

# The Vaginal Microbiota of Guinea Pigs

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The vaginae of four guinea pigs were swabbed and samples cultured aerobically on horse blood agar, in 5 per cent carbon dioxide on MRS agar or anaerobically on anaerobic horse blood agar. Vaginal microbiota consisted almost exclusively of gram-positive bacteria including *Corynebacterium*, *Streptococcus*, *Enterococcus*, *Staphylococcus* and *Lactobacillus* species.

KEY WORDS: guinea pigs; vaginal microbiota; vaginal vaccines

## INTRODUCTION

Infections of the genital mucosa such as non-gonococcal urethritis, gonorrhoea, syphilis and AIDS are a serious worldwide problem. The availability of vaccines against genital tract infections would, therefore, be a major addition to preventive health care systems. One area of investigation in the design of vaccines for inducing secretory immunity in the reproductive tract is the concept of using strains of commensal microorganisms as the vaccine vehicles to deliver antigen directly to the female reproductive tract (FRT).<sup>19</sup> For this to be a feasible option, the microbiota of the healthy vagina needs to be known. Several qualitative and semi-quantitative studies of the human vaginal microbiota have been done.<sup>8,10,20</sup> Few studies, however, have attempted to quantitate the vaginal bacteria of laboratory animals.<sup>5,11</sup> An understanding of the vaginal ecosystem is essential if these animals are to be used as models for human sexually transmitted diseases, as it is the commensal bacteria that are the primary defence against invasion and they may be useful as vaccine delivery vehicles.

The predominant organisms isolated from the normal human vagina are members of the genus *Lactobacillus*.<sup>2,3,12,13,16</sup> Quantitative studies of the rat vagina have shown that *Lactobacillus* spp. are not the dominant bacterial genus.<sup>11</sup> The most

frequently isolated bacteria in this study were  $\alpha$ -haemolytic streptococci, *Pasteurella* spp., non-haemolytic streptococci and *Corynebacterium* spp. Similar studies of the bacterial populations of the mouse reproductive tract<sup>5</sup> showed that streptococci, staphylococci, corynebacteria and lactobacilli were consistently isolated. *Corynebacterium* spp. appear to predominate in the monkey vagina, although *Lactobacillus* spp. were also found.<sup>9</sup> To date, there have been no studies that describe the significance of *Lactobacillus* in the guinea pig vagina, or indeed describe the guinea pig vaginal microbiota, in general. The bacteriology of the guinea pig vagina is described, for the first time, in the present study.

This paper outlines results of an investigation into the normal microbiota of the vagina of the guinea pig, the animal model often used for the study of human female genital tract infection by *Chlamydia trachomatis*.<sup>15</sup> The microbial ecology of the female guinea pig reproductive tract was investigated and the major bacterial genera present were identified.

## MATERIALS AND METHODS

### Experimental animals

Sexually mature outbred female guinea pigs, housed in groups of two, were used in all studies (QUT Biomedical Ethics Committee Approval Number 638).

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### *Sample collection and determination of the stages of the oestrous cycle*

For the collection of vaginal samples, a sterile cotton-tipped aluminium swab was inserted approximately 2 cm into the vagina and the walls of the vagina were swabbed vigorously. The vaginal swab was collected into 1 ml of phosphate buffered saline (PBS). At stages of the oestrous cycle other than oestrus, a swab was first used to puncture the vaginal closure membrane. Swabs were smeared directly on to a slide, fixed in methanol for 2 min and stained using 10 per cent Giemsa in phosphate buffer pH 6.8. The smears were evaluated using standard cytological criteria for diagnosis of the stage of the oestrous cycle.<sup>7</sup>

### *Culture, enumeration and identification of vaginal bacteria*

Horse blood agar (HBA) was used for the isolation and cultivation of facultatively anaerobic bacteria. CDC anaerobic blood agar (ANHBA) was used for the isolation and cultivation of anaerobic bacteria. MRS agar and broth (Oxoid) were used for the isolation and culture of *Lactobacillus*. For the culture of vaginal facultatively anaerobic bacteria, the vaginal swab was vortexed vigorously for 30 s to dislodge bacteria. Ten-fold dilutions of this sample were made in PBS and 0.1 ml aliquots spread on to HBA and MRS plates and incubated in an atmosphere of 5 per cent CO<sub>2</sub> at 37°C for 72 h. Counts were expressed as colony forming units (CFU)/g of vaginal swab material, using 20 mg as the average mass of vaginal material collected. For anaerobes, the swab was collected into pre-reduced media and placed into an anaerobic chamber (10.2 per cent H<sub>2</sub>, 80.1 per cent N<sub>2</sub>, 9.7 per cent CO<sub>2</sub>) as soon as possible after sample collection. The contents of the collection tube were pipetted repeatedly, in preference to vortexing. Ten-fold dilutions were made and 0.1 ml of these were spread on to ANHBA plates. Plates were incubated in an anaerobic chamber for 5–7 d. Bacterial types were counted and categorised on the basis of colonial morphology. Five colonies of each colony type were subcultured and identified to ensure that colony morphology was a reliable indicator of bacterial type. Gram stain morphology was used to further categorise the isolates. Various tests (catalase, growth on MRS at pH 5.2 and nitrate reduction) were done to classify the gram-positive isolates to genus level. Gram-negative facultatively anaerobic bacteria were not

