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NOTE

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An abnormal birth in bovine suspected of being caused by Peaton virus first occurred in Shikoku region, Japan

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ABSTRACT. Peaton virus (PEAV) is a type of arthropod-borne virus (arbovirus) belonging to the genus *Orthobunyavirus*, much like Akabane virus and Aino virus. These arboviruses cause stillbirth and congenital malformations of fetuses in ruminants. In Japan, abnormal birth in bovine caused by PEAV were reported in Okinawa, Kyushu, and Chugoku regions, but it has never been reported in Shikoku region. The abnormal birth occurred in 2020 in Ehime Prefecture (Shikoku region) and suspected of being caused by PEAV from results of clinical signs, pathological findings, and virus neutralization test using PEAV. However, PEAV was not detected and isolated. This report describes the case of abnormal birth in bovine suspected of being caused by PEAV first occurred in Shikoku region, Japan.

KEY WORDS: abnormal birth, arbovirus, *Orthobunyavirus*, Peaton virus, Shikoku region

Peaton virus (PEAV) belongs to the order *Bunyavirales*, family *Peribunyaviridae*, genus *Orthobunyavirus*, Simbu serogroup, and is a negative-sense, single-stranded, enveloped RNA virus. PEAV was initially isolated from *Culicoides brevitarsis* and bovine blood in 1976 in Australia [14]. In Japan, PEAV was isolated from the blood of sentinel cattle in Nagasaki Prefecture (Kyushu region) and the *Culicoides* biting midges in Miyazaki Prefecture (Kyushu region) in 1999 [9]. PEAV is a type of arthropod-borne virus (arbovirus), such as Akabane virus (AKAV, species *Akabane orthobunyavirus*) and Aino virus (AINOV, species *Shuni orthobunyavirus*), belonging to the same genus and serogroup. These arboviruses cause stillbirth and congenital malformations of fetuses in ruminants depending on the pregnancy stage, causing severe economic losses in the livestock industry [3, 5, 8]. Parsonson *et al*. reported that experimental infections of pregnant ewes with PEAV, such as AKAV and AINOV, caused arthrogryposis and hydranencephaly in fetuses [12]; however, this has not been experimentally proven in bovines.

In Japan, antibodies against PEAV had been detected in mainly Kyushu and Chugoku regions by the surveillance of arboviruses infection in sentinel cattle every year and abnormal birth in bovine caused by PEAV were reported in these regions [4, 10]. However, cases of PEAV infection were less than other arboviruses infections, such as AKAV. Moreover, in Shikoku region, abnormal birth caused by PEAV has never been reported, although antibodies against PEAV had been detected in a part of region. This report describes the case of abnormal birth in bovine suspected of being caused by PEAV first occurred in 2020 in Shikoku region, Japan.

The stillbirth of a calf (gestational age: 285 days) occurred in February 2020 in Ehime Prefecture (western Shikoku region), Japan, and the breed of the stillborn calf was an F1 hybrid (Japanese Black bull × Holstein cow). Twenty-six farm-bred cows (Holstein) were housed in a tie stole cowshed. However, the dam (age: 41 months) of the stillborn calf was slaughtered immediately after stillbirth due to low milk production, although it had not been observed clinical symptoms in pregnant periods. The dam of the stillborn calf had been bred in this farm since birth (September, 2016). Furthermore, these cows had not been vaccinated for preventing arbovirus infections, such as PEAV, AKAV, AINOV, Chuzan virus (CHUV), Ibaraki virus (IBAV), and bovine ephemeral fever virus (BEFV).

For detection of viral gene, tissue specimens of the stillborn calf obtained from a part of its brain tissue (probably considered olfactory bulb), spinal cord, heart, lungs, liver, spleen, and kidney were minced and homogenized in serum-free Eagle's minimum essential medium (EMEM) (Nissui, Tokyo, Japan). Four sera of cattle (Holstein) bred on the same farm were also collected

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10 days after stillbirth instead of the dam of the stillborn calf. These cattle had been bred beside the dam of the stillborn calf in pregnant periods and bred in this farm at least since October, 2018 (Table 1). Total RNA was extracted from the supernatant of 10% homogenate of each tissue, body fluid (ascites) of the stillborn calf, and four sera of cattle bred on the same farm using RNeasy Mini Kit (QIAGEN, Hilden, Germany). Multiplex RT-PCR for arboviruses and orthobunyaviruses and RT-PCR for bovine viral diarrhea virus (BVDV) were performed using the PrimeScript One-Step RT-PCR Kit Ver.2 (Takara, Kusatsu, Japan) with the primer sets used in a previous study [11, 13, 15]. Cycling conditions were as follows: 50°C for 30 min and 94°C for 2 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min. PCR products were electrophoresed on 1.5% agarose gel and visualized by staining with Midori green Xtra (Nippon Genetics, Tokyo, Japan).

