PROJETO

“Pesquisa de “Gastrospirillum hominis” Tipo 1 e Tipo 2 na Mucosa Gástrica de Seres Humanos e de Animais

Relatório Técnico Final

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Mouse inoculation for the detection of non-cultivable gastric tightly spiralled bacteria

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Abstract

In the present study we compared the inoculation of swine gastric mucosa into the stomach of mice, the urease test and carbol-fuchsin-stained smears for the diagnosis of the infection with "Gastrospirillum suis" ("Helicobacter heilmannii" type 1), an uncultivated tightly spiralled gastric bacterium. Fragments obtained from the antral and oxyntic mucosa of the stomach of 50 slaughtered pigs were used for urease test, for carbol-fuchsin-stained smears and for obtaining scrapings of mucus for mouse inoculation. The mice were killed by spinal dislocation 10 days after inoculation and fragments of the antral and oxyntic mucosa were used for spiral bacterium identification (urease test and carbol-fuchsin-stained smears). Among the methods employed for the diagnosis of "H. heilmannii" infection, the inoculation of gastric mucosa into the stomach of mice was the most sensitive and demonstrated bacterial positivity in 31 (62%) of the 50 pigs studied. Direct examination showed tightly spiralled bacteria in the gastric mucosa of only 4 (8%) of the 50 pigs studied. Among them, 3 (6%) presented a positive performed urease test. Spiral bacteria were not seen in the gastric mucosa of any control mice. These results show that the use of the mouse inoculation method improved the detection of "H. heilmannii" in swine.

Key words
- Helicobacter
- Helicobacter heilmannii
- Gastrospirillum suis infection diagnosis
- Gastric spiral bacteria
- Helicobacter infection diagnosis
- Mouse inoculation

Although spiral bacteria have been observed in the stomach of animals since the end of the 19th century (1,2), the interest in gastric bacteria has increased only after the isolation of Helicobacter pylori from the gastric mucosa of human patients with gastritis and peptic ulcer. Since then, several spiral organisms have been described in the gastric and intestinal mucosa of man and other animals (3). Among them, a tightly spiralled bacterium, named "Gastrospirillum hominis", was described by Dent et al. (4) and McNulty et al. (5) in the stomach of patients with gastritis and peptic ulceration. Recently, 16S rRNA sequencing studies have demonstrated that this bacterium, in fact, represents at least two new species in the genus Helicobacter: "H. heilmannii" type 1 and "H. heilmannii" type 2 (6). Another organism, morphologically similar to "H. heilmannii" and provisionally named "Gastrospirillum suis", has been observed in the gastric mucosa of swine (7-9). More recently, it was shown that this bacterium is
also a Helicobacter and is 90.5% similar to "H. heilmannii" type I. This level of similarity indicates that "H. heilmannii" type I and "C. suis" are members of a single species and allowed us to hypothesize that pigs could be a reservoir host for human "H. heilmannii" type I infection (10).

Several methods have been used for diagnosing the presence of helicobacters in the gastric mucosa of man and other animals. Among them, rapid methods such as the urease test and several staining procedures have been used for presumptive diagnosis (11). Of the methods involving collection of tissue fragments, however, culture is, in experienced hands, one of the most sensitive for detecting some helicobacters, especially H. pylori. Despite several attempts, however, culture of tightly spiralled bacteria in artificial media has not been successful, except for H. felis (12) and H. bizzozzeri (13).

For this reason, the diagnosis of the presence of these organisms has been based on methods such as the urease test and examination of stained smears and histological sections (7,9,14,15). Based on early studies by Salmon (2), Dick et al. (14) have proposed the inoculation of mice with scrapings of gastric mucus as a way of maintaining and isolating these organisms in vivo. Using the same method, Moura et al. (15) have succeeded in colonizing mouse stomach with "H. heilmannii" type I employing scrapings of the gastric mucosa of bacterium positive swine.

Although urease test, stained smears and histological sections of gastric mucosa have been widely used for the diagnosis of infection with uncultivated tightly spiralled bacteria, especially in humans (14,16), the accuracy of these methods and gastric mucus inoculation into the stomach of mice has not yet been evaluated.

