Teat papillomatosis in dairy herds: First detection of bovine papillomavirus type 10 in China

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ABSTRACT. Teat papillomatosis is one important infectious disease affecting cattle health and results in significant economic losses especially in the dairy industry. Although there is a large number of commercial cattle herds in China, limited information is available for molecular epidemiological investigation of bovine papillomaviruses (BPVs). In October 2017, an outbreak of teat papillomatosis occurred in the Shandong Province of China. Samples were collected and diagnosed with PCR, and 3 full-length viral genomes were amplified from tissue samples collected from 3 outbreak farms. Analysis results revealed that the outbreak was associated with BPV type 10. This is the first report of BPV-10 infection in China and will contribute to the molecular epidemiological study of the disease.

KEY WORDS: bovine papillomavirus type 10, dairy herd, teat papillomatosis

Bovine papillomatosis is a benign proliferative tumor that manifests into several forms, including cutaneous fibropapillomas, genital fibromas, urinary bladder tumors, upper gastrointestinal tract warts, and teat warts. It has been reported as a common health problem in cattle herds worldwide [1]. For cutaneous warts, although most of them rarely cause problems in cattle, large warts may cause bleeding and potentially lead to secondary infections. Moreover, teat warts can cause mastitis and interfere with suckling and milking. Especially in dairy herd, it can predispose cows to mastitis, cause bleeding during milking, which result in significant economic losses in the dairy industry [1]. Bovine papillomatosis is caused by bovine papillomavirus (BPV), a group of non-enveloped, circular double-stranded DNA viruses of the family *Papillomaviridae*. BPVs have been reported worldwide and caused a number of problems in cattle industry. Despite the growing concerns about BPV, limited information about BPV infection in China is available. Here we report an outbreak of teat papillomatosis occurred in dairy farms in the Shandong Province of China 2017, in the present study, BPV-10 was also detected in teat lesions. This is the first report of BPV-10 in China.

The flat-and-round type teat papillomatosis (Fig. 1) was found in 3 holstein dairy farms which ranged from 1 to 5 km apart. All 3 farms were operated by the same owner and managed by the same veterinarian team, and formed an interconnected network where technical staff and veterinarians moved from one farm to another. Twelve papilloma tissue samples and 36 swab samples were collected from the teats of individual affected holstein dairy cows at three farms (4 tissue samples and 12 swab samples from each farm) in which 117 cows showed symptoms among total 442 cows (69/202, 22/163 and 26/77, respectively). Part of each tissue sample were fixed with 10% formalin and routinely embedded in paraffin. And then stained with hematoxylin and eosin (HE). Morphological lesions of neoplastic tissues were almost identical, the neoplasm consisted of hyperkeratotic squamous epithelial cells arranged in papillary pattern.

DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to tissue and swab protocols in the manufacturer's instructions. Then Rolling Circle Amplification (RCA) was performed using TempliPhi 100 Amplification Kit (GE Healthcare, Buckinghamshire, U.K.). Subsequently, using RCA product as template, PCR assays were performed with primer pairs FAP59/FAP64 [3] and MY09/11 [6] respectively. All tissue and swab samples (n=48) were PCR positive with primer pair FAP59/FAP64 but negative with MY09/MY11. The positive PCR products were gel-purified with a QIAquick Gel Extraction Kit (Qiagen)

Fig. 1. Clinical symptoms of teat papillomatosis in dairy cattle infected with bovine papillomavirus type 10 (BPV-10).

Table 1. Oligonucleotides for whole genome amplification and cloning

Name	Sequences (5′-3′)	Location (KF017607)
BPV10-1F	AGCCCAGACTGTTGCACCGA	7195–7214
BPV10-1R	AGTGCCGTCTAGTCCATTTC	2204–2223
BPV10-2F	GGGCTGTTAAAGGGAAAGTG	2069–2088
BPV10-2R	GACCAATCGGGATGGATGTG	4594–4613
BPV10-3F	CCTGACAGTGCCTTTAGAAC	4494–4513
BPV10-3R	GGGTCTTCTTTCTCTGGTTG	6667–6686
BPV10-4F	CAAGACACTTACACTGCCTC	6424–6443
BPV10-4R	TCCCATCCATTCAAGTCATC	207–226

BPV: bovine papillomavirus.

and cloned into the pMD19-T vector (Takara, Dalian, China), then were Sanger sequenced using the ABI 3730XL sequencer at Sangon Biotech (Shanghai, China). FAP59/FAP64 PCR products were confirmed to be 428 base pair (bp) in length. Sequence alignment of all samples revealed 99.5 to 100% nucleotide sequence identity. Sequence similarity analysis with the BLAST tool of the National Center for Biotechnology Information (NCBI) demonstrated 99 to 100% identity with BPV-10 (AB331651) [5] and BPV-10 MY55 (KF017607) [11]. Previous studies reported different types of BPVs simultaneously presented in the same lesion [2]. Therefore it is necessary to confirm the sole presence of BPV-10. For detecting reported BPVs, PCR was performed with type-specific primers [2]. The result showed no other BPVs were detectable. Since most of PV types were initially detected by the consensus primer set FAP59/FAP64, the primer set is useful to confirm whether or not there are other types of BPV. Moreover, the RCA product was used as a PCR template, which can make the detection more sensitive. The PCR product (FAP59/FAP64) was sequenced directly with FAP59/FAP64 primers, and there was no overlapping peak in sequencing electropherogram. Therefore, the result suggested that there were no other types of BPV detected in the samples. The outbreak of teat papillomatosis was associated with BPV-10 infection.

