Short communication

Treponema species in the subgingival microflora of ovine periodontitis

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DENTAL changes are one of the main reasons for slaughtering sheep early before the end of their regular breeding period, resulting in increased costs due to the need to purchase new animals for the flock. Within this context, excessive dental wear and periodontal disease are key disorders affecting sheep teeth and bearing structures (West and Spence 2000). Periodontitis manifests as gingivitis, periodontal pocket formation, gingival recession, bleeding on probing, suppuration, food debris retention, teeth loosening and loss of teeth. Studies have reported similarities between oral microflora in sheep and human periodontitis (Frisken and others 1989, Ismaiel and others 1989, McCourtie and others 1990). Riggio and others (2013) demonstrated the prevalence of *Mannheimia ruminalis* and *Moraxella caprae* in sheep with broken mouth periodontitis. Among the microorganisms considered periodontopathogenic, those belonging to Socransky's red complex (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) are of recognised importance in several forms of periodontitis in humans and a few other animal species (Hardham and others 2005, Booij-Vrieling and others 2010, Yamasaki and others 2012). In advanced cases of periodontitis, spirochaetes may comprise more than 50 per cent of the subgingival microflora (Chu and Holt 1994), enabling their adherence to skin cells and, consequently, the invasion of adjacent tissues, and thus linking disease incidence and severity with large numbers of oral treponemes (You and others 2013). The aetiopathogenesis of sheep periodontitis, as well as the detailed microbiota composition associated with this disease, are still unknown. To increase knowledge on the microorganisms related to sheep periodontitis, the current study aimed to detect, through PCR, species of spirochaetes belonging to the genus *Treponema* within subgingival biofilm samples of sheep with and without periodontitis.

The clinical status of the sheep was determined after intra-oral and periodontal examinations, taking into consideration the Ethics Committee on Animal Experiment criteria (Process FOA no 2015-00280) during all stages of the examinations. The oral cavity was examined with the aid of a mouth-opening device and periodontal pocket depth was determined by using a graduated periodontal probe. Samples of the periodontal pocket (n=14) and the gingival sulcus (n=20) were collected from each sheep reared in farms located in endemic and non-endemic areas. Gingival sulcus or periodontal pocket material sampling procedures were undertaken in accordance with the recommendations described by Gaetti-Jardim Jr and others (2012). After supragingival bacterial biofilm removal with a sterile gauze pad, samples were loaded into a paper cone and left for about 60 seconds. Then, the cone was transferred to a tube containing 1 ml of sterile ultrapure water and stored at −80°C until DNA extraction. The bacterial DNA of each sample was extracted by a commercial DNA extraction kit (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma). Specific primers were used to identify the species *Treponema amylovorum*, *T denticola*, *T maltophilum*, *T medium*, *T pectinovorum*, *T socranskii* and *T vincentii*. The PCR procedures are described by Gaetti-Jardim Jr and others (2012).

Through this analysis we observed that, among the periodontal pocket samples, *T amylovorum* was present in 78.6 per cent (11/14) of samples, *T denticola* in 78.6 per cent (11/14), *T maltophilum* in 7.1 per cent (1/14), *T medium* in 21.4 per cent (3/14) and *T pectinovorum* in 64.3 per cent (9/14) of samples. *T socranskii* and *T vincentii* were not detected in any of the diseased samples. In addition, none of the tested species was found in the gingival sulcus of periodontally healthy sheep.

Determination of the presence of *T amylovorum*, *T denticola*, *T maltophilum*, *T medium* and *T pectinovorum* in the periodontitis lesions of sheep provides a major contribution to the understanding of the role played by these potentially pathogenic bacteria as a causative factor of this disease. Even though periodontitis is regarded as a multibacterial disease (Holt and Ebersole 2005) in which various species are organised in complexes (Socransky and others 1998), a few species of microorganisms are recognised as being of outstanding importance due to a series of cumulative findings. Spirochaete species have been associated with periodontal breakdown through molecular techniques or solely based on test for antibodies. According to quantitative studies of human oral microbiota, when prevalent and at high levels in cases of severe periodontitis, *T denticola* plays an important role in disease progression (Tanner and others 1994, Choi and others 1996, Riviere and others 1999). Against this background, *T denticola* and *T sockranskii* are related to the severity of human periodontal tissue destruction (Takeuchi and others 2001). *T denticola* and other members of the red complex are present in high numbers in pockets deeper than 3 mm (Ximénez-Fyvie and others 2000); therefore, in general, the best way to differentiate between healthy and diseased tissues is by determination of the prevalence, counts and proportion of species of this complex (Socransky and others 1998). According to Song (2005), besides its ability to detect small cell numbers, the PCR technique also has the advantage of being specific, contributing to the identification of species and to improved understanding of their roles in the disease.

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Microorganisms of the genus *Treponema* are commonly found in large numbers in deeper pockets. Spirochaetes are rarely found or exist to a lesser extent in healthy periodontal sites. On the other hand, inflamed gingiva sites without insertion loss exhibit low to moderate levels of these bacteria (Moore and others 1982). Characterising these microorganisms as periodontal-specific pathogens is difficult because of their inability to grow in vitro. In general, verification of the presence of *Treponema* species in oral microbiota linked to periodontitis is restricted when classic culture media and isolation methods are employed. These microorganisms are most often found in periodontal pockets of diseased cattle rather than in healthy animals (Borsanelli and others 2015). Determination of the presence of *T amylovorum*, *T denticola*, *T maltophilum*, *T medium* and *T pectinovorum* in periodontitis lesions of sheep represents an original contribution to studies on the aetiopathogenesis of sheep periodontitis.

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