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Alveolar echinococcosis in slaughter pigs: estimation of prevalence and identification of risk factors

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Key words
Echinococcus multilocularis, pigs, risk factor analysis, prevalence, multiplex PCR, histology, serology

Aim of the study
The aims of this study were (1) to estimate the prevalence of *Echinococcus multilocularis* in slaughtered pigs in Switzerland, (2) to describe *E. multilocularis* lesions morphologically and by histopathology, (3) to establish a serological test for anti-*E. multilocularis* antibodies in pigs, (4) to search for potential regional clusters of infection, and (5) to find factors associated with infection in pigs.

Material and methods
During a twelve-month sampling period, the six largest abattoirs that slaughter about 95% of all pigs in Switzerland collected one liver per batch of pigs with visible lesions suggestive of *E. multilocularis* infection. For each case batch, a control liver from an unaffected batch of pigs slaughtered on the same day was sampled. All submitted livers were tested by multiplex PCR and histopathology for *E. multilocularis* / cestode infection. In parallel, a piece of diaphragm from the same pigs was submitted and meat juice was recovered. Different ELISA protocols with a well-established diagnostic *E. multilocularis* antigen (Em2) as well as a Western blot were assessed as serological tools to detect anti-*E. multilocularis* antibodies in pigs. Case and control farms included in the study were mapped using GIS to identify potential regional clusters. Furthermore, 100 case and 110 control farms were contacted and a standardized questionnaire to assess factors associated with infection was completed during a telephone interview. Risk factors were analyzed with logistic regression analysis.

Results and significance
In total, 456 pig livers with lesions suggestive of *E. multilocularis* infection from 450 batches of pigs were submitted. Of those, 200 livers with characteristic white lesions ranging in diameter from 1-21 mm were positive for *E. multilocularis* DNA by multiplex PCR. These 200 pigs originated from 179 farms. A further five livers had lesions caused by *Taenia taeniaformis* (n=2), *T. polyacantha* (n=2), and *T. martis* (n=1). We received 411 livers from healthy control batches. None of these livers were positive in multiplex PCR. Of the 200 positive pigs, 177 were fattening pigs, and 22 mother sows. Using the numbers of pigs slaughtered in the study period (2'123'542 fattening pigs and 20'454 sows & boars) the prevalence was 0.008% for fattening pigs and 0.1% for sows, respectively. Histopathology was done on livers submitted as “cases” only. A laminated layer characteristic for *Echinococcus* spp. infection was present in 105 cases, and in one case a germinal layer was additionally present. No protoscoleces were observed in any of the lesions, meaning that the pig was a dead-end host for the parasite in all our cases. The most commonly observed features in truly positive lesions were abscesses (n=174), presence of lymphoid follicles (n=136), calcification (n=130), and presence of laminated layer (n=105). The attempts to establish a serological protocol to detect anti-*E. multilocularis* antibodies in pigs did not lead to a method that could differentiate between truly infected and uninfected pigs. This approach was thus abandoned. Infection was present in all regions sampled which represented the important pig rearing areas of

Switzerland, with no obvious geographical clusters. Factors positively associated with infection were "presence of other animals in the stables" (OR=3.0), “having two stable buildings compared to having three” (OR=2.7) "outdoor feeding" (OR=2.1), and "feeding grass" (OR=1.9). Factors negatively associated with infection were “no foxes observed on the premises” (OR=0.17), "deworming of sows twice a year" (OR=1.8), “presence of a hygiene barrier" (OR=0.42), "rodent control" (OR=0.65), and "having own dogs on the farm" (OR=0.66).

The results of this study confirmed the endemic presence of *E. multilocularis* throughout Switzerland. They also supported the hypothesis that pigs are dead-end hosts for the parasite. This finding is very important, as it underlines that pigs and pork do not pose a risk for human infection. The identified factors positively or negatively associated with *E. multilocularis* infection in pigs are mostly explained by the biology of the parasite. Many of the factors can quite easily be addressed by changes in management. The results may potentially also be used to make recommendations to prevent human infection with *E. multilocularis*.

Publications, posters and presentations

Meyer-Schülke, A. (2019): Alveoläre Echinokokkose beim Schlachtschwein. Oral presentation. Laborleitertagung, 24th October 2019, CH-3097 Bern Liebefeld

Meyer-Schülke, A. (2020): Alveoläre Echinokokkose beim Schlachtschwein. Oral presentation. Tierärztetagung, 5th-7th May 2020, CH-4000 Basel

Meyer-Schülke, A., Olias, P., Schüpbach-Regula, G., Hentrich, B., Gottstein, B., Henzi, M., Frey, C.F. (2020): *Echincoccus multilocularis* infection in pigs in Switzerland. Scientific publication. In preparation.

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