

## ADHERENCE AND EXPERIMENTAL INFECTION OF BACTERIA ASSOCIATED WITH PERIODONTAL INFECTIONS OF YOUNG CATTLE IN BRAZIL ("CARA INCHADA")<sup>1</sup>

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**ABSTRACT-** Grassmann B., Döbereiner J., Dutra I.S., Kopp P.A. & Blobel H. 1997. Adherence and experimental infection of bacteria associated with periodontal infections of young cattle in Brazil ("Cara inchada"). *Pesquisa Veterinária Brasileira* 17(3/4):123-125. Embrapa-CNPAB/Projeto Saúde Animal Embrapa/UFRRJ, Km 47, Seropédica, Rio de Janeiro 23851-970, Brazil.

*In vitro*- and *in vivo*-assays were conducted, to study the possible role of streptomycin and actinomycin-producing soil actinomycetes for the pathogenesis of "Cara inchada" in cattle (CI). Adherence of *Bacteroides* spp. to epithelial cells of the bovine gingiva, known to be associated with the progressive lesions of CI, was significantly increased by the addition of streptomycin, actinomycin or antibiotic culture supernatants of the soil actinomycetes. Applications of these mixtures together with *Actinomyces pyogenes* to the marginal gingiva of the upper premolar teeth of about 1 month old Holstein Friesian calves did not lead to progressive lesions of CI. Only one calf exhibited a slight diarrhea and a temporary retraction of the gingiva at the site of application.

**INDEX TERMS:** "Cara inchada", cattle, periodontal infection, *Bacteroides* spp., streptomycin, actinomycin, adherence.

**SINOPSE.-** [Aderência e infecção experimental de bactérias associadas com infecções peridentárias de bovinos jovens no Brasil ("cara inchada").] Foram realizados ensaios *in vitro* e *in vivo* para estudar o possível envolvimento de actinomicetos do solo como produtores de estreptomicina e actinomicina na patogênese da periodontite da "cara inchada" dos bovinos (CI). A aderência de *Bacteroides* spp. às células epiteliais da gengiva marginal de bezerros, dos quais se sabe estarem associados às lesões progressivas da CI, aumentou significativamente através a adição de estreptomicina, actino-

micina ou de antibióticos produzidos pelo cultivo de actinomicetos do solo. Aplicações de *Bacteroides* spp. e *Actinomyces pyogenes*, junto com os antibióticos, na gengiva dos dentes premolares maxilares de bezerros da raça holandes preto-branco, de cerca de 1 mês de idade, não provocaram lesões progressivas da CI. Somente um bezerro mostrou leve diarréia e retração temporária da gengiva no local da aplicação.

**TERMOS DE INDEXAÇÃO:** "Cara inchada", bovinos, infecção peridentária, *Bacteroides* spp., estreptomicina, actinomicina, aderência.

### INTRODUCTION

*Bacteroides* spp. and *Actinomyces* (*A.*) *pyogenes* play a decisive role in the pathogenesis of "Cara inchada" in cattle (CI) (Blobel et al. 1984a,b, Blobel et al. 1987). It is of further interest that CI occurred in former forest areas and in the savanna ("cerrado") recently cleared for pasture and other agricultural use after "liming" that led to a modification of the soil microbiota with an increase of streptomycin-producing actinomycetes (Baldani et al. 1982, Döbereiner 1990, Dutra et al. 1993). Streptomycin was shown to increase the adherence of

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*Bacteroides* spp. to the epithelial cells of the bovine gingiva significantly *in vitro* (Kopp et al. 1996).

In this study we investigated the influence of actinomycin and culture supernatants of soil actinomycetes with antibiotic activities on the adherence of *Bacteroides* spp. to the epithelial cells *in vitro*. Then these studies were extended to determine if streptomycin and actinomycin could also promote infection under *in vivo*-conditions.