For virus isolation, the supernatant of 10% homogenate of each tissue, body fluid (ascites) of the stillborn calf, and four sera of cattle bred on the same farm were used. These specimens were inoculated into monolayer cultures of hamster lung (HmLu-1) cells. After incubation at 37°C for 1 hr in a humidified 5% CO_2 incubator, the supernatant was replaced with EMEM containing 2% fetal bovine serum (FBS). After incubation at 37°C for 7 days, cell culture fluids were subinoculated into freshly prepared cell cultures twice. We observed the cytopathic effect (CPE) of cell cultures and detected PEAV, AKAV, AINOV, CHUV, and IBAV using cell culture fluids using the aforementioned methods of RT-PCR.

For virus neutralization (VN) test using bovine arboviruses (PEAV: strain KSB-1/P/06, AKAV: strain OBE-1, AINOV: strain JaNAr28, CHUV: strain C31, IBAV: strain No.2, and BEFV: strain YHL), body fluid (ascites) of the stillborn calf and four sera of cattle bred on the same farm were used. Moreover, we collected 148 sera from 37 sentinel cattle (four sera per cow) in June, August, September, and November 2019 in Ehime Prefecture, Japan. These specimens, inactivated at 56°C for 30 min, were serially diluted two-fold with EMEM containing 2% FBS in 96-well cell culture microplates. Then, 50-µl of each sample dilution was mixed with an equal volume of 200 $TCID_{50}$/0.1-ml virus and incubated at 37°C for 1 hr. Next, 100-µl HmLu-1 cells suspended in EMEM containing 2% FBS were added to each well. After incubation at 37°C for 7 days, the antibody titers were determined as the reciprocal of the highest serum dilution showing 50% inhibition of the CPE. VN titers ≥2 were considered positive for neutralizing antibodies to the virus. In addition, four sera from each sentinel cow seroconverted against PEAV were used to detect arboviruses and orthobunyaviruses and virus isolation using the aforementioned methods.

For histopathological examination, tissue samples, skeletal muscle and other organs, were collected from the stillborn calf. Collected tissue samples were fixed in 10% neutral buffered formalin, routinely embedded in paraffin blocks, and sectioned for hematoxylin and eosin (HE) staining. However, a part of its brain tissue and spinal cord were not collected because these tissues were lysed and inappropriate for histopathological and immunohistochemical examinations.

As results, arthrogryposis, spinal curvature, hydranencephaly (remaining part of the brain considered the olfactory bulb), and brainstem and cerebellar hypoplasia was observed as clinical signs in the stillborn calf (Fig. 1). The loss of skeletal muscle fibers accompanied by adipose replacement was observed as a pathological finding (Fig. 2). We observed no other pathological changes in other tissue samples. Arboviruses, orthobunyaviruses, and BVDV were not detected and PEAV, AKAV, AINOV, CHUV, and IBAV were not isolated. Antibodies against AKAV, AINOV, CHUV, IBAV, and BEFV were not detected (less than 2). Furthermore, antibodies against PEAV were detected from body fluid (ascites) of the stillborn calf (VN titers: 8), from three of four sera of cattle bred on the same farm (VN titers range: 32–128) (Table 1), and from 15 of 148 sera from 10 of 37 sentinel cattle in September and November 2019 (VN titers range: 8–128) (Table 2).

Our results clearly showed that incursion of PEAV in Shikoku region occurred in around August 2019 by detecting seroconversion against PEAV in sentinel cattle. Moreover, we detected antibodies against only PEAV from stillborn calf and not detected antibodies against other arboviruses. However, PEAV was not detected and isolated from stillborn calf and sentinel cattle. De Regge *et al.* reported that detection of viral genes and virus isolation are challenging in malformed calves because the virus disappears from the infected fetuses during gestation [2]. Kato *et al.* reported that viremia of bovine arboviruses is short (less than one week) and the occasion of isolation is highly limited from sentinel cattle [6, 7]. Therefore, we assume that PEAV was not detected and isolated in this case by these reasons. We observed arthrogryposis and spinal curvature that are distinctive clinical signs in arbovirus infections in stillborn calf. We also observed the pathological findings of the loss of skeletal muscle fibers accompanied by adipose replacement that had been observed frequently in previous reports [1, 4, 10]. Therefore, the abnormal birth of this case was suspected of being caused by PEAV.