For these reasons, we undertook the present study in order to compare the inoculation of gastric mucus of swine into the stomach of mice, the urease test and the carbolufuchsinsin-stained smears, as suitable methods to detect the presence of non-cultivable helicobacters in the gastric mucosa of swine.

In the first part of this study, stomachs of 50 consecutive pork-weight pigs, slaughtered at about 6 months of age, were studied. The organs were opened longitudinally along the greater curvature and thoroughly washed with tap water. One tissue sample (about 1.5 cm²) was then taken from the gastric antrum in the lesser curvature at approximately 2 cm from the pylorus, and another fragment of the same size was obtained from the oxyntic mucosa in the greater curvature of the stomach, for microbiological examination and inoculation into the stomach of mice. The fragments were transported to the laboratory in individual bags.

Fragments of approximately 1 cm² were removed from the antral and oxyntic mucosa samples, obtained as described before, and used for identification of spiral bacteria (urease test and carbolufuchsins-stained smears). Approximately half of each fragment was placed in a tube containing Christensen’s urea broth to detect preformed urease (7). The other half was blotted on a piece of filter paper to remove surface mucus and then smeared on a glass slide, heat fixed and stained with carbolufuchsia (7).

Scravings of both remaining fragments of the antral and oxyntic mucosa from each pig were homogenized in the same flask containing 3 parts of 0.85% saline and 0.2 ml of the mixture was inoculated with a sterile stomach tube into two 4-6-week-old gastric spiral bacteria-free BALB/c male mice. Each group of animals was maintained under the same conditions in separate cages and had free access to water and to commercial pelleted diet. The control group, consisting of 20 4-6-week-old BALB/c male mice, was inoculated with 0.2 ml of 0.85% saline and maintained under the same conditions as described above, in groups of 5 animals. The animals were killed by spinal dislocation 10 days after inoculation. The stomachs were
removed, opened along the greater curvature and washed in sterile 0.85% saline. Fragments of the antral and oxyntic mucosa were taken for microbiological examination as described above.

In the second part of the study, in order to investigate if the sensitivity of the methods employed could be influenced by the uneven distribution of the microorganism, we took four fragments of approximately 1.0 x 2.0 cm from the antral, oxyntic and cardiac regions of the stomach of 10 swine. Two fragments of the antrum were obtained along the lesser curvature at about 2 cm from the torus pyloricus and the other 2 along the greater curvature. All fragments of the gastric body were obtained along the middle portion of the greater curvature. The fragments of the cardiac mucosa were obtained in the upper portion of the greater curvature (N = 2) and adjacent to the pars esophagae (N = 2). One third of each fragment was used for the urease test, one third for the preparation of carbol fuchsin-stained smears, and the remaining fragment was ground in a tissue grinder, homogenized in 3 parts of saline and inoculated into the stomach of 2 gastric spiral bacteria-free BALB/c male mice.

Among the methods employed for the diagnosis of "H. heilmannii" type 1 infection, the inoculation of gastric mucus into the stomach of mice was the most sensitive and demonstrated bacterial positivity in 31 (62.0%) swine. Among the mice inoculated with mucus from the 31 "H. heilmannii" type 1 positive pigs the bacterium was seen in the antrum and corpus of 22, only in the antrum of 4, and only in the corpus of 5. The urease test was positive in gastric fragments from 3 (6.0%) pigs and tightly spiralled bacteria were observed in carbol fuchsin-stained smears of the antral and oxyntic mucosa of 4 (8.0%) animals. Although the direct examination and urease test were less sensitive, in no instance were their results in disagreement with those of inoculation into the stomach of mice, i.e., when their results were positive the inoculation into mice was also positive. Tightly spiralled bacteria were not found in the antral or oxyntic mucosa of any control animal.

In the second part of the study, the mouse inoculation method detected the organism in the stomach of 8 (80%) swine. The urease test and the examination of carbol fuchsin-stained smears were positive in 2 (20%) swine: in one pig, both tests were positive in the same fragment obtained from the body of the stomach and, in the other, the urease test was positive in 3 fragments of the oxyntic region and the carbol fuchsin-stained smears were positive in 2 fragments of the antrum.

