INVESTIGATION ON *CYCLOSPORA CAYETANENSIS* IN FRESH PRODUCE IN ITALY

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In the USA, large outbreaks of cyclosporiasis have increasingly been linked to various types of fresh produce imported from South-American countries. Despite some outbreaks of cyclosporiasis have been recorded in Germany and Sweden, *C. cayetanensis* has received much less attention as a foodborne pathogen in Europe compared to the U.S. In Italy, the high prevalence of *C. cayetanensis* DNA detected in water, soil, vegetables, and in humans indicate the need to investigate more in depth the public health significance of *Cyclospora* in this country.

The aim of this study is to investigate the prevalence of *C. cayetanensis* as a contaminant of fresh produce -ready to eat (RTE) mixed salads and berries- sold on the Italian market, using validated methodologies.

The sampling regime has been based on testing samples in pools with an expected prevalence of 0.6 %, 95 % confidence and 1.15 % of precision. To estimate the prevalence, we chose a pool size of 9 packages of fresh produce each month, for a total of 54 samples per month and 72 pools per one year. RTE mixed salads and berries packages were bought from Italian food stores. After collection, they were transferred to the laboratory and then washed before their expiry date using the FDA washing procedure (BAM 19b). After concentration, the pellets were subjected to molecular analyses for the detection of *C. cayetanensis* whereas an aliquot of each pool was examined by microscopy.

Two real-time *q*PCR assays, one based on the 18S ribosomal RNA gene, according to the BAM 19b (*Assay 1*) and, a new, specific *q*PCR assay, targeting the ITS1 region (*Assay 2*), were set up. Both these methods showed correct amplification from the internal amplification control as well as the positive control, with consistent Ct values across the replicates.

So far, a total of 27 RTE mixed salad packages, belonging to three industrial brands, and 27 berries packages (blueberries and blackberries imported from Perù and Mexico, respectively, and raspberries grown in Italy) have been collected and washed. Six pools and a total of 54 aliquots (9 per pool) were obtained. Four samples (two from RTE mixed salads belonging to two different brands and two from raspberries and blueberries) were tested in duplicate in *q*PCR using *Assay 2* molecular analysis.

Preliminary microscopy and molecular results will be presented. Once the study is completed, we anticipate that the molecular tools that have been developed cooperatively in different partner labs will provide not only data on the prevalence of *Cyclospora* in fresh produce in Italy but also a shared methodological direction for wider monitoring of fresh produce at European level.