Table 1. Virus neutralization (VN) titers of Peaton virus (PEAV) from body fluid (ascites) of the stillborn calf and sera of cattle bred beside the dam of the stillborn calf in the same farm

Origin	Breed	Age (Month)	Vaccination history	Breeding history in the farm	Specimen	VN titers of PEAV
The stillborn calf	F1 hybrid (Japanese Black bull × Holstein cow)	(Gestational age: 285 days)	(The dam of the stillborn calf: None)	(The dam of the stillborn calf: Since Sep-16)	Body fluid (ascites)	8
Cow A	Holstein	16	None	Since Oct-18	Serum	128
Cow B	Holstein	18	None	Since Aug-18	Serum	64
Cow C	Holstein	82	None	Since Apr-15	Serum	32
Cow D	Holstein	74	None	Since Mar-16	Serum	<2

None: not vaccinated for prevention of any arbovirus infection.

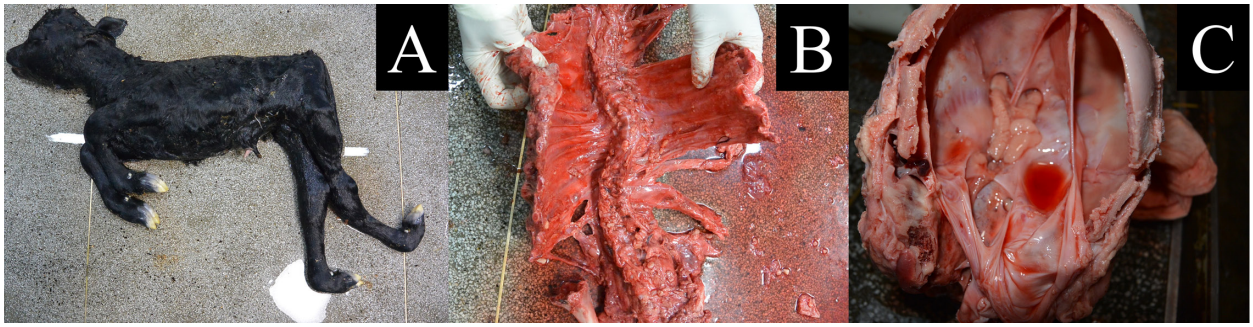

Fig. 1. (**A**) Arthrogryposis, (**B**) spinal curvature, and (**C**) hydranencephaly with brainstem and cerebellum (remaining part of the brain was considered the olfactory bulb) hypoplasia as clinical signs of the stillborn calf suspected of being caused by Peaton virus infection (gestational age: 285 days).

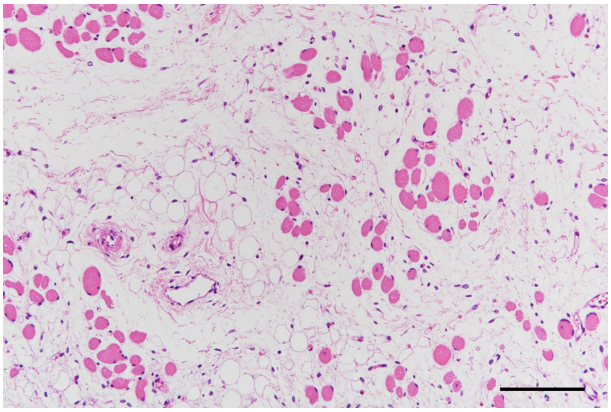

Fig. 2. The loss of skeletal muscle fibers accompanied by adipose replacement as a pathological finding of the stillborn calf suspected of being caused by Peaton virus infection. Hematoxylin-eosin. Bar=100 μm.

On the other hand, we observed hydranencephaly with brainstem and cerebellar hypoplasia that findings had never been reported. A hydranencephaly caused by PEAV was observed in only one case in Israel [1]. Behar *et al*. reported that hydranencephaly was caused by novel strains (MH331909–MH331911) detected from their case [1]. However, these strains were close relative the Japanese strain (KSB-1/P/06) isolated in Kagoshima Prefecture, Japan.

