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NOTE

Pathology

Congenital cutaneous fibropapillomatosis without evidences of papillomavirus infection in a Holstein-Friesian calf

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ABSTRACT. A male Holstein-Friesian calf was born with multiple, cauliflower-like, pale pink cutaneous masses on the head and limbs. On histopathological examination, the cutaneous masses were diagnosed as congenital cutaneous fibropapillomatosis. Those lesions involved focal proliferation of sebaceous gland in the dermis. There were no histological findings to suggest bovine papillomavirus infection, such as the presence of intranuclear inclusion bodies, large keratohyalin granules, and koilocytosis. Furthermore, papillomaviral antigens and DNA were not detected by immunohistochemistry and polymerase chain reaction, respectively. These results suggested that there was no association between these cutaneous lesions and bovine papillomavirus infection, and the lesions were considered as harmartomatous changes.

KEY WORDS: calf, congenital, dermatopathology, fibropapillomatosis

In various animal species, acquired cutaneous papillomatosis and fibropapillomatosis are associated with papillomavirus (PV) infection [7]. Acquired bovine cutaneous papillomatosis and fibropapillomatosis are also considered to be caused by bovine PV (BPV) infection [7]. A previously described case of congenital papillomatosis in a calf was suspected to be due to BPV infection during pregnancy [3]. On the other hand, congenital papillomatosis and fibropapillomatosis without evidences of PV infection have been reported in horses [4, 11] and pigs [8, 10]. This is a first case of bovine congenital cutaneous fibropapillomatosis without evidences of PV infection.

A male Holstein-Friesian calf was born with multiple, cauliflower-like, pale pink masses on the skin. Severe lesions were distributed on the head (Fig. 1), and milder lesions were observed on the carpal region of the left forelimb, tarsal region of the left hindlimb, muzzle, lower lip, and neck. No other significant abnormalities were detected on clinical examinations, including hematological examination and serum biochemical analyses. The calving of this calf was normal, and no lesions suggesting BPV infection were detected clinically in its dam. Based on the owner's request, the calf was subjected to necropsy at 9-day-old, and systemic organs and tissues including cutaneous masses at multiple locations were collected for histopathological examination. Collected tissues were fixed in 15% neutral buffered formalin. A part of a mass on the head was collected in 99% ethanol for polymerase chain reaction (PCR). Formalin-fixed tissues were routinely processed and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin.

Immunohistochemistry (IHC) using anti-human PV rabbit polyclonal antibody (1:200, YLEM, Rome, Italy) was performed to assess the involvement of PV in these lesions. Briefly, sections were pretreated with citrate buffer pH 6.0 (98°C, 20 min). Endogenous peroxidase was blocked with 3% H_2O_2 (room temperature, 10 min), followed by an incubation with the primary antibody (4°C, overnight). MAX-PO polymer reagent (Nichirei Bioscience, Tokyo, Japan) was used as the secondary antibody (room temperature, 30 min). Immunoreactivity was visualized by 3,3′-diaminobenzidine, and the sections were counterstained with Lillie-Mayer's hematoxylin. Non-immunized rabbit blood serum (Dako, Glostrup, Denmark) was used as the negative control in IHC. DNA was extracted from the ethanol-fixed tissue using the NucleoSpin tissue kit (MACHEREY-NAGEL, Düren, Germany) in accordance with the manufacturer's protocol for PCR. The primer pair MY09 and MY11 was used to detect PV DNA [6]. This

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PCR primer pair has been shown to amplify the L1 gene of almost all human papillomavirus types [2]. Also, the primer pair detects the L1 gene of most BPV types, including BPV-1, -2, and -8 that have been associated with bovine cutaneous fibropapilloma [1, 7]. The PCR reaction consisted of 1 µl DNA template, 25 µl Gflex PCR buffer, 0.2 µM each of the forward and reverse primers, and 1 µl Tks Gflex DNA polymerase (Takara Bio, Kusatsu, Japan), in a total reaction mixture of 50 µl. The sample was heated to 94°C for 2 min, followed by 45 cycles at 98°C for 10 sec, 55°C for 15 sec and 68°C for 30 sec, and a final extension at 68°C for 1 min on a Mastercycler gradient (Eppendorf, Tokyo, Japan). PCR products were confirmed by agarose gel electrophoresis. A bovine cutaneous fibropapilloma in which the presence of BPV antigen or DNA had been confirmed was used as the positive control for IHC and PCR.