identified further. Gram-negative anaerobes were classified by Gram stain morphology.

## RESULTS

### *Enumeration of vaginal bacteria throughout the oestrous cycle*

The vaginal facultatively aerobic, anaerobic and MRS recovered bacteria of four guinea pigs were recorded over a period of 57 d (Figure 1). Examination of Giemsa-stained vaginal smears revealed that large numbers of bacteria were observed in the oestrous stage of the cycle and correspondingly fewer numbers of polymorpho-nuclear cells (PMNLs) and nucleated squamous epithelial cells (SEC). However, the di-oestrous stage contained far fewer bacteria and larger numbers of PMNLs, nucleated SEC and non-nucleated SECS.

Recovery of bacteria on ANHBA and HBA plates was consistently higher than from MRS medium, with bacterial concentrations cyclical and generally highest at oestrus. Total ANHBA and HBA recoveries were typically in the range of 10<sup>7</sup> to 10<sup>8</sup> CFU/g of swab material. Both facultatively anaerobic and obligate anaerobic bacteria grew on ANHBA plates. In contrast, the bacterial recovery on MRS agar ranged from 10<sup>4</sup> to 10<sup>7</sup> CFU/g. The low recovery of bacteria from MRS medium obtained from all animals suggests that in contrast to humans, *Lactobacillus* species are not the dominant bacterial genus in the guinea pig vagina.

The cyclical variation in the bacterial concentrations from the four guinea pigs is illustrated graphically in Figure 1. In general, the highest recovery of bacteria from ANHBA and HBA media corresponded either to the oestrous or to the pro-oestrous stages of the oestrous cycle. In contrast, recoveries on MRS medium were not consistently elevated at oestrus or pro-oestrus. Although only four animals were examined, variation between animals, in terms of types of bacteria present and responses to the hormonal changes associated with oestrous cycle may be occurring. MRS counts were found to be reliable indicators of total lactobacilli by sampling two animals over an 8 d period and noting that total lactobacilli correlated well with total MRS plate counts (data not shown).

### *Classification and enumeration of vaginal bacteria*

Bacterial genera found in the lower reproductive tracts of four guinea pigs, and the approximate

