK; Akabane virus.

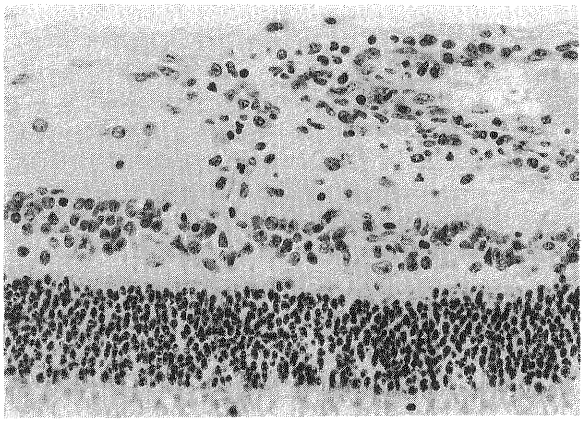

Fig. 3. Suppurative retinitis. Mild perivascular accumulation of lymphocytes and neutrophils in the stratum neurofibrarum. Case 48. HE stain. × 100.

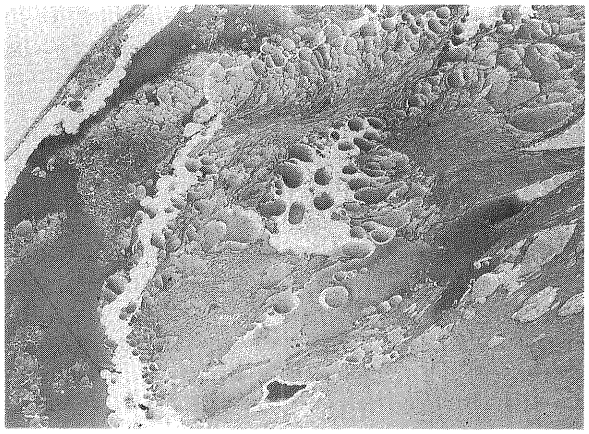

Fig. 4. Total cataract. Severe swelling and vacuolar changes of both cortical and nuclear lens fibers with a large number of Morganian globules Case 3. HE stain. × 12.

moderate infiltration of lymphocytes and neutrophils infiltrated in the perivascular area of the retina and uvea (Fig. 3). This was sometimes accompanied by perivascular edema and fibrinous exudate. The anterior chamber was filled with fibrinous materials and aggregated neutrophils. In the posterior chamber, neutrophils and a large amount of fibrin accumulated around the lens capsule and the adjacent lens showed mild degeneration. Both of these calves had suffered frequently from suppurative fibrinous acroarthritis and one (case 48) had also been exposed to systemic mucor infection (Table 1). In addition, *Staphylococcus sp.*, *Corynebacterium sp.*, and *Enterobacter cloacae* were isolated from the liver, lymph nodes, and lung of this case (case 48).

Cataracts: The diagnoses of cataract were based upon the irregular arrangement, swelling, and vacuolar changes to the lens fibers and the presence of a large number of Morganian globules of varying sizes (Fig. 4). The degenerative changes in the lens were classified into three categories of cataract; cortical, nuclear and mature. In cortical cataracts (cases 13, 24, 46), changes in the lens were localized to the cortex, but the nuclear area was intact. Conversely, the nuclear cataract detected in case 44 was characterized by severe swelling and vacuolar changes to the lens fibers in the nucleus. In the posterior area, there was marked edema of the posterior capsule and projection of the degenerating nuclear lens. In the mature cataract (cases 3, 10, 25), severe degenerative changes to the lens fibers were distributed in both the cortex and nucleus. In the most severely affected case (Case 3), capsular rupture of the anterior surface had resulted in severe infiltration by eosinophils and histiocytes into the anterior chamber and lens (Fig. 5). In this case, macrophages and eosinophils had also infiltrated the cornea, uvea and retina. Of the seven cases diagnosed as cataract, four calves had bilateral and three had hemilateral lesions. In addition to the ocular lesions, there was mild to severe dilation of the lateral ventricles in four cases (Table 2).

