American Journal of Obstetrics and Gynecology (2005) **192**, 554–7

ELSEVIER

AMERICAN JOURNAL of OBSTETRICS and GYNECOLOGY

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Chronic maternal and fetal *Porphyromonas gingivalis* exposure during pregnancy in rabbits

Kim A. Boggess, MD,[a,b] Phoebus N. Madianos, DDS, PhD,[b] John S. Preisser,[c] Kenneth J. Moise Jr, MD,[a] Steven Offenbacher, DDS, PhD[b]

Department of Obstetrics and Gynecology, University of North Carolina School of Medicine,[a] Center for Oral and Systemic Inflammatory Diseases, University of North Carolina School of Dentistry,[b] and Department of Biostatistics, University of North Carolina School of Public Health,[c] Chapel Hill, NC

Received for publication June 14, 2004; revised August 12, 2004; accepted September 2, 2004

KEY WORDS
Porphyromonas gingivalis
Periodontal disease
Rabbit
Pregnancy

Objective: This study was undertaken to develop a rabbit model of maternal exposure to *Porphyromonas gingivalis* and determine whether fetal or placental exposure occurs.
Study design: Subcutaneous steel chambers were implanted in 8 New Zealand White female rabbits. On day 7 of pregnancy, 4 rabbits were inoculated through the chamber with 5×10^8 CFU/mL live *P gingivalis*, and 4 rabbits with broth (controls) and sacrificed at term. Polymerase chain reaction was used to detect *P gingivalis* in maternal and fetal liver and placenta. Fisher exact test was used to compare *P gingivalis* detection between groups.
Results: Among exposed does, *P gingivalis* was detected in 33% of the maternal livers, 49% of placentas, and 34% fetal livers compared with none from controls ($P < .001$).
Conclusion: Chronic maternal exposure to *P gingivalis* results in systemic dissemination, transplacental passage, and fetal exposure. This model may be useful to study placental and fetal effects of this oral pathogen and to study microbial dissemination across the placenta.

Periodontal disease is an infectious disease manifested by the progressive change of healthy resident commensal oral microbes to pathogenic ones within the biofilm of the tooth and gingiva.[1] Maternal periodontal disease has recently been linked to several adverse pregnancy outcomes. In an early case-control study we found that maternal periodontal disease was associated with delivery of a preterm low-birth weight infant,[2] a finding confirmed by 2 large prospective studies.[3,4] We have also demonstrated that maternal periodontal disease is associated with delivery of significantly smaller infants,[4] and with the development of preeclampsia.[5] Systemic dissemination of oral microbes with subsequent maternal, fetal, and/or placental inflammatory responses has been suggested as a possible causal mechanism.[6] Data from a pregnant mouse model of infection demonstrate that oral organisms translocate to the placenta and adversely affect fetal growth.[7,8] Furthermore, in a large cohort study, we found that 317 (58%) of 546 umbilical cord samples collected were positive for immunoglobulin M (IgM) to 1 or more

Supported by 5 K12 HD01441-03 and 1 K08 HD043284-01.
Presented at the Annual Meeting of the Infectious Diseases Society for Obstetrics and Gynecology, Banff, British Columbia, Canada, August 2002.
Reprints not available from the authors.
E-mail: kboggess@med.unc.edu

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doi:10.1016/j.ajog.2004.09.001

specific oral pathogens,[9] suggesting systemic dissemination and fetal exposure.

The purpose of this investigation was to create a rabbit model of chronic maternal exposure to *Porphyromonas gingivalis*, an oral pathogen associated with periodontal disease, to determine an appropriate inoculum for maternal exposure without systemic illness, and to determine whether systemic dissemination occurs and results in placental or fetal exposure.

Materials and methods

The Institution Animal Care and Use Committee of the University of North Carolina approved this study. Reproductive-aged female, *Pasteurella*-free, New Zealand White rabbits (*O cuniculus*) were purchased from a local breeder (Robinson Services, Greensboro, NC). Rabbits were maintained in an animal housing facility in stainless steel cages and fed antibiotic-free Purina Laboratory Chow (Purina Mills, Inc, St Louis, Mo). For breeding, a rabbit buck was placed into the cage with the female for 5 minutes on 2 consecutive days.

After acclimatization, animals were transported to the animal surgical facility, anesthetized with intramuscular ketacet (25 mg/kg) and domitor (0.5 mg/kg). Cylindrical surgical grade steel chambers, each 2 cm in length and 0.75 cm in diameter (Figure 1, *A*), were then surgically implanted subcutaneously on the dorsal side, between the scapulae (Figure 1, *B*). The skin was then closed in 1 layer with poliglecaprone 25 suture (Ethicon Inc, Somerville, NJ). Anesthesia was reversed with subcutaneous antisedan (0.5 mg/kg). Animals were observed for 4 hours and then daily.

