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Persistence of STEC in grain, milling by-products, flour, and raw follow up products

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Key words
STEC, flour, grain, detection, persistence

Aim of the study
In this study, 100 samples of wheat grain were analyzed for the presence of STECs. Samples were collected from different locations in Switzerland during the harvest season in 2019. The persistence of *E. coli* was determined in wheat grain, wheat flour, raw pizza-, cookie-, and pastry dough. In addition, a protocol for the quantification of STECs from grain samples was established. The data add to a better understanding of the prevalence and persistence of *E. coli* in wheat grain, flour, and doughs.

Material and methods
Qualitative detection of STECs from natural wheat grain samples was performed after selective enrichment (TB-broth) followed by standard qPCR, targeting *stx1*, *stx2*, and *eae* genes. To analyze the persistence of *E. coli* in wheat flour, grain, and raw pizza-, cookie-, and pastry doughs, samples were artificially contaminated with 10^6 cfu/g and stored at room temperature. Viable cell counts of *E. coli* O28, O157, and three additional strains isolated from wheat flour (unknown serotype) were monitored over a period of 56 days (flour and grains) and 7 days (doughs), respectively. For the quantitative detection of *E. coli* O157, selective and non-selective enrichments were performed, followed by an MPN approach and standard qPCR.

Results and significance
The analysis of natural wheat grain revealed presence of *E. coli* in 39 % of all tested samples. None of these samples was tested positive for *stx1*-, *stx2*- and *eae* genes. However, the high prevalence of *E. coli* indicates that pathogenic strains of this organism are likely also associated with grain.
In wheat flour samples, all tested strains of *E. coli* (5/5) were detectable after 56 days of storage at room temperature. 14 days after inoculation viable cell counts dropped by ca. 2 logs and remained stable for an additional 14 days. Another 14 days later viable cell counts again dropped by 0.5 – 2 logs, respectively, depending on the strain used. After 56 days of storage cell counts reached ca. 10 – 100 cfu/g. In whole grain samples, all tested strains of *E. coli* (5/5) were also detectable after 56 days of storage. However, viable cell counts decreased slightly faster compared to flour samples. In the tested doughs cell counts of *E. coli* remained stable over a period of 7 days. These findings suggest that grains and flour can likely contain viable cells of both, pathogenic and non-pathogenic *E. coli*. Because viable cell counts remain stable in raw dough, *E. coli* can easily be transmitted to susceptible consumers. If the infection dose of a pathogenic strain is low, e.g. 10 – 100 bacteria, consumption of raw dough can lead to an outbreak.
In order to detect low contamination levels of *E. coli* O157 in grain and flour a selective enrichment (TB-broth) was compared to a non-selective enrichment (BPW), followed by an MPN approach and qPCR. The data revealed that a selective enrichment leads to an underestimation of viable *E. coli* O157 counts. Hence, MPN/qPCR should be performed using a non-selective enrichment.

Publications, posters and presentations
The results of this research project have not been published yet. However, a poster or oral presentation at a scientific meeting is intended. In addition, a manuscript is in preparation.

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