Table 2. Summary of calves with cataract *(n=7)*

Histopathologic diagnosis	Case No.	Age (days)	*Breed	Localization	Major lesions beside eye	**Antibody titer
Cortical cataract	13	4	JB	Right	–	N.E.
(n=3)	24	31	C	Right	–	N.E.
	46	1	HF	Right	–	N.E.
Nuclear cataract *(n=1)*	44	37	HF	Bilateral	Mild hydrocephalus	AK>256
Mature cataract	3	30		Bilateral	Mild hydrocephalus	–
(n=3)	10	3		Bilateral	Severe hydrocephalus	–
	25	25	JB	Bilateral	Severe hydrocephalus	AIN>256

*Breed: HF; Holstein-Friesian, JB; Japanese Black, C; cross breed between HF and JB.
**Antibody titer: N.E.; not examined, -; negative; AK; Akabane virus, AIN; Aino virus.

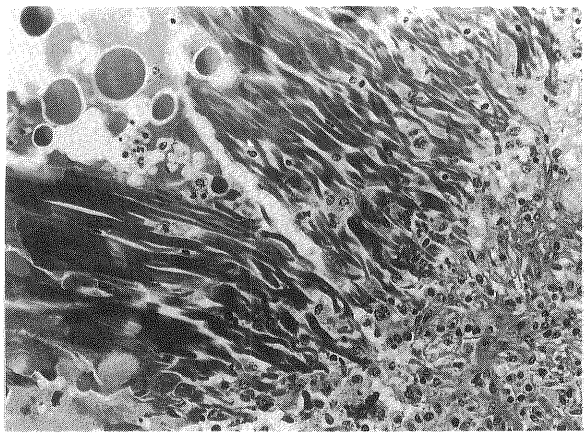

Fig. 5. Cataract with granulomatous inflammation. Degenerative lens fibers with severe infiltration of histiocytes and eosinophils. Case 10. HE stain. × 70.

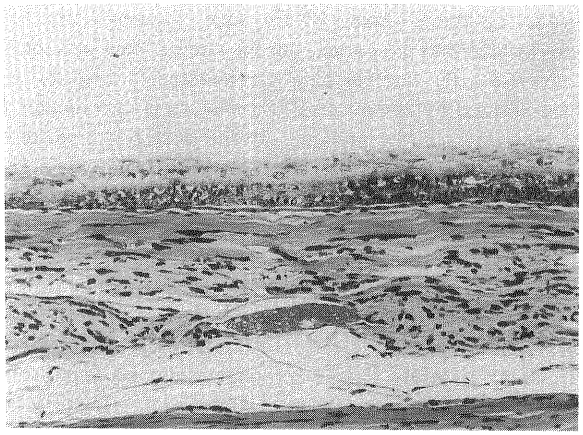

Fig. 6. Retinal atrophy. Severe diffuse hypoplasia and irregular cell-arrangement of whole layer of the retina. Case. 31. HE stain. × 50.

Retinal atrophy: Histopathologically, retinal atrophy was characterized by total thinning of the retina and irregular arrangement of the retinal cells (Fig. 6). The depletion of the inner and outer nuclear cell layer was prominent in the hypoplastic area. In some cases the rods and cones had disappeared completely. In the most severely affected cases, the sensory retina atrophy had caused glial scarring of the photoreceptor layer. Of these five cases, three had focal lesions mainly located in the posterior area of optic disc (cases 26, 32, 39) and two had diffusely extended lesions from the optic disc to the orbiculus ciliaris (cases 31 and 33).

Neutralization antibodies: The examination of precolostral sera and/or pleural effusion fluid collected at necropsy revealed a significant increase in neutralization antibodies for the Akabane virus in three cases (cases 33, 44, 47), for the Aino virus in one case (case 25) and for both the Akabane and Aino viruses in two cases (cases 26, 31). Of the cases with a high titer against the Akabane virus, one showed suppurative keratitis with uveitis, one showed nuclear cataract and three retinal atrophy. Of the cases with a high titer against the Aino virus, two each had total cataract and two had retinal atrophy. These results are shown in Tables 1–3.

