Enterobacter cloacae
Escherichia
coli(O128)　　　　　　　　　　　　1

	The journal of veterinary medical science
ISSN	09167250
	, , , 　, , ,
	Japanese Society of Veterinary Science
/	69　4
	p. 445-448
	2007　4

A Neonatal Calf with Concurrent Meningoencephalitis by *Enterobacter cloacae* and Enteritis by Attaching and Effacing *Escherichia coli* (O128)

Yukio M. SEIMIYA[1], Makiko MURAKAMI[1], Maki TAKAHASHI[1], Koji SASAKI[1], Hiroshi MIYAZAKI[1] and Kenji KAWASHIMA[2]

[1]*Iwate Prefecture Central Livestock Hygiene Service Center, 390–5 Sunagome, Takizawa-mura, Iwate 020–0173 and* [2]*Tohoku Research Station, Environmental/Enzootic Diseases Research Team, National Institute of Animal Health, 31 Uminai, Shichinohe-machi, Aomori 039–2586, Japan*

ABSTRACT. A neonatal calf developed nervous symptoms followed by diarrhea. The principal pathological changes were fibrinopurulent meningitis with necrosis of the cerebral parenchyma, and attaching and effacing lesions in the intestine. Cerebral necrosis was frequently associated with vascular changes. *Enterobacter cloacae* was isolated from the brain and *Escherichia coli* (O128) from the intestinal content. These suggest that cerebral and intestinal lesions were caused by the isolated organisms, and that most necrosis of the cerebral parenchyma might be infarctive.
KEY WORDS: Attaching and effacing *Escherichia coli* (O128), *Enterobacter cloacae*, neonatal calf.

Bacterial meningitis in neonatal calves is virtually limited to calves less than two weeks of age [4] and usually preceded by septicemia [3]. The pathological changes are characterized by fibrinopurulent inflammation of the meninges, choroid plexuses, and ventricular walls with rare secondary involvement of the central nervous system (CNS) parenchyma [3]. The disease is most often caused by *Escherichia coli* [3, 9], and occasionally by *Pasteurella* [20], *Streptococcus pneumoniae* [9], *S. bovis* [19], or *Klebsiella oxytoca* [17]. Although meningitis [11] and meningoencephalitis [10, 12] of neonatal calves due to *Enterobacter cloacae*, an opportunistic pathogen in immunologically compromised animals and humans such as neonates [1, 16], have been also reported, the lesions of CNS parenchyma have not been sufficiently characterized.

Attaching and effacing *E. coli* (AEEC) strains are attached closely to intestinal epithelia and efface the microvilli, causing diarrhea in humans and animals [13]. AEEC infection in calves has been reportedly associated with *E. coli* strains classified into serotypes O5 [2, 14, 21], O15 [23], O26 [7, 11, 22], O111 [22], and O119 [22]. Although several strains of the serotype O128 are known to possess the *E. coli* attaching and effacing (*eaeA*) gene [5], there seem to be few reports describing AE lesions associated with the organism in calves. This study describes the pathological and bacteriological findings in a neonatal calf concurrently infected with *E. cloacae* and AEEC (O128).

A neonatal calf was affected on a dairy farm raising 25 Holstein-Friesian cows or calves in Iwate Prefecture. The calf was born at full term after dystocia in September 1999, and had made a little attempt to suck colostrum from the dam. Since birth, the animal had shown anorexia, depression, and difficulty in standing and walking, and impaired vision was indicated by the frequency of bumping its head against the wall. The body temperature was not examined. After worsening day by day, the animal developed swollen cubital joints and excreted loose feces with mucus at the age of eight days, and was euthanatized two days later. There was no medical treatment attempted during the disease course.

At necropsy, the principal lesions were confined to the CNS and digestive tract. There was an increased amount of cloudy cerebrospinal fluid along with dilation of the ventricles and mesencephalic aqueduct, especially both of the lateral ventricles. The meninges of the brain and spinal cord were congestive and cloudy with petechiae. On frontal sections of the CNS, necrotic foci ranging from 2 to 10 mm in diameter were scattered throughout the corpus striatum and thalamus, and occasionally in the cerebral parietal lobe. The small and large intestinal walls were flaccid with small amounts of fluid in the lumina. Mucus was found on the colonic and rectal mucosa, and blood-tinged mucus was present on the congestive cecal mucosa. Other findings were fibrinous exudate on the peritoneal surface, an increased quantity of cloudy synovial fluid in many limb joints, especially the cubital joints, and atrophic thymus.

