Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO
Research Management

Section **Fields (of activity)**

Style sheet (Bitte die Vorlage direkt mit Ihrem Text überschreiben / veuillez substituer le texte du modèle par votre texte)

Improving methods for the assessment of risk of infection with taeniid eggs in food in Switzerland

[1]Cristian A. Alvarez Rojas, [1]Annina Guggisberg, [1]Nadia Miranda, [1]Peter Deplazes
[1]Institute of Parasitology, University of Zürich, CH-8057, Zürich

Key words (Formatvorlage Überschrift 2)
Echinococcus multilocularis, alveolar echinococcosis, lettuce, detection

Aim of the study (Formatvorlage Überschrift 2)
This study was performed to 1) standardize a simple and feasible method to isolate taeniid eggs from food (vegetables, fruits and others), 2) investigate methods to detect the viability of *E*. *multilocularis* eggs and 3) use the proposed methods to investigate the presence and viability of *E*. *multilocularis* eggs in different food produced in Switzerland to improve surveillance for this important parasite.

Material and methods (Formatvorlage Überschrift 2)
We recovered mature taeniid eggs (*Taenia polyacantha* and *E*. *multilocularis*) from fox faeces. Also, eggs were harvested from adult specimens of *Echinococcus multilocularis* isolated from foxes which were hunted in the canton of Zurich (during the official hunting season). Eggs were used for spiking experiments of lettuce to determine the minimum number of eggs (detection limit) of the sieving system (objective 1) used to isolate taenidd eggs from vegetables from in Switzerland (objective 3). The water used to wash the lettuce spiked with *Taenia polyacantha* and *E*. *multilocularis* eggs included the detergent Tween20 (0.3%). Different volumes of washing water and the weight of salads were tested. For achieving objective 3, lettuce heads were purchased in markets of the city of Zürich including Helvetiaplatz, Burkiplatz and Oerlikon. Nine lettuce heads were purchased per vendor each time, the origin of lettuce was recorded and letuce were washed with the method proposed in objective 1 ; positive samples to E. multilocularis were used for molecular diagnostic and for viability assessment in an in vivo mice model. For achieving the objective 2, we selected genes highly expressed in activated oncospheres including em95 (spanning exons 1 and 2), em95 (spanning exons 2 and 3), major antigen egg p40, ETS transcription factor and amyloid beta A4 protein. Total RNA was isolated from a decreasing number of oncospheres (after treatment of eggs with sodium hypochlorite) from 50, 40, 30, 20, 10, 5 and 2 eggs for cDNA synthesis. Simultaneously eggs were incubated at 37°C, 55°C, 65°C and 85°C and mRNA was isolated (from sodium hypochlorite resistant oncospheres) for cDNA synthesis. cDNA synthesis was used for conventional and qPCR experiments. At the same time, eggs incubated at 37°C, 55°C and 85°C were used for in vivo experiments after treatment of eggs with sodium hypochlorite ; resistant oncospheres were injected into mice subcutaneously and the development of a lession was expected in a maximum period of three months.

Results and significance (Formatvorlage Überschrift 2)
We were able to develop a simple method for the isolation of taeniid eggs from lettuce purchased in markets from the city of Zürich. The sieving method had a limit of detection of 2 eggs of *E*. *multilocularis*. We used PET bottles for sieving, aiming for easy implementation of the method. For the objective 3 we detected taeniid DNA in 10 of 157 lettuce samples including *Hydatigera taeniaeformis* (Syn. *Taenia taeniaeformis*) (four samples), *T*. *polyacantha* (three), *T*. *martis* (one) and *E*. *multilocularis* (two). Two samples positives to *E*. *multilocularis* were used for assessment of viability with an in vivo model, however there was no growth of parasitic lesion in mive. For the objective 2 it was possible to detect mRNA (converted to cDNA) from a minimum of 5 oncospheres using the genes em95 (spanning exons 2 and 3), major antigen egg p40 and the amyloid beta A4 protein. Unfortunately, when incubating eggs at temperatures of 37°C, 55°C, 65°C and 85°C, it was not possible to obtain good quality mRNA for gene expression with the genes mentioned above. Therefore it was not possible to use

the genes mentioned above as markers of viability. Furthermore, oncospheres resistant to sodium hypochlorite, from eggs incubated at 37°C, 55°C and 85°C were not successful in producing infection with the mice in vivo model. The in vivo method (inoculation of mice), produced infection with the control groups. However, the development of the lesion was slower as usual suggesting that the batch of eggs produced was not optimal which hampered the results of the in vivo markers of the viability of eggs from *E*. *multilocularis*.

Publications, posters and presentations (Formatvorlage Überschrift 2)

Guggisberg A., Alvarez Rojas C. A., Kronnenberg P. A., Miranda N., Deplazes P. (2020). A sensitive, one-way sequential sieving method to isolate helminths' eggs and protozoal oocysts from lettuce for genetic identification. Pathogens, 9, 0624

Guggisberg A., A scientific presentation by the doctoral student showing the preliminary results was performed in September 2019 as part of a 'Blockkurs' with the topic parasitology for Biology students from the University of Zurich.

Alvarez Rojas C. A., Preliminary results of the study were shown as part of an oral presentation related with "transmission of *Echinococcus* eggs" at the XXVIII World Congress on Echinococcosis, Lima, Peru, October 29-31, 2019.

Project *4.19.01*

Project duration *March 2019- November 2020*