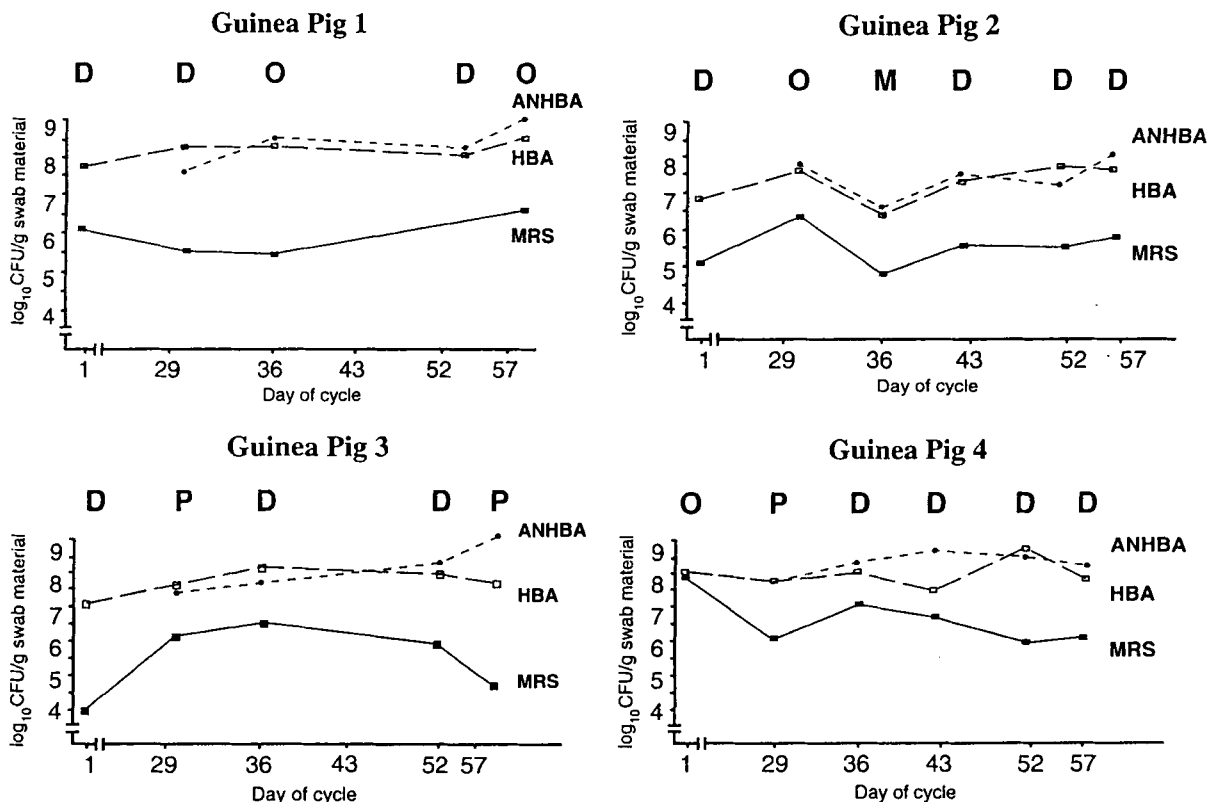


Figure 1. Fluctuation in concentrations of bacteria as determined by recovery on ANHBA, HBA and MRS throughout the oestrous cycle of four guinea pigs. D=di-oestrus; P=pro-oestrus; O=oestrus; M=met-oestrus

numbers recovered from each animal expressed as CFU/g swab material, are presented in Table 1. Guinea pig vaginal microbiota was found to consist almost exclusively of gram-positive bacteria. The facultatively anaerobic/microaerophilic bacteria isolated included *Corynebacterium* spp., *Streptococcus* spp., and *Lactobacillus* spp. Some animals contained more than one representative of each genus, as determined by distinctive Gram stain and colony morphologies. For example, guinea pig 1 contained two different *Corynebacterium* and three *Lactobacillus* types. These may represent different species within these genera. *Corynebacterium* spp. and *Streptococcus* spp. were the dominant microaerophilic bacteria isolated from the vaginae of all four guinea pigs. Both *Propionibacterium* spp. and *Bifidobacterium* spp. were isolated in high numbers from one guinea pig.

These results confirm the earlier observation that lactobacilli are not numerically dominant in the guinea pig vagina. The dominant bacteria of the guinea pig vagina are *Corynebacterium* spp.

and *Streptococcus* spp., with variation in other components of the microbiota occurring between animals.

### DISCUSSION

The female reproductive tract is susceptible to infection with numerous pathogenic microorganisms, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, HSV, HPV and HIV. It is the autochthonous bacteria which naturally inhabit the vagina that are fundamental in prevention from these infections.<sup>17</sup> These bacteria are found in layers attached to the vaginal epithelium, and therefore they effectively shield the mucous membrane from invasion by sexually transmitted pathogens. The human vagina is colonised by a relatively homogeneous population of bacteria. Predominant among these are representatives of the genus *Lactobacillus*. Lactobacilli have long been recognised as beneficial in the human vagina, as well as at other niches throughout

Table 1. The major bacterial types isolated from the vaginae of four guinea pigs and the approximate numbers of each per gram of swab material

Organism	CFU/g swab in guinea pigs 1-4			
	1	2	3	4
Facultative anaerobes				
<i>Corynebacterium</i> sp. 1 <sup>a</sup>	10 <sup>6</sup>	3 × 10 <sup>5</sup>	3 × 10 <sup>6</sup>	5 × 10 <sup>6</sup>
<i>Corynebacterium</i> sp. 2 <sup>a</sup>	10 <sup>7</sup>	— <sup>c</sup>	—	—
<i>Enterococcus</i> spp.	10 <sup>7</sup>	5 × 10 <sup>6</sup>	—	—
<i>Streptococcus</i> spp.	5 × 10 <sup>6</sup>	1 × 10 <sup>6</sup>	2 × 10 <sup>6</sup>	5 × 10 <sup>5</sup>
<i>Staphylococcus</i> spp.	10 <sup>4</sup>	5 × 10 <sup>4</sup>	2 × 10 <sup>5</sup>	5 × 10 <sup>4</sup>
<i>Lactobacillus</i> sp. 1 <sup>a</sup>	3 × 10 <sup>6</sup>	1 × 10 <sup>5</sup>	2 × 10 <sup>6</sup>	—
<i>Lactobacillus</i> sp. 2 <sup>a</sup>	1 × 10 <sup>6</sup>	5 × 10 <sup>3</sup>	5 × 10 <sup>3</sup>	4 × 10 <sup>3</sup>
<i>Lactobacillus</i> sp. 3 <sup>a</sup>	1 × 10 <sup>6</sup>	—	—	—
<i>Proteus</i> spp.	—	10 <sup>4</sup>	—	—
Obligate anaerobes				
<i>Propionibacterium</i> spp.	10 <sup>7</sup>	nd	nd	nd
<i>Bifidobacterium</i> spp.	5 × 10 <sup>5</sup>	nd	nd	nd
Gram-negative bacillus <sup>b</sup>	4 × 10 <sup>4</sup>	nd	nd	nd

