J Clin Periodontol 2010; 37: 888–893 doi: 10.1111/j.1600-051X.2010.01593.x

Journal of Clinical Periodontology

# Association between periodontal disease, bacterial vaginosis, and sexual risk behaviours

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*Zabor EC, Klebanoff M, Yu K, Zhang J, Nansel T, Andrews W, Schwebke J, Jeffcoat M. Association between periodontal disease, bacterial vaginosis, and sexual risk behaviours. J Clin Periodontol 2010; 37: 888–893. doi: 10.1111/j.1600-051X.2010.01593.x.*

***Abstract***

**Background:** Both periodontal disease and bacterial vaginosis may cause adverse pregnancy outcomes. This study evaluated the association between periodontal disease and bacterial vaginosis.

**Materials and Methods:** Data from 3569 women enrolled in the Longitudinal Study of Vaginal Flora were used. Periodontal disease, defined as greater than three sites with $\geq 4$ mm attachment loss, was assessed by specially calibrated hygienists at baseline. Positive bacterial vaginosis status was based on a Nugent Gram stain score $\geq 7$. Pairs of independent variables were compared with Pearson's $\chi^2$ and risk ratios were calculated through log-binomial regression.

**Results:** Twenty-eight per cent of women with bacterial vaginosis had periodontal disease compared with 22% without , corresponding to 1.29 (95% CI: 1.13, 1.47) times greater risk of periodontal disease among women with bacterial vaginosis. In adjusted analysis the risk ratio dropped to 1.23 (95% CI: 1.08, 1.40). Receptive oral sex with an uncircumcised partner was associated with 1.28 times (95% CI: 0.97, 1.69) the risk for periodontal disease compared with receptive oral sex with a circumcised partner, though the association is not statistically significant.

**Conclusions:** In this population, there is a small but significant association between periodontal disease and bacterial vaginosis and a possible trend between receptive oral sex with an uncircumcised partner and periodontal disease.

Key words: bacterial; bacterial infections; epidemiology; periodontal diseases; sexual behaviour; vaginosis

Accepted for publication 1 May 2010

Adverse pregnancy outcomes are a significant cause of neonatal morbidity and mortality in the United States. In 2007, the preterm birth rate was 12.7% and the rate of low birth weight was 8.2% (Hamilton et al. 2009). While these numbers are down slightly from the 2006 figures, they continue to constitute a major public health concern.

**Conflict of interest and source of funding statement**

The authors declare that there are no conflicts of interest in this study.
The Longitudinal Study of Vaginal Flora was supported by intramural funds (Z01-HD002535-10), and by contract (N01-HD-8-3293) from the NIH, NICHD.

Periodontitis is a condition in which dental bacterial plaque cause an inflammatory response that leads to loss of connective tissue attachment, ultimately resulting in loss of affected teeth (Tatakis & Kumar 2005). Periodontal disease is prevalent among 3.6% of US adults, with significant differences in prevalence by race and socioeconomic status (Borrell & Crawford 2008). Symptoms include bleeding when brushing or flossing, inflammation at the site, and tooth loosening and ultimately tooth loss.

Bacterial vaginosis is a highly prevalent condition with unknown aetiology. Bacterial vaginosis affects nearly 30% of women between the ages of 14 and 49, with significant differences by age, race, and income (Allsworth & Peipert 2007). Bacterial vaginosis is characterized by a decrease in the normally predominant Lactobacillus bacterial species, and a corresponding increase in a diversity of anaerobic microbes (Fredricks et al. 2005). Bacterial vaginosis is typically asymptomatic, but if symptoms are present, the most common complaints are discharge and odour (Klebanoff et al. 2006).

Both periodontal disease and bacterial vaginosis have been linked to adverse pregnancy outcomes such as pre-term birth and low birth weight in numerous studies (Holst et al. 1994, Hillier et al. 1995, Offenbacher et al. 1996, Jeffcoat et al. 2001, Goepfert et al. 2004, De Seta et al. 2005, Dortbudak et al. 2005), yet the pathological mechanisms through which they may induce an immune

response resulting in pre-term birth remain unclear. While not identical, microbiological similarities between the oral and vaginal environments in the presence of periodontal disease and bacterial vaginosis, respectively, may indicate a common pathophysiology, such as a deficient host response to infection (Genco 1992, Cauci 2004). However, the relationship between periodontal disease and bacterial vaginosis remains largely unexamined. The present study aims to evaluate the association between periodontal disease and bacterial vaginosis in a large cohort of non-pregnant women, and to further examine possible risk factors, particularly sexual risk behaviours, for periodontal disease.