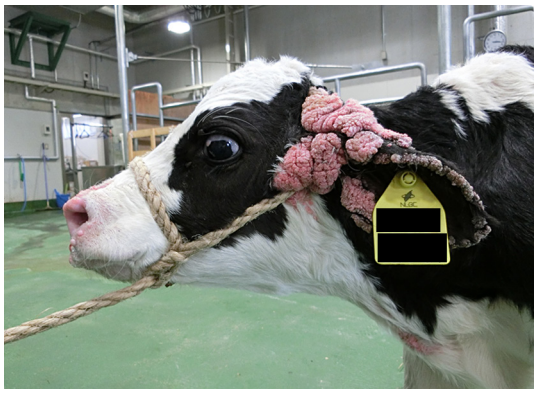

Fig. 1. Pale pink, multiple, cauliflower-like cutaneous masses are scattered on the left ear, neck, and muzzle.

On histopathological examination of the cutaneous masses, the lesion was characterized by epidermal papillary outgrowths with subjacent proliferation of immature fibroblasts (Fig. 2a). Hyperkeratosis and parakeratosis were observed on the cornified layer. A few lymphocytes and plasma cells infiltrated the dermis. Some keratinocytes exhibited hydropic degeneration. However, koilocytosis, which is characterized by swollen cells with a perinuclear halo and pyknotic nucleus [5], was not observed (Fig. 2b). There were no giant keratohyalin granules and intranuclear inclusion bodies. Hyperplasia of matured sebaceous glands was also observed focally in the dermis (Fig. 2c). IHC did not demonstrate PV antigen (Fig. 2d), and PCR did not reveal an amplicon (Fig. 3). Gross examination or histopathological examination of the other collected organs and tissues did not reveal any abnormalities.

Based on the results of clinical and histological examinations, the present case was diagnosed as congenital cutaneous fibropapillomatosis. In general, bovine cutaneous papillomatosis and fibropapillomatosis are caused by BPV infection [7]. Since BPV is mainly transmitted by animal-to-animal contact or fomites, the majority of lesions are