DISCUSSION

Pathological and serological findings suggest a relationship between congenital arboviral infections and some ocular diseases, especially retinal atrophy. Interestingly, keratitis associated with arbovirus infections has been reported in affected calves with Akabane and BVD-MD viruses [3, 17]. In this study, suppurative keratitis was considered not to be caused by hematogenic infections, but by direct injury and bacterial infections. We considered that suppurative fibrinous keratitis and uveitis were a more progressive form of the disorder. Since a few calves with suppurative keratitis also exhibited hydrocephalus or cerebellar hypoplasia, rarely with an increase in neutralizing

Table 3. Summary of calves with retinal atrophy *(n=7)*

Histopathologic diagnosis	Case No. No.	Age (days) (days)	*Breed	**Localization	Major lesions beside eye	***Antibody titer
Retinal atrophy *(n=5)*	26	15	HF	N.E.	Severe hydrocephalus	AK>256, AIN>256
	31	42	JB	N.E.	Severe hydrocephalus	AK>256 AIN>256
	32	13	JB	N.E.	Severe hydrocephalus	–
	33	18	JB	N.E.	Severe hydrocephalus	AK>256
	39	118	JB	N.E.	Severe hydrocephalus	–

*Breed: HF; Holstein-Friesian, JB; Japanese Black.
**Localization: N.E.; not examined.
***Antibody titer: –; negative, AK; Akabane virus, AIN; Aino virus.

antibody for Akabane virus, congenital viral infections would appear to be a likely cause of keratitis in these cases. However, there was no evidence for this in most of keratitis cases in this study. Thus, congenital viral infections might have only a limited role on the pathogenesis of these suppurative ocular lesions. Aldbridge *et al.* [1] reported that of 25 calves with bacterial septicemia, three exhibited ocular signs, but histopathological examination was not performed. In our study, two calves with suppurative retinitis and uveitis without corneal lesions, suffered also from suppurative fibrinous arthritis, suggesting that these suppurative ocular changes might be caused by hematogenic bacterial infections. Although a case (case 48) also involved in systemic mucor mycosis, the ocular lesions were quite different from those of a case reported previously [24]. Since *Staphylococcus sp.*, *Corynebacterium sp.*, and *Enterobacter cloacae* were isolated from the liver, lymph nodes, and lung of this case, it seems most likely that the ocular lesions were associated with these bacteria rather than mucor infection.

Bovine cataracts caused by congenital BVD-MD infections, with inheritance as a simple autosomal recessive trait and nutritional disturbance, have been reported previously [3, 7, 11]. Bistner *et al.* [3] reproduced BVD-MD infection in three calves by infecting pregnant cows and described bilateral cortical cataract and retinal atrophy with cerebellar hypoplasia in all calves. Of the 7 calves diagnosed as having cataracts, four presented little evidence to indicate congenital viral infections. There have been several reports on inherited primary congenital cataracts in cattle [7, 9] as well as in other animals, such as mice [21], dogs [10], horses [2] and sheep [6]. It is possible that genetic factors may have played a role in the pathogenesis of the cataracts in these cases. Since a significant elevation of the neutralization antibody for the Akabane virus was found in one case of nuclear cataract (case 44), congenital Akabane infection might be cause of the cataract. A similar hypothesis for an association with congenital Aino virus infection might also be possible as the basis of ocular disease in case 25, which had a mature cataract with a high antibody titer for Aino virus. In addition, as all cases with nuclear or mature cataract also had mild to severe hydrocephalus, these types of cataract might be more closely associated with congenital arbovirus infections rather than cortical cataract.

Retinal atrophy is a common ocular abnormality in congenital BVD-MD infections [3, 15, 20]. It has been reported that the retinal lesions are characterized by atrophy of the photoreceptor cells with marked thinning of the inner and outer nuclear cell layer and a decrease in rods and cones [3, 15, 20]. It has been suggested that retinal atrophy is caused by BVD-MD infection during development of the retina. The facts that three of five calves with retinal atrophy had severe hydrocephalus and significant elevation of the neutralizing antibodies for either the Akabane or Aino viruses, might indicate that the retinal lesions in these calves were related to congenital infections with these viruses. On the other hand, there is little evidence for congenital viral infections as the basis of retinal atrophy in the remaining two cases. Since retinal atrophy has also been reported in calves with vitamin A deficiency [13, 23], these nutritional factors should also be considered as a background.

In conclusion, this study has indicated that congenital arbovirus infections might play a role in the pathogenesis of some bovine ocular diseases, especially retinal atrophy. Further studies of a larger number of cases will be needed to confirm this hypothesis. However, the current paper provides a sound basis for further study of the incidence and pathogeneses of ocular diseases in young calves in southern Kyushu, Japan.

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