## Materials and Methods

### Participants

Study participants were enrolled through the Longitudinal Study of Vaginal Flora, which has previously been described in detail (Klebanoff et al. 2004). In brief, 3620 non-pregnant women between the ages of 15 and 44 were recruited between August 1999 and February 2002 when presenting for routine care at 12 health clinics in Birmingham, AL, USA. The purpose of the Longitudinal Study of Vaginal Flora was to assess the natural history of bacterial vaginosis. Those with severe medical or gynaecological conditions were excluded, as well as anyone planning to become pregnant or move from the area in the next 12 months. All participants provided written informed consent. This study was approved by the Institutional Review Boards of the University of Alabama at Birmingham, the Jefferson County (Alabama) Department of Health, and the NICHD. All women provided written informed consent.

### Interviews and exams

In-person interviews by trained female research staff were conducted to collect standard data on demographic characteristics, sexual risk behaviours, feminine hygiene practices, and vaginal symptoms at baseline and up to four quarterly follow-up visits. A full dental examination and interview regarding dental health behaviours were performed at the baseline visit. Specially trained and calibrated dental hygienists examined gingiva for redness, swelling, bleeding, and recession, defined as the distance from the cemento-enamel junction to the gingival margin. Each tooth was probed at six sites (three buccally and three lingually) using a North Carolina probe to measure probing pocket depth, defined as the distance between the gingival margin and the base of the pocket. Clinical attachment level was then calculated as probing depth plus recession. As there is no consensus in the epidemiological literature as to how periodontal disease is defined (Savage et al. 2009), here we characterize periodontal disease as three or more sites with $\geq 4$ mm clinical attachment loss.

Bacterial vaginosis status was determined through Gram stain evaluation of vaginal fluid obtained by cotton swab, scored using the Nugent criteria (Nugent et al. 1991). In this study, bacterial vaginosis was defined as a Nugent score $\geq 7$, normal flora as a Nugent score 0–3, and intermediate flora as a Nugent score 4–6.

### Statistical analysis

We tested for associations of demographic characteristics, oral hygiene behaviours, and sexual risk factors with a woman's bacterial vaginosis and periodontal disease status. We also examined the association between periodontal disease status and bacterial vaginosis status. Potential confounders for adjusted analysis were determined a priori and included race, age, education, marital status, cigarette smoking, brushing, flossing, vaginal sex, receptive oral sex, and insertive oral sex. Receptive oral sex is the act of a woman giving oral sex to a male partner, while insertive oral sex is the act of a woman receiving oral sex from a partner. Interactions that were of interest a priori included age by education and race by smoking. Through likelihood ratio tests, we determined that the interaction terms were not significant and did not include them in any further analyses.

Finally, we tested the association between receptive oral sex, partner's circumcision status, and periodontal disease. The predictor was defined with three levels: no receptive oral sex, receptive oral sex with a circumcised partner, and receptive oral sex with an uncircumcised partner. Since a woman could have more than one sex partner during the investigated time period, the predictor was assigned on a hierarchical basis, where receptive oral sex with an uncircumcised partner was considered the highest risk and no receptive oral sex was considered the lowest risk.

Pearson's $\chi^2$-test was used to test for independence between pairs of categorical variables. Student's *t*-test was used to test for independence between groups when the variables were continuous. Risk ratios and confidence intervals were calculated using either univariate or multivariate log-binomial regression. Risk ratios were utilized instead of odds ratios as this was a cross-sectional analysis and periodontal disease is a common outcome, affecting 25% of the study population (McNutt et al. 2003).

## Results

### Summary of study participants

Fifty-one participants were excluded because they did not have a dental exam, resulting in a total of 3569 participants for analysis in this study. Demographic and behavioural characteristics of these women and their associations with periodontal disease and bacterial vaginosis are presented in Table 1. Participants with periodontal disease tended to be older, more frequent smokers, currently or formerly married, and brushed their teeth less frequently. Participants with bacterial vaginosis tended to be black, never married or formerly married, moderate smokers, and less educated. Both women with bacterial vaginosis and women with periodontal disease were less likely to have regular dental checkups or professional cleanings and also flossed less frequently. Finally, women with bacterial vaginosis had more frequent vaginal sex than women with normal vaginal flora. Of the 3569 women included in this analysis, 26% had periodontal disease and 40% had bacterial vaginosis at their baseline visit.