## MATERIALS AND METHODS

### Bacterial strains and culture conditions

*Bacteroides* spp., originally isolated from the gingiva of CI-lesions in young cattle (Blobel et al. 1984a,b, 1987), were cultured on CDC-blood agar plates (Dowell et al. 1977) under anaerobic conditions for 5 days at 37°C. For fluid cultivation a modified brain heart infusion (BHI) (Eley et al. 1985) was used. After adjustment to 10% transmission at 620 nm (i.e. 10<sup>9</sup> bacteria/ml) *Bacteroides* suspensions were kept at -20°C until use.

*A. pyogenes* was cultivated on blood agar plates under microaerobic conditions for 48 h at 37°C (Blobel et al. 1984a,b, 1987). Subsequently, one colony of these cultures was suspended in 1 ml phosphate buffered sodium (0.15 mol/l NaCl, 0.05 mol/l disodiumhydrogenphosphate-dihydrate; pH 7.5).

Actinomycetes, isolated from soil samples of farms with a high prevalence of CI, were kindly provided by Carlos Gava (Embrapa-CNPAB, Seropédica, Rio de Janeiro, Brazil). For antibiotic production these actinomycetes were cultivated in fluid APM-medium (Berwick 1988) under aerobic conditions for 7 days at 30°C. Biochemical characterization of these strains was conducted according to the methods of Pridham & Gottlieb (1948), Perlman & O'Brien (1956), Berd (1973), Pridham & Tresner (1974), Gordon (1974) and Land (1992). Culture supernatants could be obtained by centrifugation for 10 min at 10.000x g with subsequent sterile filtration (0.45 µm, Millipore, Bedford, Massachusetts, USA).

### Adherence assays (in vitro)

Adherence assays with *Bacteroides* spp. and bovine epithelial cells were conducted as previously described by Kopp et al. (1996). Acti-

nomycin was added in increasing concentrations from 5 to 25 µg/ml. Culture supernatants of the actinomycetes contained 0.1 mg/ml protein according to the method of Lowry et al. (1951). All adherence assays were repeated 3 times.

Exposure of calves to the bacteria in the presence or absence of the antibiotics/culture supernatants (in vivo)

For the experimental infections 5 Holstein Friesian calves, 18 to 35 days of age, were used. Mixtures of the bacteria (*Bacteroides* spp. and *A. pyogenes*) with streptomycin and actinomycin as well as the antibiotic supernatants from actinomycetes in dosages indicated in Table 1 and 2 were applied to the marginal gingiva of the upper premolar teeth at the site of the interdental papilla 3 times daily. Calves were examined daily for periodontal lesions and diarrhea indicating CI.

## RESULTS

### In vitro-adherence assays

Addition of actinomycin in concentrations of 5 to 25 µg/ml increased the adherence of *Bacteroides* spp. to the oral epithelial cells of calves for up to 335% (Fig. 1).

Adherence was also increased significantly by antibiotic culture supernatants of soil actinomycetes from farms with a high prevalence of CI (Fig. 2).

### Exposure of calves

After experimental infections of the 5 calves no progressive periodontal lesions developed. Only 1 (no. 4688) of the 5

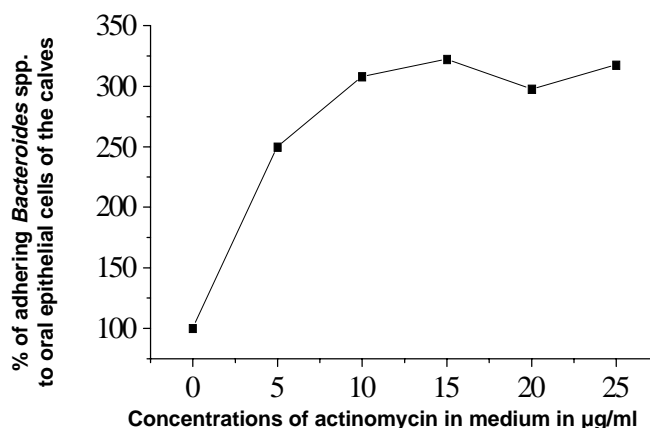


Fig. 1. Adherence of *Bacteroides* spp. to oral epithelial cells of calves after addition of actinomycin in increasing concentrations.