Tissue blocks collected throughout the body were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of tissue were stained with hematoxylin and eosin (HE). Selected sections of the CNS and intestine were stained with Gram (Brown and Brenn method), phosphotungstic acid hematoxylin (PTAH), periodic acid Schiff (PAS), azan, Masson trichrome, and Weigert's fibrin as well as the streptavidin-biotin-peroxidase (SAB) method. The SAB method was applied using a SAB kit (Nichirei Co., Tokyo) and rabbit hyperimmune sera against *E. coli* O128 (Denka Seiken Co., Tokyo) or against *E. cloacae* [11]. Specimens from the ileum fixed in 10% buffered formalin were postfixed in 1% osmium tetroxide-phosphate buffer, embedded in epoxy resin, sectioned, and stained with uranyl acetate and lead citrate for transmission electron microscopy. The specimens of osmium fixed-ileum were dehy-

drated through graded alcohol solution, dried by the t-butyl alcohol freeze-drying method, mounted on stubs, and coated with gold for scanning electron microscopy.

Histologically, marked fibrinopurulent inflammation extended along the meninges from the cerebrum to the lumbar parts of the spinal cord. The changes consisted of congestion, perivascular hemorrhages, fibrinous exudate, and infiltration of neutrophils, macrophages, and lymphocytes. Involvement of the ventricular walls and choroid plexuses included occasional desquamation of the ependymal and epithelial cells with edema and mild infiltration of neutrophils and macrophages. There were small amounts of fibrinopurulent exudate in the ventricular lumina. Fibrinopurulent exudate was also found in the perineural tissues of the trigeminal ganglia and the subleptomeningeal spaces of the optic nerves. There was no apparent involvement of intraocular tissues.

Necrosis of the CNS parenchyma was rather clearly demarcated, and located at some distance from the ventricles in the corpus striatum and thalamus as well as smaller lesions at the cortico-medullary junction of the cerebral parietal lobe. The lesions consisted of loosening of neuropil, neuronal degeneration, perivascular fibrinopurulent exudate and hemorrhages, and infiltration of neutrophils and macrophages. These lesions were frequently associated with nearby vascular changes such as fibrinous thrombi and loosening or fibrinoid necrosis of the walls in small to middle sizes of vessels (Fig. 1-A, B). Swelling and proliferation of capillary endothelial cells were seen at the peripheral areas of several lesions. Gram-negative bacilli or *E. cloacae* antigens were present in and around macrophages and neutrophils infiltrating into the meninges (Fig. 1-C) and parenchyma. There were no *E. coli* (O128) antigens detected in the CNS.

Characteristic AE lesions were observed in the ileum and large intestine. Gram-negative bacilli were multifocally attached to the luminal surface of the intestinal epithelia, with higher frequency in the ileum. There were no bacilli associated with any cryptal epithelia in the intestinal mucosa. *E. coli* (O128) antigens showed a similar distribution (Fig. 2-A). The epithelia colonized by the bacilli were shrunken, showed increased eosinophilic affinity of the cytoplasm, and frequently followed by desquamation. The nuclei of degenerated epithelia were often pyknotic. Neutrophils multifocally infiltrated the superficial lamina propria. There was mucus in the dilated crypts in the colonic and rectal mucosa. The medullary sinus of the jejunal and cecal lymph nodes was dotted with foci of neutrophils.

Other findings included fibrinopurulent peritonitis, pleuritis, and epicarditis, hepatitis, and mucosal erosions of the omasum. There were no vascular changes such as thrombosis and degeneration of the vascular walls in any organs other than the CNS. None of the joints were examined histologically .

Scanning and transmission electron microscopical examinations of the intestinal epithelia in the ileum demonstrated that numerous bacilli were closely attached to the surface of epithelia with effacing microvilli. The plasma membranes formed cup-like invaginations and pedestal-like protrusions with accumulation of dense fibrillar materials beneath the attached bacteria (Fig. 2-B, C).