### Periodontal disease and bacterial vaginosis

Overall, 28% of women with bacterial vaginosis had periodontal disease and 22% of women without bacterial vaginosis had periodontal disease. This difference corresponds with 1.29 (95% CI: 1.13, 1.47) times the risk of periodontal disease among women with bacterial vaginosis in unadjusted analysis. After adjusting for race, age, education, marital status, cigarette smoking, toothbrushing, tooth flossing, vaginal sex, insertive oral sex, and receptive oral sex, the association remained significant and indicated 1.23 (95% CI: 1.08, 1.40) times the risk of periodontal disease

*Table 1* Factors associated with periodontal disease (PD) and bacterial vaginosis (BV) at the first study visit

| | *N* | % PD ($n = 912$) | *p*-value* | % BV ($n = 1434$) | *p*-value* |
|---|---|---|---|---|---|
| Nugent score | | | <0.001 | | |
| Normal (0–3) | 1277 | 21.9 | | – | |
| Intermediate (4–6) | 858 | 26.5 | | – | |
| BV (≥ 7) | 1434 | 28.2 | | – | |
| Periodontal disease | | | | | <0.001 |
| Yes | 912 | – | | 44.4 | |
| No | 2657 | – | | 38.7 | |
| Race | | | 0.92 | | <0.001 |
| Black | 2849 | 25.5 | | 44.2 | |
| Other | 720 | 25.7 | | 24.2 | |
| Age | | | <.0001 | | 0.13 |
| 14–19 | 888 | 13.0 | | 36.0 | |
| 20–24 | 1185 | 20.6 | | 41.7 | |
| 25–29 | 686 | 29.7 | | 41.8 | |
| 30–45 | 810 | 43.1 | | 41.1 | |
| Education | | | 0.74 | | <0.001 |
| ≤12 | 2645 | 25.7 | | 42.2 | |
| >12 | 918 | 25.2 | | 34.5 | |
| Marriage status | | | <0.001 | | <0.001 |
| Never married | 2507 | 22.9 | | 41.8 | |
| Currently married | 573 | 30.2 | | 31.1 | |
| Other† | 489 | 34.0 | | 42.3 | |
| Cigarette smoking‡ | | | <0.001 | | <0.001 |
| Never | 2368 | 23.4 | | 38.3 | |
| ≤10/day | 776 | 27.8 | | 47.0 | |
| >10/day | 400 | 33.5 | | 38.8 | |
| Regular dental check ups | | | <0.001 | | <0.001 |
| Yes | 1388 | 18.7 | | 34.7 | |
| No | 2173 | 29.9 | | 43.6 | |
| Professional dental cleaning | | | <0.001 | | <0.001 |
| Past year | 1009 | 16.8 | | 34.0 | |
| 1–3 years | 1194 | 23.8 | | 39.0 | |
| More than 3 years ago | 1178 | 34.7 | | 45.0 | |
| Never | 174 | 27.0 | | 51.7 | |
| Toothbrushing | | | 0.03 | | 0.19 |
| < Daily | 61 | 37.7 | | 44.3 | |
| daily | 1394 | 26.8 | | 42.1 | |
| Twice daily | 2109 | 24.4 | | 38.8 | |
| Tooth flossing | | | <0.001 | | 0.04 |
| Never | 1386 | 29.7 | | 42.8 | |
| < Once/week | 515 | 25.4 | | 38.8 | |
| Weekly | 679 | 22.4 | | 36.1 | |
| At least once/day | 984 | 22.0 | | 40.0 | |
| Vaginal sex‡ | | | 0.41 | | <0.001 |
| Never | 473 | 26.0 | | 31.3 | |
| ≤ Once/week | 1503 | 24.4 | | 40.3 | |
| > Once/week | 1593 | 26.5 | | 42.7 | |
| Receptive oral sex‡ | | | 0.76 | | <0.001 |
| Never | 2689 | 25.4 | | 42.0 | |
| ≤ Once/week | 631 | 26.6 | | 33.6 | |
| > Once/week | 249 | 24.5 | | 37.0 | |
| Insertive oral sex‡ | | | 0.76 | | 0.07 |
| Never | 2203 | 25.2 | | 41.0 | |
| ≤ Once/week | 911 | 26.5 | | 37.0 | |
| > Once/week | 455 | 25.5 | | 42.4 | |

*$p$-values determined based on Pearson $\chi^2$ statistic.
†Includes separated, divorced, and widowed.
‡Frequencies were reported for the previous 6 months.

among women with bacterial vaginosis. Furthermore, in adjusted analysis women with intermediate vaginal flora were 1.19 (95% CI: 1.02, 1.37) times more likely to have periodontal disease than women with normal vaginal flora, suggesting that an intermediate level of bacterial vaginosis is associated with an intermediate level of risk for periodontal disease (Table 2).