Table 1. Application-modus with Streptomycin

Calf number	<i>Bacteroides</i> spp. 10 <sup>9</sup> /ml	<i>A. pyogenes</i> 10 <sup>9</sup> /ml	Streptomycin
4688	400 µl	400 µl	20 µg/ml
4689	400 µl	400 µl	200 µg/ml
4690	400 µl	400 µl	2 mg/ml
4691	400 µl	400 µl	20 mg/ml
4692	400 µl	400 µl	200 mg/ml

Table 2. Application-modus with streptomycin, actinomycin and culture supernatants of soil actinomycetes with antibiotic activities

Calf number	<i>Bacteroides</i> spp. 10 <sup>9</sup> /ml	<i>A. pyogenes</i> 10 <sup>9</sup> /ml	Supernatants of streptomycetes	Combinations Streptomycin/ Actinomycin	Streptomycin 200µg/ml	Actinomycin 200µg/ml
4688	400 µl	400 µl	/	/	100 µl	/
4689	400 µl	400 µl	/	/	/	100 µl
4690	400 µl	400 µl	/	/	/	/
4691	400 µl	400 µl	100 µl	/	/	/
4692	400 µl	400 µl	/	100 µl each	/	/

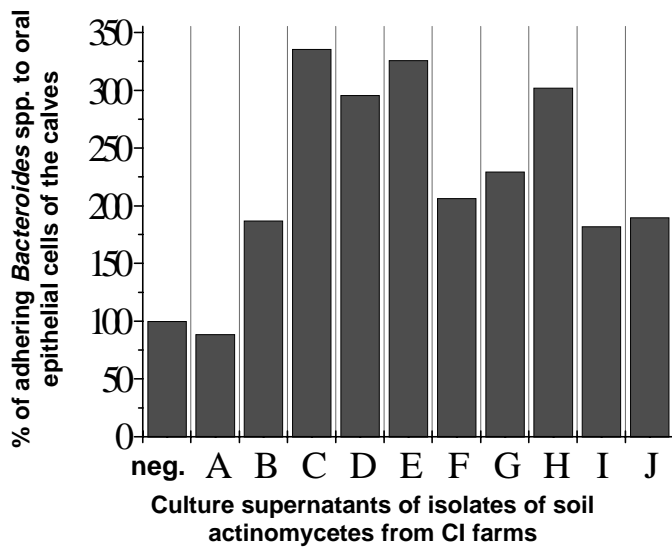


Fig. 2. Adherence of *Bacteroides* spp. to oral epithelial cells of the calves after addition of different culture supernatants from soil actinomycetes (neg. = without supernatants).

calves exhibited a slight retraction of the gingiva at the site of application and a diarrhea that persisted for a period of 6 days. Bacteriological examination of the feces of this calf revealed black pigmented colonies (*Bacteroides* spp.) on CDC-blood agar plates after cultivation under anaerobic conditions.

## DISCUSSION

In the *in vitro*-assays actinomycin significantly increased the adherence of *Bacteroides* spp. to the oral epithelial cells of the calves as reported previously for streptomycin (Kopp et al. 1996). Both antibiotics (streptomycin and actinomycin) are produced by soil actinomycetes, isolated in increased numbers in soils of regions with a high CI-prevalence (Baldani et al. 1982, Döbereiner 1990, Dutra et al. 1993). Thus, there appears to be a correlation between the antibiotic activities in the soil and the development of CI-lesions in calves. Most of the actinomycetes isolated from soil of CI-farms were biochemically identified as streptomycetes. The antibiotics produced by these actinomycetes increased *in vitro* the adherence of *Bacteroides* spp. to the oral epithelial cells of the calves significantly. On the other hand, *in vivo*-exposure of calves to streptomycin, actinomycin or the antibiotic supernatants together with *Bacteroides* spp. and *A. pyogenes* within an observation period of 20 days did not lead to any progressive lesions in the periodontium. This could be explained by the relatively short time of exposure and low frequency of applications as well as possibly insuitable antibiotic

concentrations in comparison to natural conditions. Nevertheless, it was confirmed *in vitro* that antibiotics produced by soil actinomycetes enhanced the adherence of CI-associated bacteria to the host epithelial cells and probably play a role as determinant factor in the pathogenesis of CI.