Using extracted DNA as a template, full-length genome sequences were amplified from three positive tissue samples named as P316, P332 and P348 (one for each farm) with 4 pairs of oligonucleotide primer (Table 1) designed according to published BPV-10 genome sequences in Genbank. The amplicons were gel-purified and 4 overlapping fragments cloned into the pMD19-T vector (Takara). The recombinant clones were sequenced with the same procedure as the diagnostic PCR described above. Sequences were spliced using Seqman software in Lasergene Package (DNA-STAR, Madison, WI, U.S.A.). Full-length viral genomes of P316 (MH251867), P332 (MH251868) and P348 (MH251869) have been successfully amplified from three tissue samples collected from three farms with outbreaks. Sequence analysis results show that three genomes shared 99.9 to 100% nucleotide identity and have the same length of 7,270 bp. With the addition of nucleotide sequence identity of the PCR product (FAP59/FAP64), the whole genome identity revealed that viruses associated with the outbreak were derived from same BPV-10 strain, which was designated BPV10/CN2017SD. Meanwhile, the phylogenetic analysis based on the full-length viral genomes showed that all three strains were tightly close to BPV-10 (AB331651) and BPV-10 MY55 (KF017607) (Fig. 2).

Teat papillomatosis is one important disease affecting cattle health and causing economic losses especially in the dairy industry [1]. To date, BPV-3, −6, −7, −9, −10, and −11 have been reported to be associated with teat papillomatosis [4, 7, 10]. BPV-10 was first detected and characterized in Hokkaido, the northern island of Japan, where it caused an outbreak of bovine teat papillomatosis
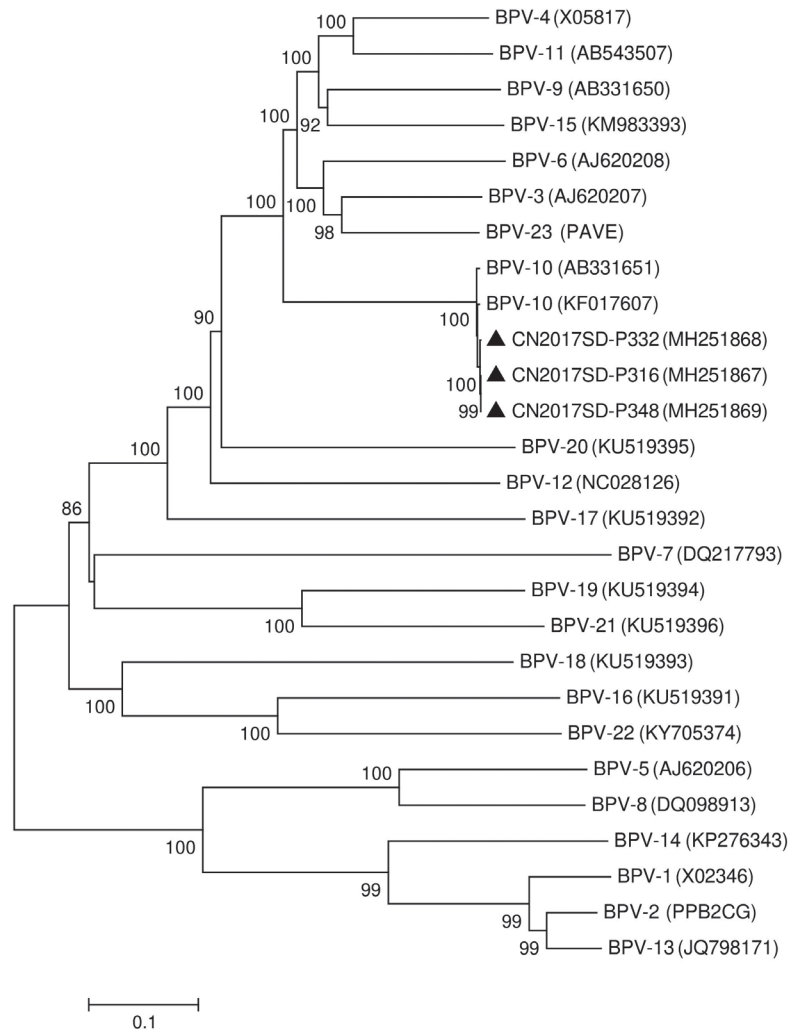
Fig. 2. Phylogenetic tree based on the complete genomes of all 23 bovine papillomavirus (BPV) types. The tree was conducted using p-distance-based neighbour-joining method in MEGA softare (version 7.0). Bootstrap values were calculated with 1,000 replicates. The virus strains identified in this study were indicated by black solid triangles.

along with BPV-6 and BPV-9 [5]. Then it was found to be associated with teat, udder, and lingual papillomatosis in dairy and beef cattle herds in Brazil, India, Italy and Japan [8–11]. A large number of commercial cattle herds in China and papillomatosis outbreaks are often monitored by managers and feeders in farms. However, limited information is available for molecular epidemiological investigation of BPVs in China. This is the first report of BPV-10 infection in association with an outbreak of teat papillomatosis in dairy herds and will contribute to the molecular epidemiological study of the disease.

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