NOTE

Virology

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

Nobuki YOSHIZAWA1), Michiko SHINOTO2), Akiho KATAYAMA1), Riko BEKKU3) and Kenichi INATANI3)

1) Ehime Prefectural Livestock Disease Diagnostic Center, 743-1 Tanokubo, Toon, Ehime 791-0212, Japan
2) Livestock Division, Agriculture, Forestry and Fisheries Department, Ehime Prefectural Government, 4-4-2 Ichibancho, Matsuyama, Ehime 790-8570, Japan
3) Ehime Nanyo Livestock Hygiene Service Center, 1-18-3 Gotanda, Yawatahama, Ehime 796-8010, Japan

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 Peribunyviridae, 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-stall 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.

*Correspondence to: Yoshizawa, N.: nyoshizawa.vet@gmail.com
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Our results clearly showed that incursion of PEA V in Shikoku region occurred in around August 2019 by detecting seroconversion against PEA V in sentinel cattle. Moreover, we detected antibodies against only PEA V from stillborn calf and not PEA V , AKA V , AINOV , CHUV , and IBAV using cell culture fluids using the aforementioned methods of RT-PCR.

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 PEA V , AKA V , AINOV , CHUV , and IBAV were not isolated. Antibodies against AKA V , AINOV , CHUV , IBAV , and BEFV were not detected (less than 2). Furthermore, antibodies against PEA V 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 PEA V in Shikoku region occurred in around August 2019 by detecting seroconversion against PEA V in sentinel cattle. Moreover, we detected antibodies against only PEA V from stillborn calf and not detected antibodies against other arboviruses. However, PEA V 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 PEA V 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 PEA V.

Table 1. Virus neutralization (VN) titers of Peaton virus (PEA V) 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 PEA V |
|--------|-------|-------------|---------------------|-----------------------------|----------|-------------------|
| 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.
On the other hand, we observed hydranencephaly with brainstem and cerebellar hypoplasia that findings had never been reported. A hydranencephaly caused by PEA V 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 PEA V 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 PEA V 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 PEA V 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 PEA V infection because there are few cases of abnormal birth suspected of being caused by PEA V. 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.

ACKNOWLEDGMENTS. We would like to thank Dr. Shogo Tanaka and Dr. Hiroaki Shirafuji of the Kyushu Research Station, National Institute of Animal Health, NARO, for technical advice and for kindly providing arboviruses. We also thank Yasuko Toku-naga of the Ehime Chuyo Livestock Hygiene Service Center for technical advice and all members of the Ehime Prefectural Livestock Disease Diagnostic Center and the Ehime Nanyo Livestock Hygiene Service Center for technical supports.
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             | <2            |
| 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 / - / - | <2             | 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 / - / - | <2 / - / -     | 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             | <2            |
| 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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