#### Risk markers for periodontal disease

The primary risk marker for periodontal disease identified in this study is age. Risk of periodontal disease progressively increases with age. In unadjusted analysis, women aged 30–45 were 3.33 (95% CI: 2.76, 4.02) times more likely to have periodontal disease than women aged 14–19. Other significant factors identified in unadjusted analysis included marital status and cigarette smoking. Toothbrushing and flossing were both associated with significantly lower risk of periodontal disease (Table 2). In multivariate analysis, age became an even stronger risk factor for periodontal disease, while cigarette smoking no longer had a significant association. We found that education and tooth flossing were associated with significantly lower risk of periodontal disease in multivariate analysis (Table 2).

#### Receptive oral sex and partner circumcision

A total of 139 women were missing data on whether they had receptive oral sex with a partner, on the partner's circumcision status, or both, leading to a sample size of 3430 for this sub-analysis, the results of which are presented in Table 3. Of the 3430, 3% had receptive oral sex with an uncircumcised partner, 16% had receptive oral sex with a circumcised partner, and 81% did not have receptive oral sex. In unadjusted analysis, women who had receptive oral sex with an uncircumcised partner were 1.37 (95% CI: 1.01, 1.84) times more likely to have periodontal disease as compared with women who had receptive oral sex with a circumcised partner. This risk ratio decreased to 1.28 (95% CI: 0.97, 1.69) after adjustment for race, age, education, marital status, cigarette smoking, toothbrushing, tooth flossing, vaginal sex, and insertive oral sex.

## Discussion

Our results indicate that bacterial vaginosis is associated with periodontal disease. Even after adjusting for other commonly cited risk factors and possible sexual risk behaviours, we found a significant 23% increase in risk of periodontal disease among women with bac-

*Table 2* Risk ratios for periodontitis in the subset of women who had a dental exam at their first visit in the Longitudinal Study of Vaginal Flora

| | RR* | 95% CI | | aRR† | 95% CI | |
|---|---|---|---|---|---|---|
| Nugent score | | | | | | |
| Normal (0–3) | Ref | | | Ref | | |
| Intermediate (4–6) | 1.21 | 1.04 | 1.40 | 1.19 | 1.02 | 1.37 |
| BV (≥7) | 1.29 | 1.13 | 1.47 | 1.23 | 1.08 | 1.40 |
| Demographic characteristics | | | | | | |
| Race | | | | | | |
| Other | Ref | | | Ref | | |
| Black | 0.99 | 0.86 | 1.14 | 0.95 | 0.81 | 1.11 |
| Age | | | | | | |
| 14–19 | Ref | | | Ref | | |
| 20–24 | 1.59 | 1.30 | 1.95 | 1.67 | 1.35 | 2.05 |
| 25–29 | 2.30 | 1.87 | 2.82 | 2.47 | 2.00 | 3.06 |
| 30–45 | 3.33 | 2.76 | 4.02 | 3.68 | 3.00 | 4.51 |
| Education | | | | | | |
| ≤12 | Ref | | | Ref | | |
| >12 | 0.98 | 0.86 | 1.11 | 0.85 | 0.74 | 0.97 |
| Marital status | | | | | | |
| Never married | Ref | | | Ref | | |
| Currently married | 1.32 | 1.14 | 1.53 | 0.97 | 0.84 | 1.13 |
| Other‡ | 1.49 | 1.29 | 1.71 | 0.88 | 0.75 | 1.02 |
| Health behaviours | | | | | | |
| Cigarette smoking§ | | | | | | |
| Never | Ref | | | Ref | | |
| ≤10/day | 1.19 | 1.04 | 1.36 | 1.04 | 0.92 | 1.19 |
| >10/day | 1.43 | 1.22 | 1.67 | 1.17 | 0.99 | 1.37 |
| Toothbrushing | | | | | | |
| <Daily | Ref | | | Ref | | |
| Daily | 0.71 | 0.51 | 0.99 | 0.94 | 0.69 | 1.26 |
| Twice daily | 0.65 | 0.47 | 0.90 | 0.88 | 0.65 | 1.20 |
| Tooth flossing | | | | | | |
| Never | Ref | | | Ref | | |
| <Once/week | 0.86 | 0.72 | 1.01 | 0.91 | 0.78 | 1.07 |
| Weekly | 0.75 | 0.64 | 0.89 | 0.77 | 0.66 | 0.91 |
| At least once/day | 0.74 | 0.64 | 0.85 | 0.75 | 0.65 | 0.86 |
| Sexual behaviours | | | | | | |
| Vaginal sex§ | | | | | | |
| Never | Ref | | | Ref | | |
| ≤Once/week | 0.94 | 0.79 | 1.12 | 0.90 | 0.76 | 1.07 |
| >ONCE/week | 1.02 | 0.86 | 1.21 | 0.92 | 0.77 | 1.10 |
| Receptive oral sex§ | | | | | | |
| Never | Ref | | | Ref | | |
| ≤Once/week | 1.05 | 0.91 | 1.21 | 0.91 | 0.76 | 1.08 |
| >Once/week | 0.96 | 0.77 | 1.21 | 0.87 | 0.66 | 1.14 |
| Insertive oral sex§ | | | | | | |
| Never | Ref | | | Ref | | |
| ≤Once/week | 1.05 | 0.92 | 1.20 | 1.05 | 0.91 | 1.23 |
| >Once/week | 1.01 | 0.85 | 1.20 | 1.04 | 0.84 | 1.27 |