The brain, cerebrospinal fluid, liver, spleen, kidney, heart, lung, synovial fluid from the cubital joint, jejunal lymph node, and small intestinal content were cultured aerobically on tryptic soy agar supplemented with 5% sheep blood and DHL agar, or anaerobically on GAM agar using a gas generating kit (Oxoid Ltd., England) at 37°C for 48 hr. The isolates were identified using a commercial system
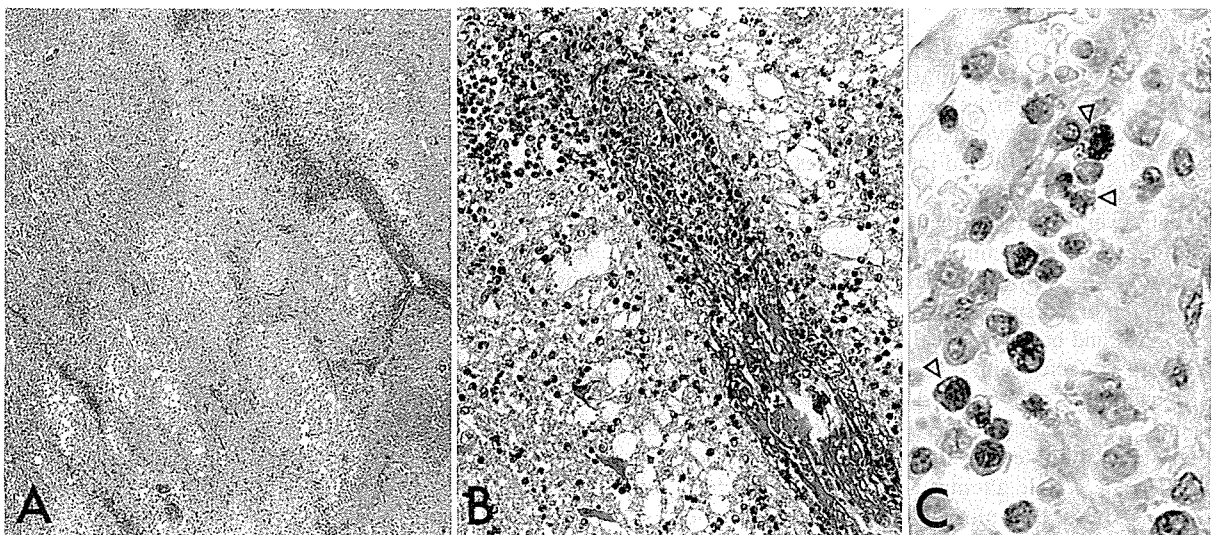