## REFERENCES

- Baldani J.L., Baldani V.L.D., Xavier D.F., Body R.M. & Döbereiner Joh. 1982. Efeito da calagem no número de actinomicetos e na porcentagem de bactérias resistentes à estreptomicina na rizosfera de milho, trigo e feijão. *Revta Microbiol.*, S. Paulo, 13: 250-263.
- Berd D. 1973. Laboratory identification of clinically important aerobic actinomycetes. *Applied Microbiol.* 25: 665-681.
- Berwick P.G. 1988. Beta-lactam and aminoglycoside production from streptomycetes. *J. Appl. Bacteriol.* 64: 9-15.
- Blobel H., Döbereiner J., Lima F.G.F. & Rosa I.V. 1984a. Bacterial isolations from „Cara inchada“-lesions in cattle. *Pesq. Vet. Bras.* 4(2):73-77.
- Blobel H., Döbereiner J., Lima F.G.F. & Rosa I.V. 1984b. Bakteriologische Aspekte bei der „Cara inchada“ von Jungrindern in Brasilien. *Giessener Beiträge zur Entwicklungsforschung, Reihe I, Band 10, JLU Giessen.*
- Blobel H., Döbereiner J., Rosa I.V., Lima F.G.F. & Dutra I.S. 1987. Bakteriologische Untersuchungen an der „Cara inchada“, einer periodontalen Erkrankung bei Rindern in Brasilien. *Tierärztl. Umschau* 2: 152-157
- Döbereiner J. 1990. Towards the etiology of „Cara inchada“, a periodontal disease of young cattle in Brazil. *Dtsch. Tierärztl. Wschr.* 97: 482-490.
- Dowdy Jr. V.R., Lombard G.L., Thompson F.S. & Armfield A.Y. 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. US Department of Health and Human Services, Centers for Disease Control, Atlanta, Georgia.
- Dutra I.S., Matsumoto T. & Döbereiner J. 1993. Surtos de periodontite em bezerros („cara inchada“) associados ao manejo de solo. *Pesq. Vet. Bras.* 13(1/2):1-4.
- Eley A., Greenwood D. & O'Grady F. 1985. Comparative growth of *Bacteroides* species in various anaerobic culture media. *J. Med. Microbiol.* 19: 195-201.
- Gordon M.A. 1974. Aerobic pathogenic *Actinomycetaceae*, p. 175-188. In: Lennette E.H., Spaulding E.H. & Truant J.P. (ed.) *Manual of Clinical Microbiology*. 2nd ed. American Society for Microbiology, Washington, D.C.
- Kopp P.A., Dutra I.S., Döbereiner J., Schmitt M., Grassmann B. & Blobel H. 1996. Streptomycin increases the adherence on oral epithelial cells of *Bacteroides melaninogenicus* involved in the periodontal lesions of „Cara inchada“ in cattle. *Pesq. Vet. Bras.* 16(2/3):53-57.
- Land G.A. 1992. Identification of the aerobic actinomycetes, p. 4.0.1-4.1.9. In: Isenberg H.D. (ed.) *Clinical Microbiology Procedures Handbook*. Vol. 1, 1st ed. American Society for Microbiology, Washington, D.C.
- Lowry O.H., Rosenbrough N.J., Farr J.A. & Randall R.J. 1951. Protein measurement with the folin reagent. *J. Biol. Chem.* 193: 265-275.
- Perlman D. & O'Brien E. 1956. Utilization of carbohydrates by strains of *Streptomyces griseus*. *J. Bacteriol.* 72: 214-218.
- Pridham T.G. & Gottlieb D. 1948. The utilization of carbon compounds by some actinomycetales as an aid for species determination. *J. Bacteriol.* 56: 107-114.
- Pridham T.G. & Tresner H.D. 1974. *Streptomycetaceae*, p. 747-881. In: Buchanan R.E. & Gibbons N.E. (ed.) *Bergey's Manual of Determinative Bacteriology*. 8th ed. Williams and Wilkins Company, Baltimore.