*Risk ratios based on univariate log-binomial regression.

†Adjusted risk ratios based on a multivariate log-binomial regression model that included all variables listed in the table.

‡Frequencies were reported for the previous 6 months.

§Includes separated, divorced, and widowed.

RR, risk ratio; CI, confidence interval; aRR, adjusted risk ratio.

terial vaginosis. Our results support those from a recent prospective study of 180 non-pregnant women, which found that women with a diagnosis of bacterial vaginosis were more likely to have gingivitis, a condition that is often a precursor to periodontal disease (Persson et al. 2009).

Periodontal disease and bacterial vaginosis may be linked through certain microbial species. Analysis of serum antibodies from 823 women enrolled in a clinical trial investigating the association between periodontitis and pregnancy outcomes showed that women who experienced pre-term deliveries had significantly higher antibody levels to oral *Fusobacterium nucleatum* bacteria, a species that is also implicated in bacterial vaginosis (Ebersole et al. 2009). Microbial species such as *F. nucleatum* may be common to both bacterial vaginosis and periodontal disease through either haematogenous spread or oral–genital transfer (Hill 1993).

In a cross-sectional study that included 411 women, Harville et al. (2004) found that receptive oral sex was associated with gum problems such as bleeding gums, a common indicator of gum disease. While the results of the present study did not detect a significant association between receptive oral sex and a woman's periodontal disease status, we did find a significant association between insertive oral sex and bacterial vaginosis, which suggests the possibility of male to female oral–genital transfer of bacteria. A review article indicates that while the issue is still controversial, there are cases where bacterial species associated with bacterial vaginosis have been found in the mouths or throats of male partners who had performed insertive oral sex on a female partner identified as carrying the same bacterial species vaginally (Edwards & Carne 1998). Infection in either direction, though, is possible. As we did not have data on the periodontal disease status of male sex partners, the relationship could not be investigated further here and future research is warranted.

While bacterial vaginosis is not generally considered to be a sexually transmitted disease, it is closely tied to sexual behaviours such as having new or multiple sex partners, having female sex partners, and condom use (Fethers et al. 2008). Because of the strong evidence for a relationship between sexual practices and bacterial vaginosis but in the absence of evidence for causation, Verstraelen (2008) suggests that bacterial vaginosis be referred to as a sexually enhanced disease. The hypothesis that sexual contact antagonizes an environment that is already prone to infection is a plausible argument in relation to periodontal disease as well.