Fig. 1.   A) Focal necrosis associated with thrombosis is found in the thalamus. HE stain. × 33. B) Magnification of A) shows fibrinous thrombi, cellular infiltration, neuronal degeneration, and loosening of neuropil. HE stain. × 164. C) *E. cloacae* antigens (arrowheads) are present in macrophages and neutrophils infiltrating into the cerebral meninges. SAB method. × 660.
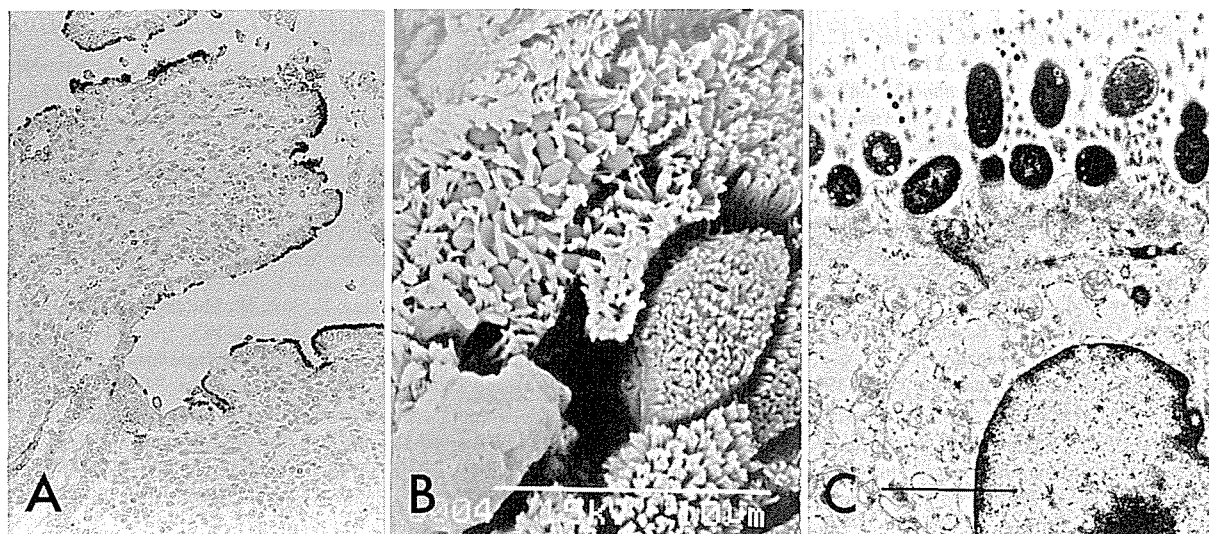
Fig. 2.   Ileal mucosa. A) Numerous *E. coli* (O128) antigens are attached to the epithelia covering the intestinal villi of ileum. SAB method. × 164. B) Scanning electron micrograph of intestinal epithelia. Heavy colonization by bacilli is found on the surface of epithelia with effacing microvilli. Bar=10 μm. C) Transmission electron micrograph of an intestinal epithelium. Numerous bacilli are closely attached to the plasma membrane forming cup-like invaginations and pedestal-like protrusions. Bar=2 μm.

(API 20E, bioMerieux, France). The isolated *E. coli* was examined by slide agglutination with rabbit hyperimmune sera against 43 serotypes of *E. coli* including O128 (Denka Seiken, Co., Tokyo) for serotype, and by polymerase chain reaction (PCR) for *eaeA*, heat-stable toxin (ST), heat-labile toxin (LT), and verotoxin (VT) genes as described elsewhere [8, 15].

Both aerobic and anaerobic cultures yielded *E. cloacae* from all materials except the liver, lung, and small intestinal content, and *E. coli* from the small intestinal content. The isolated *E. coli* was identified as the serotype O128, positive for *eaeA* and VT genes, and negative for ST and LT ones.

The pathological and bacteriological examinations of a neonatal calf with nervous symptoms and subsequent diarrhea demonstrated fibrinopurulent or purulent changes in the meninges and several other organs and isolation of *E. cloacae* from those lesions. The meningeal lesions were accompanied by parenchymal necrosis frequently associated with vascular changes. These findings suggest that meningitis was preceded by septicemia associated with *E. cloacae*, and that most necrosis of the CNS parenchyma might be infarctive.

The quality and distribution of the CNS lesions observed were closely similar to those in neonatal calves [18] demonstrating meningitis with cerebral infarct due to *E. coli*. However, there was a difference between those *E. coli*-infected calves and the present calf with respect to the appearance of vascular changes in organs other than the CNS. Changes were often present in the digestive tracts of the former calves, but not in any organs of the latter one. Necrosis of the CNS parenchyma has been found in two of three neonatal calves [10–12] with meningitis due to *E. cloacae* reported previously, and vasculitis and thrombosis

with *E. cloacae* antigens had been observed in one [10] of those calves. The previous findings appear to support the present speculation that several strains of *E. cloacae* might be able to cause vascular lesions followed by infarctive necrosis.

The CNS lesions in the present case might be able to be differentiated pathologically from thromboembolic meningoencephalitis due to *Histophilus somni*. Necrotic lesions with thrombosis and vasculitis in *Histophilus somni* infection are distributed throughout the brain and spinal cord [6], while those in the present calf were predominant in the corpus striatum and thalamus.

E. coli (O128) was isolated from the intestinal content of the present calf. The quality and distribution of the observed AE lesions were essentially identical to those [7, 11, 14, 21–23] due to AEEC of serotypes other than O128. The present finding suggests that several strains of *E. coli* (O128) might have the potentiality to induce AE lesions in calves.

Further studies will be needed to accumulate knowledge of the epidemiology and pathogenesis of *E. cloacae* or AEEC (O128) infection in neonatal calves.

