Overall achievement of IMPACT: Standardising molecular detection methods to improve risk assessment capacity using Cryptosporidium spp. in ready-to-eat salads

Main author: Gunita Deksne (Institute of Food Safety, Animal health and Environment BIOR)

Co-authors: Barbara Šoba Šparl, Smita Sutrave, Lucy Robertson, Tamirat Temesgen, Kristoffer Tysnes, Ian Woolsey, Marco Lalle, Simone Caccio, Rachel Chalmers, Anne Mayer-Scholl

INTRODUCTION

In recent years, several cryptosporidiosis outbreaks linked to the consumption of fresh produce have been reported across Europe. Parasites associated with ready-to-eat (RTE) bagged salad leaves and other fresh produce are of particular concern for both public health and the food industry. Currently, no rapid, reliable and standardisable molecular assay exists for analysing fresh produce for contamination with protozoan parasites. The IMPACT project aims to build capacity and exchange know-how among various research laboratories and to improve the risk assessment capacity for food safety in Europe. To achieve this goal, the objectives were to i) conduct the literature review of molecular methods for the detection of Cryptosporidium spp. oocysts; ii) develop a SOP for molecular detection of Cryptosporidium oocysts in leafy greens via real-time PCR; iii) organise a proficiency test to validate the developed SOP. The aim of the project is to increase awareness of validated diagnostic procedures in the food-testing community and to extrapolate the findings to other protozoan parasites.

METHODOLOGY

Current procedures for spiking and detection of Cryptosporidium oocysts in fresh produce were reviewed, a market survey of oocyst suppliers was conducted, and expert opinions were obtained. A SOP based on an 18S qPCR assay for the detection of Cryptosporidium in salad leaves was implemented in two laboratories. The optimised SOP, along with video tutorials, was shared with four partner laboratories for validation. A proficiency test was also organised for four partner laboratories and seven laboratories excluded from the IMPACT consortium.

RESULTS

Out of 899 screened papers, a total of 65 relevant papers were identified, encompassing four main groups of sample types that have been used for spiking and PCR method development: faeces (31 studies), environmental samples (23), food (4) and other matrices (15). Only a few of the methods described were fully validated and thus fulfilled the IMPACT requirements. Furthermore, a guidance document on spiking of salad leaves with Cryptosporidium oocysts was drafted. Feedback from four partner laboratories in which the SOP was implemented was used to refine the protocols. Validation of the resulting SOP by means of a proficiency test and the hands-on experiences of the participating laboratories will be analysed via a detailed questionnaire on the performance of various aspects of the protocol under standard operating conditions.

DISCUSSION

The results of our review highlighted the lack of sensitive and robust molecular methods for analysing fresh produce for contamination with protozoan, and on which the IMPACT SOP could be based. The SOP was developed via comparative experimental work at two of the participating laboratories, concentrating all of the necessary analytical steps, including: elution of the parasites from the food matrix, DNA extraction, and parasite identification via qualitative real-time PCR. Characteristics such as sensitivity, reproducibility, repeatability, ease of handling, availability and costs of reagents and equipment, hands-on and turn-around times were considered for each step.

The proposed SOP for Cryptosporidium spp. can be extrapolated for the detection of infective oocysts of other protozoa in food matrices. The final SOP will be disseminated throughout the network of NRLs in Europe, EFSA focal points, the COST Action Euro-FBP network, and the OHEJP network. IMPACT makes it possible to exchange knowledge between EU participants, thereby contributing to stronger food testing networks.