Unfortunately, viral antigens and genes of PEAV were not detected and isolated, we assume that the dam of the stillborn calf was infected between 90 and 110 days of pregnancy in around August 2019 and viral antigen and genes of PEAV were disappeared in the stillborn calf by the time of stillbirth. We could not get data of prove the relationship between the abnormal birth and PEAV infection.

In this case, brain malformations observed in the stillborn calf was severe than the case of Israel that observed only hydranencephaly [1]. It is unclear that the pathophysiology and pathogenicity of PEAV infection because there are few cases of abnormal birth suspected of being caused by PEAV. Therefore, it is need to collect epidemiological information for clarify the pathophysiology and pathogenicity, and it may be helpful to prevent economic losses in the cattle industry.

CONFLICT OF INTEREST. The authors have no conflicts of interest to declare.

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Table 2. Virus neutralization (VN) titers, RT-PCR, and virus isolation of Peaton virus (PEAV) from sera of sentinel cattle collected from June to November 2019 in Ehime Prefecture, Japan

Sample number	Farm ID	Breed	Month of birth	VN titers of PEAV/RT-PCR/virus isolation*			
				June, 2019	August, 2019	September, 2019	November, 2019
1	A	Holstein	Mar-19	<2	<2	<2	<2
2	A	Holstein	Dec-18	<2 / - / -	<2 / - / -	<2 / - / -	64/ - / -
3	B	Holstein	Apr-19	<2	<2	<2	<2
4	B	Holstein	Apr-19	<2	<2	<2	<2
5	B	Holstein	May-19	<2	<2	<2	<2
6	B	Holstein	Feb-19	<2	<2	<2	<2
7	B	Holstein	Feb-19	<2	<2	<2	<2
8	B	Holstein	Feb-19	<2	<2	<2	<2
9	C	Japanese Black	Mar-19	<2	<2	<2	<2
10	C	Japanese Black	Mar-19	<2	<2	<2	<2
11	C	Japanese Black	Mar-19	<2	<2	<2	<2
12	D	Holstein	Dec-18	<2	<2	<2	<2
13	D	Holstein	Dec-18	<2	<2	<2	<2
14	D	Holstein	Feb-19	<2	<2	<2	<2
15	E	Holstein	Jan-19	<2	<2	<2	<2
16	E	Holstein	Jan-19	<2 / - / -	<2 / - / -	<2 / - / -	64/ - / -
17	F	Japanese Black	Feb-19	<2	<2	<2	<2
18	F	Japanese Black	Feb-19	<2	<2	<2	<2
19	F	Japanese Black	Mar-19	<2	<2	<2	<2
20	F	Japanese Black	Apr-19	<2	<2	<2	<2
21	F	Japanese Black	Apr-19	<2	<2	<2	<2
22	F	Japanese Black	Apr-19	<2	<2	<2	<2
23	G	Japanese Black	Mar-19	<2 / - / -	<2 / - / -	64 / - / -	64 / - / -
24	G	Japanese Black	Mar-19	<2	<2	<2	<2
25	G	Japanese Black	Mar-19	<2 / - / -	<2 / - / -	128 / - / -	128 / - / -
26	H	Holstein	Nov-18	<2	<2	<2	<2
27	I	Holstein	Jan-19	<2 / - / -	<2 / - / -	8 / - / -	128 / - / -
28	I	Holstein	Apr-19	<2 / - / -	<2 / - / -	<2 / - / -	64 / - / -
29	J	Holstein	Dec-18	<2	<2	<2	<2
30	J	Holstein	Jan-19	<2 / - / -	<2 / - / -	<2 / - / -	128 / - / -
31	J	Holstein	Feb-19	<2	<2	<2	<2
32	K	Holstein	Nov-18	<2 / - / -	<2 / - / -	128 / - / -	64 / - / -
33	K	Holstein	Dec-18	<2 / - / -	<2 / - / -	128 / - / -	128 / - / -
34	L	F1 hybrid	Mar-19	<2	<2	<2	<2
35	L	F1 hybrid	Mar-19	<2 / - / -	<2 / - / -	<2 / - / -	32 / - / -
36	L	F1 hybrid	Mar-19	<2	<2	<2	<2
37	L	F1 hybrid	Mar-19	<2	<2	<2	<2

All cattle had not been vaccinated for prevention of any arbovirus infection. *RT-PCR and virus isolation were performed only to cows confirmed seroconversion against PEAV.

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