The diagnosis of the gastric tightly spiralled bacterium infection in both man and other animals has been made by the urease test and by the examination of smears and histological sections of gastric mucosa stained by several methods (7.9.14.15) depending on availability and on the preference of each author. The results of these methods, however, are dependent on the number and distribution of organisms in the gastric mucosa. In fact, it has been determined that detection of bacteria in general by direct examination is positive only when the number of organisms/ml is higher than 10^4 (17). In regard to the urease test, it was demonstrated for the diagnosis of H. pylori infection that the test may be negative when the number of viable bacteria is less than 10^4 (18). The present results show that the inoculation of mice with scrapings of gastric mucosa was more sensitive than either the urease test or direct examination for the diagnosis of "H. heilmannii" type 1 infection in pigs. The uneven distribution of the organisms within the gastric mucosa could alter the sensitivity of the methods due to sampling error but the use of scrapings of gastric mucosa for mouse inoculation in this study may have overcome the possible irregular distribution of the organism in the stomach. Also, the use of two mice and the examination of both the antral and oxyntic...
mucosa of each animal further improved the diagnostic sensitivity.

The results of the second part of the study further confirmed that the mouse inoculation was the most sensitive method and the use of several fragments from different regions, which could reduce the problem related to the uneven distribution of the microorganism, did not increase the sensitivity of the direct examination of smears and of the urease test. The higher sensitivity of the mouse inoculation method is probably due to the predisposition of mice to be colonized by gastric tightly spiraled bacteria, even when their number is low.

We conclude that the inoculation of mice with gastric mucus is the most sensitive method available thus far for diagnosing "H. heilmannii" type 1 infection in pigs. Since "H. heilmannii" type 1 also colonizes human gastric mucosa (10,18,19), the method could be used for the diagnosis of the infection in humans. In fact, Dick et al. (14) have reported heavy colonization of the stomach of mice inoculated not only with mucus scraped from the stomach of a monkey but also with biopsies obtained from the gastric mucosa of a man.

Our results showed an improvement in the detection of "H. heilmannii" in swine, suggesting that the procedure we used in this study could also improve the diagnosis of uncultivated gastric Helicobacter in the stomach of mammals.

References

NATURAL COLONISATION OF THE GASTROINTESTINAL TRACT OF MICE BY HELICOBACTER MURIDARUM

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SUMMARY

This study was undertaken in order to determine the pattern of Helicobacter muridarum colonisation in the gastrointestinal tract of mice. Twenty-five axenic CFW 4-week-old female mice were removed from the isolator and kept in a conventional animal house under ordinary conditions. Groups of 5 mice were killed by spinal dislocation 0, 2, 4, 5 and 6 months after removal from the isolator. Fragments of the antral and oxyntic gastric mucosa and of the duodenal, ileal and colonic mucosa were obtained for culture, carbolfuchsin staining smears and urease test. Stool specimens were also taken for urease test and carbolfuchsin smears. H. muridarum were not found in any region of the gastrointestinal tract of the animals killed immediately after removal from the isolator. After 2 months, the animals began to be gradually colonised by spiral bacteria in an anal-to-oral sequence. Our results suggest that H. muridarum colonises the gastrointestinal tract of mice in a characteristic sequence (anal-to-oral direction) that may depend upon the age of the animal.

INTRODUCTION

The recognition of Helicobacter pylori as an important gastroduodenal pathogen in man (Graham, 1985) has led to an increasing interest in the search for animal models to better understand the relationship between H. pylori and gastric mucosa. Unfortunately, this spiral bacterium has a very narrow host range and only gnotobiotic piglets and dogs have proved to be susceptible to the microorganism (Krakowka et al., 1987; Jorgensen et al., 1988; Radin et al., 1990). For this reason, the use of laboratory animals naturally colonised or artificially infected by gastric spiral bacteria other than H. pylori has been proposed (Fox et al., 1991; Lee et al., 1990; Moura et al., 1993).

