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Research article

Prevalence of bovine papillomavirus and *Treponema* DNA in bovine digital dermatitis lesions

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ABSTRACT

Bovine digital dermatitis (BDD) is a common infectious foot disease whose aetiology is not fully understood. Its origin is thought to be multifactorial, with treponemes being involved. Using PCR-based techniques, BDD samples from 45 affected cows and intact skin from 8 BDD-affected and 33 healthy cows were assessed for the presence of bovine papillomavirus and *Treponema* DNA. BPV DNA (mainly BPV-1/2) was detected in 22% of lesions and one skin sample from affected animals, and in 15% (BPV-1/-2) and 23% (BPV-3/4/6/9/10) of skin samples from healthy cows. Using quantitative PCR, *Treponema* DNA was demonstrated in 38/45 BDD lesions, with bacterial DNA loads ranging between 2×10^3 and 2.78×10^5 copies/40 ng of total DNA. Qualitative PCR confirmed this result and revealed *Treponema* DNA in 4 additional BDD samples, thus leading to an overall infection rate of 93.3%. Sequence analysis of amplified *Treponema* DNA revealed *T. pedis* sp. *nov*. in 51%, *T. medium* ssp. *bovis* in 37.7%, and *T. phagedenis* ssp. *vaccae* in 4.4% of lesions. *T. brennaborense* was not detected in any of the samples. Six BDD samples contained type IV oral *Treponema* strains, 6 other harboured so far unpublished *Treponema* sequences. To our knowledge, this is the first report providing information on BPV infection in BDD-affected cattle, and the *Treponema* DNA load and occurrence of type IV treponemes in BDD samples. Our findings further support an etiologic association of treponemes, particularly *T. pedis* sp. *nov*., with BDD disease, yet indicate that BPVs do not directly contribute to BDD development.

1. Introduction

Bovine digital dermatitis (BDD) is a common inflammatory claw disease in cattle which affects the epidermis proximal to the skin–horn junction in the flexor region of the interdigital space (Cheli and Mortellaro, 1974). In severe cases BDD causes considerable pain and lameness, leading to frequent recumbency, reduced ingestion and decreased performance. The incidence of BDD is increasing worldwide, thus seriously compromising the welfare of affected animals and causing substantial economic losses (Argaez-Rodriguez et al., 1997; Hernandez et al., 2001, 2002; Warnick et al., 2001; Losinger, 2006; Amory et al., 2008; Hulek et al., 2010). Considerable efforts have been made to elucidate BDD aetiology. The impact of unhygienic stabling on BDD progression and the effectiveness of antibiotics are both indicative for a causal involvement of anaerobic bacteria (Sabo et al., 1988; Blowey et al., 1994; Döpfer et al., 1997; Trott et al., 2003; Strub et al., 2007). There is increasing evidence that treponemes contribute to BDD development (Blowey et al., 1992; Read et al., 1992; Walker et al., 1995; Choi et al., 1997; Demirkan et al., 1998;

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Schrank et al., 1999; Trott et al., 2003; Demirkan et al., 2006; Strub et al., 2007; Evans et al., 2008, 2009), but only *Treponema brennaborense* has been proposed as aetiologic agent of BDD thus far (Schrank et al., 1999). To date, BDD is hence assumed to be of multifactorial origin, with treponemes and possibly other as yet unidentified infectious agents contributing to disease aetiology (Metzner, 2001; Trott et al., 2003; Laven and Logue, 2006). Recently, the presence of bovine papillomavirus type 1 (BPV-1) DNA and transcripts has been reported for canker (Brandt et al., 2010) – a chronic, hyperproliferative dermatitis of the equid hoof (Rooney and Robertson, 1996; Dietz, 2006), and equine inflammatory skin conditions (Yuan et al., 2007). These findings encouraged us to screen BDD tissue, and intact skin of affected and healthy cows for BPV DNA. In parallel, we determined *Treponema* DNA loads in BDD samples and identified respective *Treponema* types residing within individual lesions. By this, we aimed at elucidating whether BPV and treponemes act as partners in crime in the aetiology of BDD.