nd=not determined; <sup>a</sup>types were based on colonial characteristics; <sup>b</sup>morphology consistent with *Bacteroides* spp.; <sup>c</sup>less than 3 × 10<sup>2</sup> CFU/g. The detection limit using this method is 3 × 10<sup>2</sup> CFU/g.

the body, including within the gastrointestinal tract. Their contribution to human and animal health is believed to be by their production of antimicrobials, including hydrogen peroxide<sup>6</sup> and bacteriocins.<sup>1,4,18</sup> These characteristics have seen their inclusion in many health-promoting foodstuffs such as yoghurts, as well as probiotic preparations consisting of dried *Lactobacillus* cells.<sup>14</sup>

The study of human reproductive tract infections necessitates the use of various animal models of infectious processes and pathogenesis. Genital tract infection of humans, caused by *C. trachomatis* serotypes D-K, is closely paralleled in the guinea pig model by *C. psittaci* strain GPIC genital tract infection.<sup>15</sup> The aim of the present study was to describe the commensal microbiota of the guinea pig vagina and to attempt to isolate *Lactobacillus* spp. from this site.

The current work demonstrated variation in vaginal bacterial concentrations at various stages of the oestrous cycle. Numbers of ANHBA, HBA and MRS-recovered bacteria varied throughout the cycle, with the highest numbers often coinciding with the oestrous phase of the cycle. The total numbers of bacteria isolated were similar to total numbers of bacteria isolated from humans<sup>16</sup> and rats.<sup>11</sup> These results also demonstrated the relative abundance of different types of bacteria in the guinea pig vagina. A consistent observation was

that the numbers of bacteria recovered by AN-HBA and HBA were 10- to 1000-fold higher than for MRS agar. This indicates that *Lactobacillus* spp. are not the dominant bacteria in the guinea pig vagina, although they were consistently isolated from the vaginae of all guinea pigs.

The dominant bacterial genera isolated from the guinea pig vagina in the present study were *Corynebacterium*, *Enterococcus*, *Propionibacterium* and *Streptococcus*, although not all of these bacteria were present in all animals. In terms of numerical dominance and frequency of isolation, *Corynebacterium* sp. 1 and *Streptococcus* spp. appeared to be the dominant bacteria. The use of *Lactobacillus* as a live vaccine in the genital tract requires knowledge of the stability the *Lactobacillus* population. The present study has shown that the guinea pig vaginal flora, including the *Lactobacillus* population, varied throughout the oestrous cycle. However the isolation of lactobacilli from all animals, in all stages of the oestrous cycle, indicates that lactobacilli are a persistent component of the guinea pig vaginal ecosystem, in keeping with the observations in humans, although these organisms do not predominate in the guinea pig ecosystem, in contrast to the findings in humans.<sup>2,12</sup>

Studies in human females have demonstrated the predominance of *Lactobacillus* species within

the reproductive tract.<sup>20</sup> However, this is not the observation in the majority of other mammals studied. Rats, mice and monkeys have a vaginal flora that is dominated by *Streptococcus* spp. and *Corynebacterium* spp.<sup>7,9,11</sup> The present study concluded that the guinea pig vaginal microbiota is also dominated by these bacteria. In conclusion, a significant finding of the current work was the consistent presence of *Lactobacillus* within the female guinea pig reproductive tract. The presence of *Lactobacillus* in the vagina of the guinea pig indicates the validity of this animal as a model for the delivery of antigens to the human reproductive tract using recombinant lactobacilli. We are currently using *Lactobacillus* strains isolated in this study as hosts for recombinant plasmids and in heterologous gene expression studies for investigating the potential of lactobacilli as live vectors to deliver foreign antigens to mucosal surfaces.

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