Author's response to reviews

**Title:** Identification and genotyping of bacteria from paired vaginal and rectal samples from women at 35 weeks of gestation indicates similarity between vaginal and rectal microflora

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**Version:** 3 **Date:** 8 September 2009

**Author's response to reviews:** see over
Sir,

We thank both of the reviewers for their useful review.

Their comments have been addressed in the revised manuscript.

Hereby, you will find the answers of their comments.

Sincerely

Mario Vaneechoutte

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Reviewer's report

Title: Identification and genotyping of bacteria from paired vaginal and rectal samples from women at 35 weeks of gestation indicates similarity between vaginal and rectal microflora

Version: 2 Date: 1 September 2009

Reviewer: Johanna Maukonen

Reviewer's report:
El Aila et al., Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora

The manuscript describes analyses of microbial populations between two sites (vagina and rectum) among 132 pregnant women. Isolates have been obtained from two different media and thereafter identified and genotyped. About 35% of the pregnant women carried the same Lactobacillus species both vaginally and rectally, and 34 out of the 50 species pairs were identical genotypes.

In my opinion, the manuscript has greatly benefitted from the revision and is nearly acceptable for publication. However, I still have one major compulsory revision.

Major Compulsory Revisions

1. Page 17 last paragraph: It is still stated in the text that: “All these data indicate strong dynamics of the vaginal and rectal microflora, influencing each others species composition to some degree and containing several genotypes per species, which also may be exchanged between both sites, whereby different genotypes of the same species are continuously replacing each other”. First, this sentence is too long to be easily understandable. Second, I agree with the authors that having same genotypes in different locations (most likely) proofs that they originate from a same site. I do not object those conclusions. I neither object the conclusion in abstract (page 3) “These results support the hypothesis that the rectal microflora serves as a reservoir for the colonization of the vaginal econiche”. However, this study does not prove (or even indicate) that there are “strong DYNAMICS of the vaginal and rectal microflora ...” It is also possible that e.g. the rectal lactobacilli colonized the vagina already at childhood and the
identical genotypes have been present ever since. It is also possible that the migration has occurred only in the direction of rectum to vagina. With “dynamics” one understands that there is a continuous migration from one site to another and back (i.e. from vagina to rectum and from rectum to vagina). This is a cross-sectional study in which samples were taken at one time-point, so in my opinion this conclusion is not warranted from the results of this manuscript. Therefore, I would suggest that the sentence would be rephrased.

A1: We can agree with these objections of the reviewer. We have changed to “Although we did not sample the same subjects at different time intervals, this finding suggests the occurrence of changes in the composition of the vaginal microflora, whereby different strains of a limited number of species may replace each other, and may be exchanged between vagina and rectum.”

The same applies to a sentence in the beginning of the page 17 “Although we did not sample the same subjects at different time intervals, this finding suggests strong DYNAMICS of changing composition of the vaginal microflora, whereby different strains of a limited number of species replace each other CONTINUOUSLY, and are exchanged between vagina and rectum”.

A2: We agree and changed to “All these data indicate a strong correlation between vaginal and rectal microflora, not only at the species level but also at the strain level.”

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer's report
Title: Identification and genotyping of bacteria from paired vaginal and rectal samples from women at 35 weeks of gestation indicates similarity between vaginal and rectal microflora
Version: 2 Date: 31 August 2009
Reviewer: SVETLA DANOVA
Reviewer's report:
Minor Essential Revisions
Comments to the Authors
1. The authors present a simultaneous study “Identification and genotyping of vaginal and rectal bacterial microflora”. They combine a phenotypic method (the modified Gram procedure as first step to categorize the isolates), with tDNA-PCR analyses. Thus, an original and useful polyphasic approach was applied. The parallel species identification is 50% of the overall work and has a significant fundamental and scientific sound. Despite of careful edition is not easy to understand how been achieved the Lactobacillus species affiliation (in Materials and methods and in Results). Examples – species L. iners- it’s the only species that no growth on MRS agar and in addition no data for tDNA-PCR protocol for this species in added new ref 18.
I kindly advice the authors to fulfill the part Identification of isolates (page 6-Mat& Methods) with brief details of identification procedure for new reported species at
least (different from about cited in ref 18). It will add a methodological value of the work and interest for other researchers.

**A3:** We have now added, after the sentence: “Isolates were identified by means of tRNA intergenic length polymorphism analysis (tDNA-PCR) as described before [8, 17-19].”, the following sentence: “Briefly, the tRNA-intergenic spacer regions were amplified by PCR using consensus primers, applicable to most bacterial species, and the resulting fingerprints, obtained by separation of the amplified spacers by means of capillary electrophoresis on an ABI310, were compared with those of a large library of reference strains of the different species, shown in previous studies to be part of the vaginal microflora. Isolates with fingerprints that did not match fingerprints already present in the library were considered as not identifiable.”

2. page 9, lines 194: “Using tDNA-PCR, 844 could be identified” Please clearly state how much isolates have been subjected to tDNA-PCR procedure and replace this phrase.

**A4:** We changed to: “For a total of 132 women, 4 colonies each were picked from the vaginal and rectal sites, i.e. a total of 1056 colonies were picked and subjected to identification by means of tDNA-PCR. Of these, 844 could be identified.”

3. The authors discuss the presence of *L. crispatus*:

   page 13-line 277: “Taking into account vaginal and rectal colonization by more than one Lactobacillus species, 18 women (13.6%) were colonized by both *L. crispatus………*”; page 12-line280: “The four species predominant in the vagina, as established in this study, i.e. *L. crispatus, L. jensenii, L. gasseri and L. iners ……*” etc.

   How was overcame the misidentification of *L. crispatus* which is reported previously as indistinguishable from *L. amylovorus* species by tDNA-PCR (“Baele, M., M. Vaneechoutte, R. Verhelst, M. Vancanneyt, L.A. Devriese, and F. Haesebrouck. 2002. Identification of Lactobacillus species using tDNAPCR. J. Microbiol. Methods 50: 267)?

**A5:** Indeed, this is a correct observation of the reviewer: tDNA-PCR cannot differentiate between both species. However, we think there is not really a problem, since we do not know of any reports on *L. amylovorus* as a genital species. The original description was of waste-corn fermentations:


   Therefore, we think it would distract to go into more detail about this.

In addition, is really difficult to discriminate the species morphologically, due to the polymorphisms of bacterial cells (including lactobacilli), observed in the complex ecosystems and laboratory conditions.

**A6:** We agree, but that is one of the reasons we use a genotypic rapid technique, like tDNA-PCR, also with it limits, but distinguishing easily between the most important vaginal and rectal *Lactobacillus* species and others.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.