Recently, we reported the presence of a spiral bacterium, named Helicobacter muridarum by Lee and co-authors (1992), in the gastric mucosa as well as in the ileal and caecal mucosa of mice (Queiroz et al., 1992). This bacterium seems to be a normal inhabitant of the lower bowel of rodents. However, when it colonises the gastric mucosa of mice, it induces a gastric histological inflammatory re-
Table 1: Number of mice colonised by *H. muridarum* in the gastrointestinal tract

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Corpus</th>
<th>Antrum</th>
<th>Duodenum</th>
<th>Ileum</th>
<th>Colon</th>
<th>Stools</th>
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*: number of mice colonised in a total of 5 animals.

It has been suggested that a comparison between the strictly gastric helicobacters, such as *H. pylori*, and non gastric helicobacters, such as *H. muridarum*, should provide important information about the characteristics that may be essential to survival in the stomach (*Lee et al., 1992*).

For these reasons, we have studied the pattern of colonisation of *H. muridarum* in mice, by removing from the isolator mice free of spiral bacteria and allowing them to be naturally colonised by *H. muridarum*.

**METHODS**

Twenty-five axenic 4-week-old CFW (LOB) female mice obtained from the University of Notre Dame (USA) were removed from Trexler's type isolators and kept in a conventional animal house under ordinary conditions. Groups of 5 animals were sacrificed by spinal dislocation 0, 2, 4, 5 and 6 months after removal from the isolator. The stomachs were opened along the greater curvature and washed in 0.85% sterile saline to remove the contents. The ileum and colon were also opened longitudinally and washed in sterile saline.

Fragments obtained from the antral and oxyntic gastric regions and from the duodenum, ileum and colon were used for carbolfuchsin staining smears, urease test and for culture. Stool specimens were also taken for urease test and carbolfuchsin smears.

One specimen from each region was smeared onto a glass slide, heat fixed, stained with 40% carbolfuchsin and examined under an oil immersion lens for the presence of spiral bacteria. For the urease test, the specimens were inserted into Christensen's urea 2% agar and examined within 24 hours (*Queiroz et al., 1990*). Specimens for culture were scraped with a sterile inoculating loop and streaked onto the plates containing Belo Horizonte medium (*Queiroz et al., 1987*) and Skirrow's medium (*Skirrow, 1977*) supplemented with 10% calf serum and 0.2% charcoal at 37°C for up to 5 days. The isolates were identified by their morphology on Carbolfuchsin-stained smears and by biochemical and physiological characteristics (positive catalase, oxidase and rapid urease tests, negative hydrolysis of hippurate, resistance to nalidixic acid and cephalothin, lack of growth in aerobic and anaerobic conditions as well as at 25°C and 42°C).
RESULTS

Despite a detailed examination of the smears, no spiral bacteria were found in the gastric and intestinal mucosa or in stool samples from any mice sacrificed immediately after removal from the isolator. The urease tests and culture results were also negative in this group (Table 1).

After 2 months out of the isolator the animals began to be gradually colonised by spiral bacteria in an anal-to-oral sequence. After 6 months, 3 mice were colonised by *H. muridarum* in every region of the digestive tract. The last 2 were colonised in all regions but the oxyntic gastric mucosa.

DISCUSSION

Animal models are needed for our understanding of the finely balanced ecological relationship between spiral bacteria and gastric mucosa and of the changes in this balance that can result in gastroduodenal disease.

It has been proposed that urease production, a common feature of gastric spiral bacteria, is an essential factor for the colonisation of gastric mucosa (Lee, 1992). Ferrero and co-authors (1991) have shown that strictly gastric spiral bacteria (including *H. pylori*) have urease activity at the acid pH of the stomach. In contrast, *H. muridarum* urease has an optimum activity at a neutral pH. Interestingly, the natural habitat of *H. muridarum* is the intestinal tract of rodents and only occasionally can this bacterium be seen in the gastric mucosa (Queiroz et al., 1992). Lee and co-authors (1992) have suggested that the conditions in the stomach of rodents should be modified to permit *H. muridarum* to colonise the gastric pits in large numbers.

In our study, we have demonstrated the colonisation pattern of *H. muridarum* in the gastrointestinal tract of mice. It seems that this bacterium has the ability to preferentially colonise the intestinal mucosa. First, it appears in the stools and colon, and later it gradually colonises the ileum, duodenum, and so on. After 6 months, we have shown that the bacterium was present in every region of the gastrointestinal tract in almost all of the animals analysed.