Alternatively, genetic susceptibility may predispose certain women to initial infection with the bacterial species common to bacterial vaginosis and periodontal disease. A recent review article further suggests a possible gene–environmental interaction, where only women with bacterial vaginosis or periodontal disease who are genetically predisposed to mount a damaging inflammatory response to the oral or genital bacteria will experience pre-term birth or other adverse pregnancy outcomes (Pretorius et al. 2007). Offenbacher et al. (2009)

*Table 3* Association of periodontal disease with receptive oral sex and partner circumcision status in the Longitudinal Study of Vaginal Flora (LSVF)

| | *N** | RR | 95% | CI | aRR† | 95% | CI |
|---|---|---|---|---|---|---|---|
| No receptive oral sex‡ | 2784 | 0.96 | 0.83 | 1.12 | 1.01 | 0.84 | 1.20 |
| Receptive oral sex with circumcised partner‡ | 546 | Ref | | | Ref | | |
| Receptive oral sex with uncircumcised partner‡ | 100 | 1.37 | 1.01 | 1.84 | 1.28 | 0.97 | 1.69 |

*$n = 139$ women were missing data on whether they gave an oral sex to a partner, on the circumcision status of the partner, or both.
†Adjusted for race, age, education, marital status, cigarette smoking, toothbrushing, tooth flossing, vaginal sex, and receptive oral sex.
‡Frequencies were reported for the 6-month period preceding the first interview in LSVF.
RR, risk ratio; aRR, adjusted risk ratio.

suggest a model where periodontal disease causes an inflammatory response that acts as an effect modifier to the relationship between bacterial vaginosis and adverse pregnancy outcomes.

In a randomized trial of circumcision to reduce HIV incidence, Gray et al. (2009) found that the wives of men randomized to be circumcised had a 20% reduced risk of bacterial vaginosis infection. If some of the same microbial species can cause both bacterial vaginosis and periodontal disease, and having uncircumcised partners increases the risk for bacterial vaginosis, then it is possible that circumcision status may interact with receptive oral sex to increase the risk of periodontal disease through genital–oral transfer. In a trial comparing pre- and post-circumcision penile bacterial microbiota, Price et al. (2009) found significantly reduced presence of anaerobic bacteria after circumcision. As high concentrations of anaerobic bacteria are closely tied to periodontal disease, it is possible that circumcision status may lead to increased risk for periodontal disease through oral–genital contact. Our results, while not significant, were suggestive of increased risk of periodontal disease among women who give oral sex to uncircumcised male partners as compared with women who give oral sex to circumcised male partners (RR: 1.28; 95% CI: 0.97, 1.69). As only 100 women had an uncircumcised receptive oral sex partner in this study, further studies with a larger population of exposed participants are needed. Further, these results should be interpreted cautiously as male circumcision status is not random in a population, so there is high potential for the presence of unmeasured confounding in this sub-analysis.

The prevalence of both periodontal disease (26%) and bacterial vaginosis (40%) in our study population is higher than that in the most recently reported National Health and Nutrition Examination Survey (NHANES) data. This may be explained by the predominance of black women (80%) and women with less than or equal to a high-school education (73%) in the Longitudinal Study of Vaginal Flora. Borrell & Crawford (2008) report an overall prevalence of periodontitis of 3.6%, with significantly increased prevalence of 7.2% among black people, 11.7% among people with $<12$ years of education, and 5.6% among people with 12 years of education, based on NHANES 1999–2004. Allsworth & Peipert (2007) use NHANES 2001–2004 to report an overall prevalence of bacterial vaginosis of 29.2% and a prevalence of 51.6% among black women, 32.9% among women with less than a high-school education, and 33.8% among women with a high-school education.

In summary, there is a small but significant association between periodontal disease and bacterial vaginosis that needs to be examined in more depth in an effort to further understand the relationship between the two infections. There may be a relationship between periodontal disease, sexual practices, and male partner circumcision status that also warrants further investigation. Only by understanding the nature of these infections, risk factors for acquiring them, and the body's response can we begin to reduce their role in pre-term birth, which remains one of the leading causes of neonatal morbidity and mortality in the United States.

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**Clinical Relevance**

*Scientific rationale for the study*: Microbiological similarities between the oral and vaginal environments in the presence of periodontal disease and bacterial vaginosis, respectively, may indicate a common pathophysiology, which may in turn be responsible for adverse pregnancy outcomes.

*Principal findings*: There is a small but significant association between periodontal disease and bacterial vaginosis. A possible relationship between periodontal disease, sexual practices, and male partner circumcision status is observed in this study, but lacks conclusive evidence and should be addressed in future studies.

*Practical implications*: The association between periodontal disease and bacterial vaginosis suggests that both may result from similar behaviours, or from an individual susceptibility to chronic infection with anaerobic bacteria.