Reviewer's report

**Title:** Studies on a possible role of *Escherichia coli* in propagation and perpetuation of the gut chronic inflammation in ulcerative colitis.

**Version:** 1  **Date:** 10 December 2012

**Reviewer:** Serena Schippa

Reviewer's report:

The manuscript: "Studies on a possible role of *Escherichia coli* in propagation and perpetuation of the gut chronic inflammation in ulcerative colitis" by Magdalena Pilarczyk-Zurek and colleagues is a study of importance in its field. Detailed studies on the *E. coli* role in ulcerative colitis are very few, but are needed. In fact, even if an increased load of mucosa associate *E. coli* in UC and CD patients has been found, it is likely that their role in UC is different from that played in MC patients. However, I'm unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions.

**Major compulsory revisions**

1) The patients' age ranged between 18 to 72 years. The authors should take into consideration that the gut microbiota composition changes in age relations, and after age of 50 years old it is reported to be different from the ones in young people. Age-related physiological changes in the gastrointestinal tract, inevitably affect the gut microbiota. The biological processes of aging may involve a role for the gut microbiota (Elena Biagi et al. Plos One 2012).

This leads inevitably to an heterogeneous group ruled in the study. This should be considered when the authors put together comparisons and analysis data, at least by comparing subjects matching by age and sex.

2) On lines 123,124 and 125 are reported the exclusion criteria (diabetes, autoimmune disorders, severe systemic diseases, alcohol abuse, cow's milk allergy as well as non-steroidal anti-inflammatory drugs intake). Nothing is reported about antibiotic treatments, which is known to have a strong impact on intestinal microbiota composition. Usually in the recruitment of patients and control subjects are excluded those which have undergone antibiotic treatments, in the three months prior to the biopsy.

3) Usually the biopsy specimens dedicated to bacterial culture, by traditional methods, undergo treatment useful to remove the flora not strictly adhered to the intestinal mucosa, such as washing with the biopic sample with dithioerythritol DDT (Conte MP et al. GUT.2006). Briefly for the bacteriological study, specimens should be immediately processed and first washed in 500 ml of physiological saline with 0.016% dithioerythritol to remove the mucus and then washed three times in 500 ml of physiological saline by shaking for 30 seach time. Nothing is mentioned to this methodic. I wonder if such treatment was not
carried out, what kind of bacterial has been cultivated? The adherent or the luminal ones, or both? Maybe this could explain the results discrepancy obtained by authors, between cultural and molecular methods.

4) Another point that remain vague is in relation to the number of E.coli strains collected from each biotic sample. For the results interpretation it is important to find out if the study was referred to a E.coli population colonizing each subject (meaning to have collected at list 50-100 E.coli strains for each biopic samples), or to a few E.coli strains, randomly collected. Since the interspecies variability, study on E.coli population will give results with stronger impact. If not the authors should discuss the limit imposed by a study conducted on few, randomly collected, E.coli strains.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests'