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NOTE

Virology

Successful measures to prevent the spread of bovine papular stomatitis in a dairy farm

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ABSTRACT. Nasal papules and oral ulcers were observed in calves that were group-housed at a dairy farm. The calves were diagnosed with bovine papular stomatitis (BPS) due to parapoxvirus (PPV) infection based on virologic examinations using polymerase chain reaction to detect PPV. To prevent the spread of BPS, we isolated the affected calves, made procedural changes so that the affected herd was managed after the healthy herd, disinfected the bedding with slaked lime, disinfected the stalls and fences with invert soap, and changed the animals' feed to soft grass which does not damage the oral cavity. As a result, we succeeded in control the infection quickly.

KEY WORDS: bovine papular stomatitis, control measure, dairy calf, infection control, parapoxvirus

Bovine papular stomatitis (BPS) is predominately caused by infections with bovine papular stomatitis virus (BPSV) belonging to the genus *Parapoxvirus* (PPV), subfamily *Chordopoxvirinae*, family *Poxviridae*. BPS causes redness, papules, nodules, and erosion on the lips, gums, mouth, and tongue. By law, veterinarians must report cases of BPS to the authorities. Since pustules and ulcers may develop in some cases, it is necessary to differentiate BPS from similar diseases such as foot-and-mouth disease (FMD). Similar lesions form around the mouth due to a pseudocowpox (PCP) virus (PCPV) infection, a member of virus that also belongs to the PPV genus. However, depending on the initial site of infection, the name of the disease and the causative virus may not necessarily be the same [5, 7]. Regardless of which viral infection is the cause, if a lesion has formed around the mouth, it is considered to be BPS and has to be reported.

The virus particles of PPV, which is a double-stranded DNA virus, have a characteristic oval shape of 250–300 × 160–190 nm. Using negative staining electron microscope, the virus particles can appear to have a bamboo cage structure because a criss-cross filament pattern covers their surface [2, 5]. BPSV, PCPV, and viruses of the same genus cross-react serologically with each other but can be identified by analyzing the nucleotide sequence of the viral envelope gene regions [5, 7]. Infection is thought to be caused by close contact with a lesion containing PPV in affected animals or PPV-contaminated materials [5, 7]. It has been reported that 72% of cattle in Japan have PPV antibodies [14]. Both BPS and PCP are zoonotic diseases [2, 5, 7, 13] and humans can form papules on their fingers after coming into contact with PPV lesions in affected animals [2]. Therefore, gloves should be worn when handling affected animals.

In this study, we reported a case of BPS at a dairy farm in Nagano Prefecture, Japan and a successful measures to prevent the spread of the outbreak at an early stage by implementing a number of countermeasures.

The farm where the outbreak occurred is a dairy farm that houses 369 dairy cattle using a free stall-feeding style. The farm had no history of a BPS outbreak. On April 3, 2019, 2 out of 14 calves (aged one to three months) that were being group-housed (cattle Nos. 1–2) were found to have formed papules and oral ulcers. According to the Specific Domestic Animal Infectious Disease Quarantine Guidelines for foot-and-mouth disease by the Ministry of Agriculture, Forestry and Fisheries, Japan (https://www.maff.go.jp/j/syouan/douei/katiku_yobo/k_bousi/attach/pdf/index-26.pdf), as well as clinical signs (papules and ulcers) and epidemiological status (no rapid spread to the cohabiting cattle), FMD was ruled out. The 14 calves in the pen were isolated

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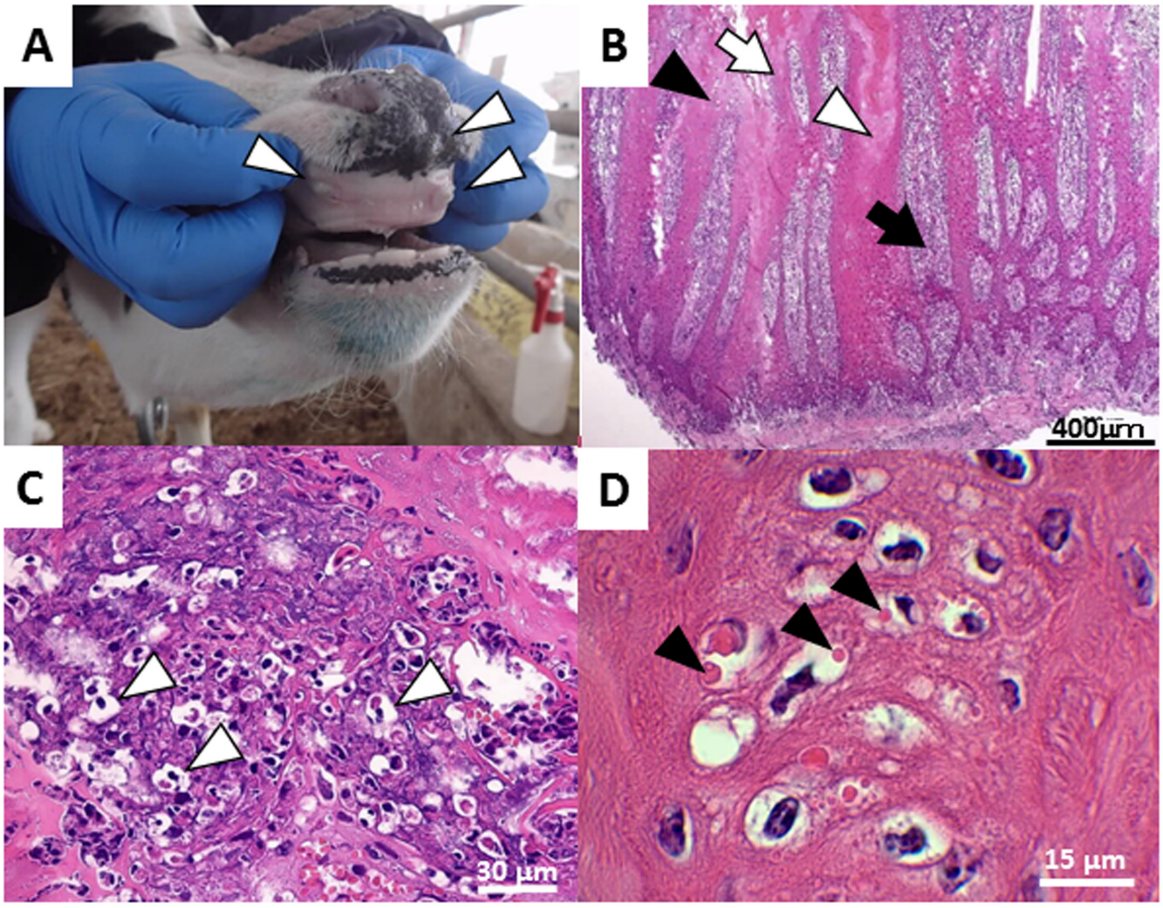

Fig. 1. Clinical signs and histological examinations. (A) Clinical signs of cattle No. 9 showing multiple papules and ulcers in the nasal speculum and the palate (white arrowheads). (B–D) Micrograph of the nasal speculum of cattle No. 3 by hematoxylin and eosin staining. (B) Papillary ridges in the lamina propria (white arrow), inflammatory cell infiltration (black arrow), thickening of the spinous layer (white arrowhead), and edema (black arrowhead) were observed. (C) Balloon-like degeneration was observed in the cells in the spinous layer (white arrowheads). (D) Eosinophilic cytoplasmic inclusion bodies were observed (black arrowheads).

from the rest of the herd, with the other cattle being prohibited from entering the affected cattle pen. Follow-up observations were conducted.

On April 19, the same clinical signs were observed in three of the isolated calves (Nos. 3–5). On April 22 and 25, two (Nos. 6–7) and two calves (Nos. 8–9) were newly confirmed to have similar clinical signs, respectively, bringing the total number of calves with confirmed clinical signs to nine (Fig. 1). These calves shared one milking robot (Fig. 2). At that time, calves were fed Sudan grass hay.

For histological examinations, tissues from the nasal speculum of the affected cattle (No. 3) were fixed with 10% neutral buffered formalin solution, embedded in paraffin, thinly sliced, stained with hematoxylin and eosin, and examined microscopically. Papillary ridges in the lamina propria, inflammatory cell infiltration, thickening of the spinous layer, edema, balloon-like degeneration, and formation of eosinophilic cytoplasmic inclusion bodies, were observed (Fig. 1).

For genetic analyses, three tissue samples from the nasal speculums and lips, as well as six swab samples from the nasal speculums and lips, were used (Table 1). The lesion swabs were collected by wiping a lesion with a sterile cotton swab. Subsequently, phosphate-buffered saline (Fujifilm Wako Pure Chemicals, Osaka, Japan) was added, and the specimens were centrifuged for 15 min at 4°C at 3,000 rpm and filtrated through a 0.45 μm pore size membrane filter (Millex-HV Filter Unit Low Protein Binding Durapore Membrane, Merck Millipore, Cork, Ireland). DNA was extracted from the tissue and swab samples using a commercial kit (Sepagene RV-R, Sekisui Medical, Tokyo, Japan). A commercial polymerase chain reaction (PCR) kit (TaKaRa Ex Taq (Mg^{2+} free Buffer), Takara Bio, Kusatsu, Japan) was used for a semi-nested PCR with a PPV consensus primer [8] that targeted the B2L gene. For virus isolation, tissues from the nasal speculum of the affected cattle (No. 3) were made into a 10% emulsion using an antibiotic containing Eagle's minimal essential medium (Nissui Pharmaceutical, Tokyo, Japan). After centrifugation at 3,000 rpm for 15 min, the supernatant was filtered, and then inoculated into primary bovine testis cells and incubated at 37°C in 5% CO_2 for three days.

The semi-nested PCR yielded a 235 bp band from all of the specimens taken from the nine affected calves (Table 1). Nucleotide

sequence of the 594 bp PCR products from these clinical samples by the first PCR was determined by direct sequencing and BPSV was identified. Therefore, we diagnosed the calves with BPS. The identified virus was named the Nagano 2019 strain, and the nucleotide sequence information was registered (accession no. LC487905). Based on a phylogenetic analysis, it was classified into the same cluster as the strains isolated in the Nara Prefecture in 2015 [3] and Iwate Prefecture in 2007 [15] (Fig. 3). Clear cytopathic effect was observed in cells (data not shown) and a PPV-specific 235 bp band was confirmed in the culture fluid of cells with a semi-nested PCR. Thus, a virus was successfully isolated.

The cattle pen where the first two calves and the 12 calves cohabiting with them were staying in was used as an isolation area. Furthermore, the order in which the cattle were reared was modified so that the affected herd was reared after the healthy herd. In addition, the cattle bedding in the pen with the affected calves was disinfected using slaked lime, and the partition and fence were disinfected using invert soap. Following this, workers' gloves were disinfected with invert soap so as not to spread the infection to areas outside the pen that contained the isolated cattle. The outbreak continued in the period feeding the hay (Sudan grass) even after countermeasures mentioned above, and it was considered possible that the hay was hard and caused small scratches in the oral cavity. Therefore, we changed the feed from hard to soft hay (Timothy grass) (Table 2). As a result of implementing these measures and carrying out follow-up observations, no new cases were found in the healthy cattle on the farm, all the affected calves were healed 40 days after the first onset, and the outbreak was stopped. Timothy grass contains more nutritive value than Sudan grass, such as crude protein and neutral detergent fiber, which are the main components of roughage [1]. In addition, the nitrate-nitrogen content, which is the cause of nitrate poisoning, is low in Timothy grass [1]. It is possible that changing to a better feed for cattle and improving the physical condition of cattle is one of the factors that stopped the outbreak.

Fig. 2. Milking robot shared by calves. (A) A total of 14 calves was group-housed. (B) A teat of milking robot shared by calves.

As PPV has strong environmental resistance and remains contagious outside of the body for a long period [9, 13], in addition to coming in direct contact with a lesion, cattle can be indirectly infected by instruments contaminated with lesion or scab tissue, as well as in breeding facilities and grazing grounds where scabs have fallen off [5, 7, 13]. PPV is thought to be transmitted from small scratches when an animal comes in direct contact with a lesion [2].

In this farm, hard hay (Sudan grass), which can cause small scratches in the oral cavity, was fed to the calves. In the cattle pen

Table 1. Summary of individual information and virological analysis of cattle

Cattle No.	1	2	3	4	5	6	7	8	9
Age (month)	3	3	3	2	3	3	3	4	4
Date of onset	3 April 2019	3 April 2019	19 April 2019	19 April 2019	19 April 2019	22 April 2019	22 April 2019	25 April 2019	25 April 2019
Sampling date	25 April 2019	25 April 2019	19 April 2019	25 April 2019	25 April 2019	25 April 2019	25 April 2019	25 April 2019	25 April 2019
Site of onset	Lips	Lips	Nasal speculum, lips	Lips	Lips	Nasal speculum, lips	Lips	Lips	Nasal speculum, lips
Sampling site	Lips	Lips	Nasal speculum	Lips	Lips	Lips	Lips	Lips	Lips
Specimen	Tissue	Swab	Tissue	Swab	Swab	Swab	Tissue	Swab	Swab
1st PCR*	+	−	+	+	+	+	+	−	−
2nd PCR*	+	+	+	+	+	+	+	+	+

*+, specific gene of parapoxvirus (PPV) was detected; –, specific gene of parapoxvirus was not detected.

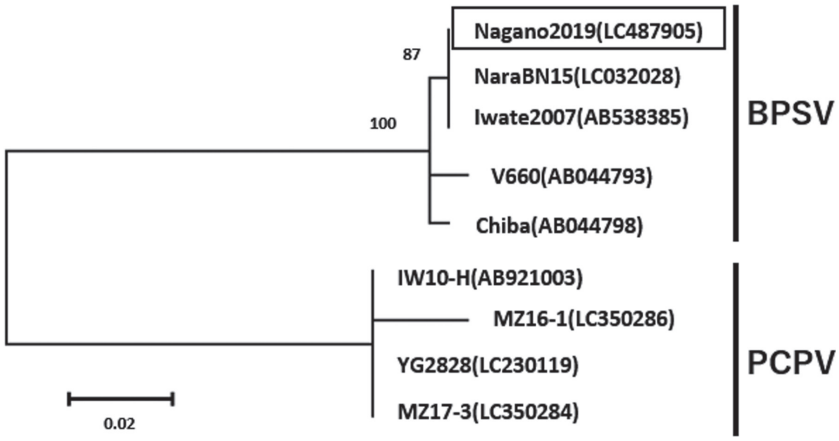

Fig. 3. Phylogenetic tree based on the nucleotide sequences of the partial B2L gene. The percentages of bootstrap values calculated from 1,000 replicates are indicated above the internal nodes. The strain detected in this study is shown in the box. BPSV, bovine papular stomatitis virus; PCPV, pseudocowpox virus.

Table 2. Measures to prevent the spread of infection

	Before implementing the measures	After implementing the measures
Segregation	Affected and their cohabiting cattle were kept in the same pen	Segregation the affected and their cohabiting cattle from healthy cattle
Workflow	Rearing affected and healthy cattle at the same time	After raising healthy cattle, raise affected cattle
Bedding	Undisinfected	Disinfection with slaked lime
Partition and fences	Undisinfected	Disinfection with invert soap
Worker	Undisinfected	Glove disinfection with invert soap after raise
Hay	Hard (Sudan grass)	Soft (Timothy grass)

where the outbreak occurred, the 14 cohabiting calves shared a single milking robot (Fig. 2) that feeds milk to calves automatically. Previous studies reported that PPV DNA was detected in residual feed and drinking water on farms [16] and in milk from cattle with clinical signs of PPV infection [12]. Moreover, it was also reported that milking machine teat clusters, udder cloths, and milker's hands were the source of spread of PPV infection from cow to cow [4]. These reports supported the possibilities that the damage in the oral cavity caused by hard hay created an environment in which the BPSV of the milking robot could easily spread and, as a result, the infection spread to the cohabiting cattle in the same pen. In addition to feed-related countermeasures, to prevent the infection from spreading from the quarantined cattle pen, the cattle bedding was disinfected with slaked lime, and the partitions and fences were disinfected with invert soap, which is effective against enveloped viruses [11]. Furthermore, the workers' gloves were also sterilized after they had finished working in the affected pen. It has been reported that the spread of infection was prevented by cauterizing the lesions caused by PPV and spraying lime in a facility that housed captive Japanese serows (*Capricornis crispus*) [6]. By implementing these countermeasures, the infection did not spread to the healthy herd, and the outbreak was quickly stopped.

There are two main reasons why the PPV outbreak at this farm was successfully stopped in a short period of time: (1) the early diagnosis of PPV and (2) the early implementation of effective countermeasures. BPS, PCP, and contagious pustular dermatitis due to PPV infection sporadically present in cattle, sheep, and goats every year in Japan. The countermeasures implemented at this farm were successful and could be used in the future to prevent the occurrence and spread of PPV without needing any special preparations or new equipment.

According to the pathological appraisal manual [10], lesion tissue is usually used as the test material. However, in this case, it was also possible to detect PPV from swabs of the lesions. In cases where it is difficult to collect lesioned tissue, it is possible to determine the infection and viral excretion status using a swab of the lesion. This method could be useful for controlling the spread of PPV.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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