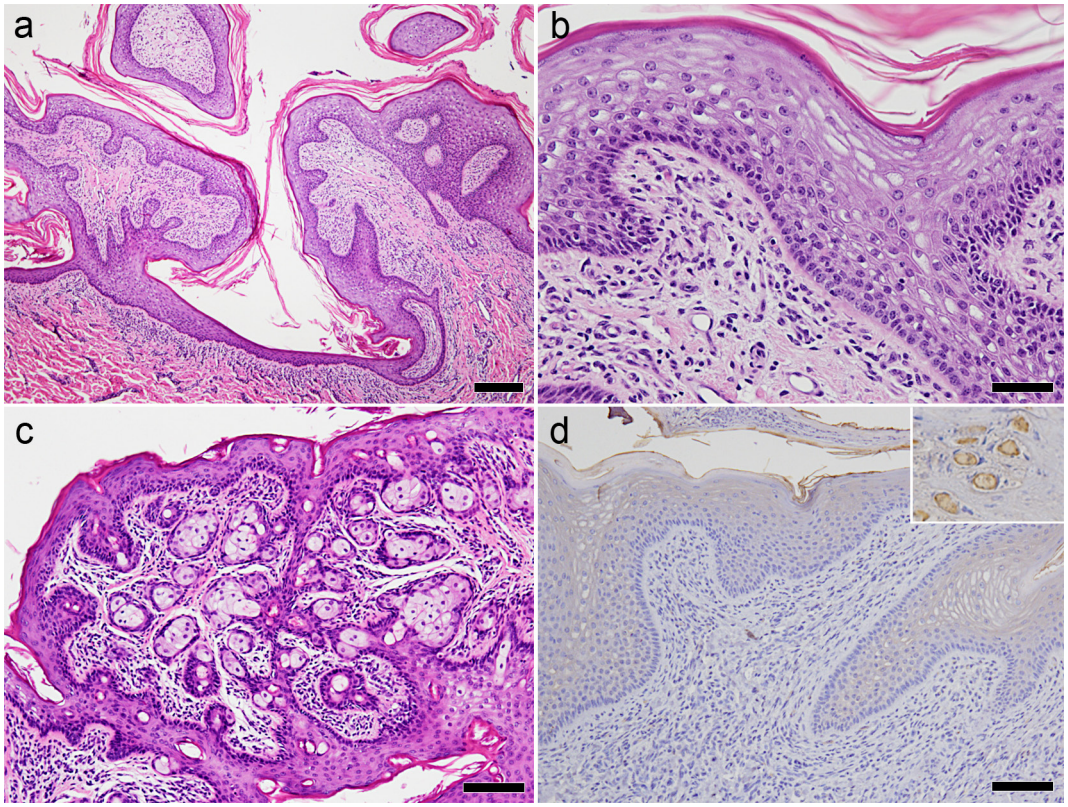

Fig. 2. **a)** Papillary outgrowths of the surface epithelium with subjacent proliferation of fibroblasts in its beneath is observed. Hyperkeratosis is also observed on the surface of the mass. Hematoxylin and eosin (HE) stain, bar=250 µm. **b)** Hydropic degeneration is found in keratinocytes, however, koilocytosis, giant and irregular keratohyalin granules, and intranuclear inclusion bodies are not observed. HE stain, bar=50 µm. **c)** Hyperplasia of the sebaceous glands is observed focally. HE stain, bar=100 µm. **d)** There are no positively stained cells with the anti-papillomavirus (PV) antibody. Immunohistochemistry, bar=100 µm. Inset: In the positive control, PV antigens are detected in the nuclei of keratinocytes.

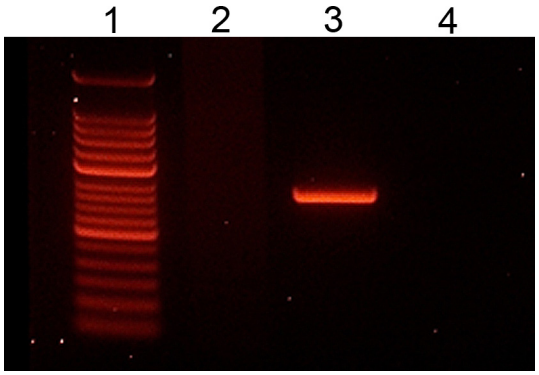

Fig. 3. Lanes 1, 2, 3, and 4 are the DNA ladder, present case, positive control, and distilled water, respectively. The amplicon is only detected in the lane of positive control.

considered to be acquired [7]. There was 1 case report about bovine congenital cutaneous papillomatosis [3]. In that case, although authors did not perform IHC or PCR to confirm the involvement of BPV, they suspected that the cause of those lesions was BPV-3 transmitted vertically because of the similarity in the macroscopic appearance of the lesion caused by BPV-3 infection [3]. In newborn lambs, the viral antigens and DNA of BPV-2 and −13 were detected in the lesions of congenital fibropapillomatosis [9]. On the other hand, the characteristic histological findings of fibropapillomatosis induced by BPV infection, such as koilocytosis of superficial stratum spinosum, intranuclear inclusion bodies, or large and irregular keratohyalin granules [5, 7], were not observed in the present case. In addition, BPV was not detected in the cutaneous lesions by IHC and PCR for PV.

Congenital papillomatosis and fibropapillomatosis have been reported in horses [4, 11] and pigs [8, 10]. In those cases, no characteristic histological findings suggesting PV infection were observed [4, 8, 10, 11]. Also, there were no evidences of PV infection by IHC or PCR examinations. Therefore, those lesions were suspected to be hamartomatous changes [4, 8, 11]. Furthermore, congenital papillomatosis in a horse involved the proliferation of matured sebaceous glands in the dermis [11]. The present case was born with the lesions, and there were no evidences of BPV infection by histopathological and PCR examinations. Also, the present lesions had hyperplastic foci of sebaceous glands. Based on the histopathological findings and their similarity to previous reports of foals [4, 11] and a piglet [8, 10], fibropapillomatosis in the present case was considered as a hamartomatous change.

CONFLICTS OF INTEREST. The authors declare no conflicts of interest with respect to the publication of this manuscript.

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