2. Materials and methods

2.1. Sample material

Animals enrolled in this study originated from 4 different farms in Lower Austria, i.e. from Breitenfurt (11 Holstein-Friesian and 2 German Jersey), Herzogenburg (14 Austrian Simmental and 1 Holstein-Friesian), St. Pölten (18 Austrian Simmental) and Berndorf (14 Austrian Simmental, 14 Austrian Brown Swiss, and 2 Holstein-Friesian). BDD diagnosis was clinically established by JK, the BDD lesions were evaluated visually and by digital palpation and the localisation was noted. Tissue (1 sample/cow; yet 2 samples from individual 10) from acute and chronic BDD lesions was obtained in the course of claw trimming. Swabs (1 swab/cow) of intact skin were collected from the neighbourhood of BDD lesions in case of 8 affected animals and from the fetlock region of 3 healthy animals. In addition, skin biopsies (1 biopsy/cow) were taken from the neck of 30 clinically normal cows following local anaesthesia using 4 mm biopsy punches according to the protocol described in Animal Trial Licence GZ 68.205/0055-II/10b/2009. Table 1 depicts the sample collection scheme.

2.2. Sample processing

Sample material was used for total DNA isolation that was performed by DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to instructions of manufacturer. DNA concentrations were determined by photometry. PCR-compatible purity of DNA isolates was evaluated by routine β-actin PCR as described previously (Brandt et al., 2008). This led to the exclusion of 2 of 86 DNA samples.

2.3. PCR for bovine papillomavirus DNA

In a next step, remaining 84 DNA samples were screened by PCR for the presence of BPV types 1–10. The reactions were performed in a final volume of 20 μl, containing 1× Phusion HF buffer (Finnzymes, Espoo, Finland), 3% DMSO, 200 μM Deoxynucleotide solution Mix (Finnzymes), 0.5 μM of sense and antisense primer (VBC Biotech, Vienna, Austria), 1 μl of DNA template and 0.4 U of Phusion® Hot Start High-Fidelity DNA Polymerase (Finnzymes). The amplification program consisted of an initial enzyme activation step at 98 °C for 2 min, followed by 45 thermal cycles {98 °C for 15 s; AT for 30 s; 72 °C for 30 s} and a final elongation step {72 °C for 5 min}. Reactions were conducted in a FlexCycler (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Primers used for detection of BPV-1/2 (BPV12f: 5′-cactacctcctggaatgaacatttcc-3′; BPV12r: 5′-ctaccttwggtatcacatctggtggtgg-3′; annealing temperature [AT]: 66 °C; amplicon length [AL]: 499 bp), BPV-3/4/6/9/10 (BPV310f: 5′-racacggatgabtttgtyacwcgmac-3′; BPV310r: 5′-cccartgytchccwtcrcargg-3′; AT: 68 °C; AL: 435 bp), BPV-5 and -8 (BPV58f: 5′-ttttatcatggagaaacagaaagactac-3′; BPV58r: 5′-caaagcctatrtccatcatatctcc-3′; AT: 63 °C; AL: 501 bp) and BPV-7 (BPV7f: 5′-ccaccaacgcagcctattgcaag-3′; BPV7r: 5′-ggtgcacaagacctgtcttcttgc-3′; AT: 57 °C; AL: 626 bp) were designed according to reference sequences with respective Genbank accession numbers X02346, AF486184, X05817, AJ620206, AJ620208, DQ217793, DQ098913, AB331650 and AB331651.

To date, more than 200 different HPV types have been identified in humans. In cattle, 10 distinct BPV types have so far been characterised and it is assumed that there are many more which still remain unknown (Campo, 2006). Therefore DNA samples from farms 1 to 3 were also analysed by using universal BPV MY and FAB primers according to the protocol described by Ogawa et al. (2004). Amplification products were gel-extracted (QiaEX II kit, Qiagen), cloned into plasmid vector pCR2.1-TOPO (Invitrogen, Lofer, Austria) and sequenced (VBC Biotech). Obtained DNA sequences were analysed by BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Quantification of Treponema DNA

Using a 16s rRNA gene-specific primer and probe system, DNA aliquots were subjected to quantitative real-time PCR (qPCR) as to determine respective concentrations of

Table 1
Sample collection scheme.

Farm	BDD tissue samples	Intact skin from BDD patients	Intact skin from healthy cows
1. Breitenfurt	11 BDD samples	0	2 skin swabs
2. Herzogenburg	16 BDD samples	6 skin swabs	1 skin swab
3. St. Pölten	18 BDD samples	2 skin swabs	0
4. Berndorf	0	0	30 skin biopsies
Total	45 BDD samples	8 skin swabs	33 swabs or biopsies