Our results clearly show a characteristic sequence in an anal-to-oral direction of *H. muridarum* colonisation in the gastrointestinal tract of mice. This pattern of colonisation may depend upon the age of the animal, as suggested by Lee (1992), possibly due to physiological changes in the conditions of the digestive tract of the animals, such as gastric alkalisation.

ACKNOWLEDGEMENTS

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LITERATURE


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Letras e Artes

Parcerias:
CNPq, FAPEMIG, CAPES

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1927-97
PRÓ-REITORIA DE PESQUISA - UFMG
2.155 - ISOLAMENTO DE LEISHMANIA BRASILIENSIS DO HUMOR AQUOSO E CORPO VÍTREO DE PACIENTE IMUNOSUPRIMIDO COM LEISHMANIOSE CUT âNEA DISSEMINADA

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CIÊNCIAS DA SAÚDE/ MEDICINA

PALAVRAS-CHAVE: LEISHMANIOSE CUTÂNEA, IMUNOSUPPRESSÃO, HUMOR AQUOSO, CORPO VÍTREO

Os autores descreveram um quadro de uveíte difusa granulomatosa em paciente imunossuprimido, portador de leishmaniose cutânea disseminada. É apresentado o caso de um paciente submetido a transplante renal, sob uso de imunossupressores, que desenvolveu leishmaniose cutânea acompanhada de manifestações sistêmicas tais como febre, leucopenia, anemia e hepatosplenomegalia, após as quais surgem os sintomas oculares (hiperemia conjuntival e dor ocular intensa). Inicialmente foram realizadas biópsias de pele e crista iliaca, com detecção de formas amastigotas de Leishmania sp e RFI1 com título positivo de 1:320. Com o surgimento das manifestações oculares procedeu-se com punções de corpo vitreo e humor aquoso, apresentando teste de crescimento em cultura positivo para Leishmania. Posteriormente, através do método de PCR, identificou-se Leishmania braziliensis tanto nas culturas de médula óssea quanto nas de humor aqueo e corpo vitreo. Assim, o comprometimento ocular provavelmente ocorreu por via hematogênica levando em conta a caracterização semelhante dos parasitas em todas as culturas pesquisadas. Estes achados devem alertar para a possibilidade do surgimento de manifestações oculares da leishmaniose em pacientes imunodeprimidos, lembrando especialmente dos transplantados e portadores de SIDA, população esta em plena expansão.

2.156 - ESTUDO ULTRA-ESTRUTURAL DE UMA NOVA ESPÉCIE DO GÉNERO HELICOBACTER

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MORFOLOGIA

CIÊNCIAS BIOLÓGICAS/ MICROBIOLOGIA

PALAVRAS-CHAVE: HELICOBACTER TROGONTUM, ULTRA-ESTRUTURA/ HELICOBACTER

O Helicobacter trogontum é uma bactéria microaerófila, urease positiva, que foi recentemente isolada do intestino de ratos. O objetivo deste trabalho foi estudar as características ultra-estruturais de H. trogontum. “Pellets” de cultura de H. trogontum (ATCC 700114) foram fixados em glutalardeído 2.5% por 1 h, lavados em cacodilato de sódio 0.1% e incluídos em agar 2%. Os blocos obtidos foram pós-fixados em tetroxido de ósmio 1% por 40 min, tratados com acetato de uranila 5% por 10 min., desidratados em gradiente de álcool e embebidos em Epon. Cortes de 60 a 90 nm foram corados com acetato de uranila 5% por 10 min. e citrato de chumbo por 5 min. Para a coloração negativa, uma suspensão da cultura foi tratada com ácido fosfotungstico 1%. O H. trogontum foi visto como uma bactéria gram negativa, fusiforme a levemente espiralada, com 4 a 6 µm de comprimento por 0.6 a 0.7 µm de largura, possuindo de 4 a 7 flagelos embeinados em ambos os polos. O citoplasma continha numerosos ribossomas, grânulos esparsos e irregulares e grânulos maiores circundados por uma membrana simples. As extremidades da bactéria possuam uma área menos densa onde foi observada a membrana polar. O H. trogontum mostrou várias características comuns ao gênero Helicobacter, sendo sua morfologia semelhante à das espécies H. rappini e H. bilis.