REFERENCES

1. Biering, G., Karlsson, S., Clark, N. C., Jonsdottir, K. E., Ludvigsson, P. and Steingrimsson, O. 1989. *J. Clin. Microbiol.* **27**:

2054–2056.

2. Chanter, N., Morgan, J. H., Bridger, J. C., Hall, G. A. and Reynolds, D. J. 1984. *Vet. Rec.* **114**: 71.
3. Cordy, D. R. 1984. *Vet. Pathol.* **21**: 587–591.
4. Gay, C. C. 1971. *Ann. New York Acad. Sci.* **176**: 336–349.
5. Giammanco, A., Maggio, M., Giammanco, G., Morelli, R., Minelli, F., Scheutz, F. and Caprioli, A. 1996. *J. Clin. Microbiol.* **34**: 689–694.
6. Humphrey, J. D. and Stephens, L. R. 1983. *Vet. Bull.* **53**: 987–1004.
7. Iijima, T., Sueyoshi, M., Yamamoto, T., Yoshioka, K. and Nakazawa, M. 1990. *Jpn. J. Vet. Sci.* **52**: 1347–1350.
8. Ito, F., Ogino, T., Ito, K. and Watanabe, H. 1992. *Nippon Rinsho* **50**: 343–347 (in Japanese).
9. Jubb, K. V. F. and Huxtable, C. R. 1993. pp. 385–390. *In*: Pathology of Domestic Animals, vol. 1, 4th ed. (Jubb, K. V. F., Kennedy, P. C. and Palmer, N. eds.), Academic Press, New York.
10. Kitano, Y., Furukawa, M., Yamaguti, H., Yamashita, S. and Sasaki, Y. 1993. *J. Jpn. Vet. Assoc.* **46**: 22–25 (in Japanese with English abstract).
11. Kurogi, A., Yamamoto, S., Sueyoshi, M., Ohta, H., Tsuneyoshi, M., Matsuda, H. and Nakazawa, M. 1996. *J. Jpn. Vet. Med. Assoc.* **49**: 9–12 (in Japanese with English abstract).
12. Matsuda, M., Nakamura, K., Kondou, M., Mukai, J., Umeshita,

T. and Tada, K. 1988. *J. Jpn. Vet. Med. Assoc.* **41**: 433–435 (in Japanese with English abstract).
13. Moon, H. W., Whipp, S. C., Argenzio, R. A., Levine, M. M. and Giannella, R. A. 1983. *Infect. Immun.* **41**: 1340–1351.
14. Moxley, R. A. and Francis, D. H. 1986. *Infect. Immun.* **53**: 339–346.
15. Nakazawa, M. and Ito, K. 1995. *J. Jpn. Assoc. Infect. Dis.* **69**: 772–776 (in Japanese with English abstract).
16. Richard, C. 1984. pp. 465–469. *In*: Bergey's Manual of Bacteriology, vol. 1 (Krieg, N. R. ed.), Williams & Wilkins, Baltimore.
17. Seimiya, Y., Ohshima, K., Itoh, H., Murakami, R. and Haritani, M. 1993. *J. Vet. Med. Sci.* **55**: 141–143.
18. Seimiya, Y., Ohshima, K., Itoh, H., Ogasawara, N., Okutomo, M. and Tanaka, S. 1992. *J. Vet. Med. Sci.* **54**: 767–768.
19. Seimiya, Y., Ohshima, K., Itoh, H., Ogasawara, N., Okutomo, M. and Tanaka, S. 1992. *J. Vet. Med. Sci.* **54**: 871–874.
20. Shand, A. and Markson, L. M. 1953. *Br. Vet. J.* **109**: 491–495.
21. Tominaga, K., Nakazawa, M., Haritani, M. and Hirata, K. 1989. *J. Jpn. Vet. Med. Assoc.* **42**: 775–779 (in Japanese with English abstract).
22. Uno, K., Takesue, K., Futo, M. and Tani, H. 1992. *J. Jpn. Vet. Med. Assoc.* **45**: 650–654 (in Japanese with English abstract).
23. Wada, Y., Nakazawa, M. and Kubo, M. 1994. *J. Vet. Med. Sci.* **56**: 151–152.