Porphyromonas gingivalis strain A7436 was grown in anaerobic blood agar (Remel, Lenexa, Kan) in a Coy anaerobic chamber for 10 days. The bacterial cells were subcultured and grown until pure, and then colonies were inoculated into Wilkins-Chalgren anaerobic broth (Remel) and grown for approximately 18 hours.

Ten to 14 days after chamber placement, rabbits were sedated with 0.9 mL (10 mg/mL) acepromazine maleate, then inoculated with *P gingivalis* through the chamber. Dosing experiments were performed to determine the concentration of *P gingivalis* that would result in chronic exposure without systemic maternal illness. Microbial exposure consisted of 2 inoculums introduced into the implanted chamber: the first, heat-killed *P gingivalis,* which was followed 14 to 21 days later by inoculation with live *P gingivalis*. For heat-killed inoculums, live organisms were boiled for 10 minutes then cooled to room temperature.[10] Central ear artery blood sampling was performed on the day of inoculation with heat-killed *P gingivalis,* then on days 1, 5, 10, 15, 20, and 25 after exposure. The blood was allowed to clot, centrifuged at 2000*g*, with the resultant serum stored in 1-mL aliquots at −80°C. To confirm sensitization, *P gingivalis*-specific IgG was assayed by using a checkerboard immunoblot, as previously described.[11]

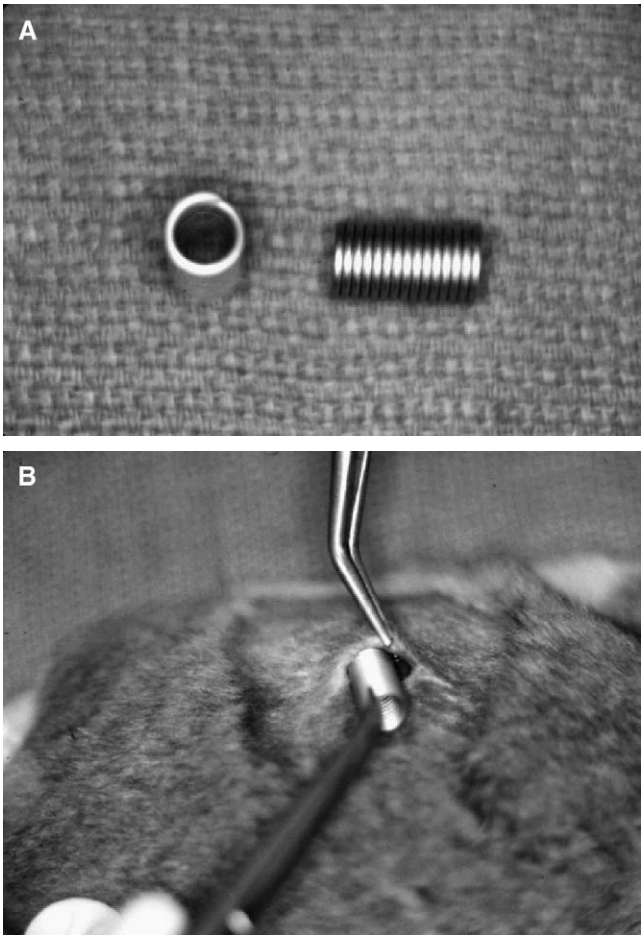

Figure 1 **A,** Surgical grade steel chambers used for *P gingivalis* inoculation. **B,** Placement of chamber into rabbit.

After testing doses of *P gingivalis* ranging from 10^9 CFU/mL to 10^7 CFU/mL, we determined that using 10^9 CFU/mL heat-killed, followed by 5×10^8 CFU/mL live *P gingivalis,* allowed for adequate maternal exposure (as demonstrated by doe serum *P gingivalis*-specific IgG) without inducing systemic illness or death.

Five female rabbits were inoculated through the chamber with 10^9 CFU/mL heat-killed *P gingivalis* and 5 were inoculated with an equal volume of sterile media (controls); all were observed daily for systemic signs of infection (temperature, feeding, behavior) for 3 to 5 days after inoculation and then mated 14 days later. On day 7 of gestation, corresponding with day of implantation, does were sedated, and those previously inoculated with heat-killed *P gingivalis* were inoculated through the chamber with 5×10^8 CFU live *P gingivalis*; controls were inoculated with sterile media. Animals were observed daily for systemic signs of infection (rectal temperature, feeding, behavior) for 5