APOIO: PRPq/UFMG, CNPq e FAPEMIG
2.121 - ANIMAIS DOMÉSTICOS SÃO FONTE DE Helicobacter PARA A INFEÇÃO DE SERES HUMANOS?

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Foi recentemente demonstrado, através do sequenciamento do rRNA 16S, que o "G. hominis" representa duas espécies distintas do gênero Helicobacter, denominadas "G. hominis" tipo 1 e tipo 2, e que o rRNA 16S do "G. suis" é aproximadamente 99,5% semelhante ao do "G. hominis" tipo 1, o que sugere que suínos constituem um reservatório do micorganismo. A fonte do "G. hominis" tipo 2 também não foi ainda investigada.

Considerando-se que bactérias espalhadas morfológicamente semelhantes ao "G. hominis" têm sido observadas no estômago de animais, desenvolvemos este estudo com o objetivo de investigar a presença de "G. hominis" tipos 1 e 2 no estômago de suínos, gatos e cães.

Foram estudados 20 suínos, 10 cães e 10 gatos. Fragmentos do estômago de cada animal foram utilizados para extração de DNA e para esfregaços (corados pela carbolúzícina). A PCR foi realizada empregando-se "primers" específicos para a detecção de "G. hominis" tipo 1 e tipo 2. Os produtos da PCR foram analisados através de eletroforese em agarose.

Bactérias espalhadas morfológicamente semelhantes a "Gastrospirillum" foram observadas no estômago de 12 suínos e de 10 cães e 7 gatos. "G. hominis" tipo 1 foi detectado, através da PCR, no estômago dos 12 suínos, mas não foi encontrado no estômago dos cães e gatos examinados. "G. hominis" tipo 2 foi detectado no estômago de 3 gatos e na mucosa gástrica de todos os cães, mas não foi encontrado no estômago dos 20 suínos estudados.

Os resultados deste trabalho demonstram que a transmissão do "G. hominis" para seres humanos ocorre, provavelmente, como uma zoonose, sendo o suíno um importante reservatório para a infeção por "G. hominis" tipo 1 e cães e gatos para a infecção por "G. hominis" tipo 2.

Aperto: CNPq / FAPEMIG

2.122 - HIV/AIDS NO BRASIL: UMA REVISÃO DE LITERATURA

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(Orientador), Francisco ACURCIO (PQ)

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Objetivo: Avaliar a prevalência/incidência da infecção pelo HIV em grupos populacionais selecionados no Brasil.

O trabalho consta de uma revisão bibliográfica a respeito do perfil epidemiológico da infecção pelo HIV no Brasil. Sendo mostradas modificações ocorridas na epidemia temporariamente, no que diz respeito aos grupos acometidos, dando enfoque ao preocupante aumento da disseminação do vírus entre a população heterossexual. Mostraremos também a distribuição regional dos casos de AIDS no Brasil.

Apesar da prevalência/incidência real da infecção pelo HIV ser desconhecida, a epidemia pode ser monitorada através de populações selecionadas, tais como: hemofílicos, prostitutas, homossexuais masculinos, usuários de drogas injetáveis (UDIs), pacientes hospitalizados, crianças, hemodializados, clínicas de DST, doadores de sangue, etc. Resultados preliminares mostraram um aumento importante da prevalência/incidência entre UDIs e heterossexuais. A variação da soroprevalência do HIV entre estados selecionados, no Brasil, foi a seguinte: doadores de sangue - 0,00 a 0,38 %, clínicas de DST - 0,00 a 0,60%, hemodializadores - 0,00 a 0,70%, grávidas - 0,00 a 12,80%, prostitutas - 0,00 a 13,30%, crianças/recent-nascidos - 1,10 a 14,00%, pacientes hospitalizados - 1,50 a 20,00%, prisioneiros - 12,50 a 28,10%, UDIs - 15,90 a 48,70%, homossexuais masculinos - 0,00 a 98,00%, hemofílicos - 9,10 a 98,00%.

Aperto: CNPq