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Combined cross-sectional and case-control study on *Echinococcus multilocularis* infection in pigs in Switzerland

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ABSTRACT

The canid tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis (AE) in humans and other intermediate hosts. Depending on the permissiveness of the intermediate host, the larval form of *E. multilocularis* (metacestode) may be either fertile, e.g. in rodents, and thus supporting the life cycle of the parasite, or infertile, e.g. in pigs, and thus interrupting the life cycle. Pigs have been shown to act as aberrant hosts for the metacestode and consequently develop liver lesions but represent a dead-end for the parasite. Routine liver inspection at slaughter provided the basis for a large-scale surveillance study on *E. multilocularis* infection in pigs. The aim of this combined cross-sectional and case-control study was to estimate the minimal prevalence of *E. multilocularis* in pigs in Switzerland, to find factors associated with infection, and to assess potential regional clusters of infection.

During the 12-month-study period, approximately 85% of all pigs slaughtered in Switzerland were assessed. In total, 450 pig livers with macroscopic lesions suggestive of *E. multilocularis* infection were analysed. Of those, 200 samples were positive by *E. multilocularis*-PCR. Thus, the overall minimal prevalence detected by molecular means was 0.009% in all slaughter pigs (200 of 2'143'996), 0.008% in finishing pigs (177 of 2'123'542), and 0.11% in breeding pigs (22 of 20'454). Histology revealed the unique presence of a laminated layer in 105 cases, and an additional germinal layer detected in a single case. Protoscoleces could not be observed in any of the lesions. Factors positively associated with infection were "foxes seen in the pig shed", "foxes on premises", "presence of other animals in the shed", "absence of a hygiene barrier", "outdoor feeding", "feeding grass", "lack of rodent control", "not having own dogs on the farm" and "infrequent deworming of sows". Infection was present in all regions sampled and was representative of the important pig rearing areas of Switzerland, without evidence of any obvious geographical cluster.

Conclusively, our study provided further evidence of widespread environmental contamination with *E. multilocularis* eggs in Switzerland. Furthermore, the absence of protoscoleces in any of the lesions supported the concept that pigs act only as a dead-end host and thus do not contribute to the life cycle of the parasite. Factors associated with *E. multilocularis* infection were in-line with parasite biology, and many can be addressed by increasing hygiene and management standards.

1. Introduction

Alveolar echinococcosis (AE) is a zoonotic parasitic disease affecting predominantly the liver of intermediate hosts such as rodents, and less frequently other mammals and humans. AE is caused by the larval stage (metacestode) of *Echinococcus multilocularis*, the small fox tapeworm. Two types of AE exist depending on the permissiveness of the intermediate host: the metacestodes may be either fertile, e.g. in rodents, and thus supporting the life cycle of the parasite, or infertile, e.g. in pigs, and thus interrupting the life cycle. The disease is a growing threat to public

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health in the northern hemisphere (Gottstein et al., 2015; Kotwa et al., 2019) and has been shown to be highly endemic in Switzerland, with the incidence of human AE significantly increasing in recent years (Gottstein et al., 2015; Lachenmayer et al., 2019). In contrast to human cases, infections in animals – either as final or intermediate hosts - are reportable in Switzerland (Anonymous, 1995). Therefore, monitoring of both definitive and intermediate or aberrant hosts is an indispensable part of disease surveillance, as stated by Deplazes and Eckert (2001).

Pigs are the most susceptible food-producing species in terms of infection with *E. multilocularis* eggs (Deplazes and Eckert, 2001). Pigs, like man, are considered to be an aberrant host because metacestodes found in pigs are infertile, i.e. they do not develop protoscoleces, and the metacestodes were also not infectious for jirds (*Meriones persicus*) (Deplazes et al., 2005). Infection in pigs typically manifests as little whitish, round structures with a creamy centre (termed "micro-abscesses") or as small white dots with poorly demarcated borders in the liver (Deplazes et al., 2005).

To date, *E. multilocularis* infection in slaughtered pigs or wild boars has been described in Japan (Hokkaido: reviewed in Ito et al., 2003; Honshu: Kimura et al., 2010), and in Europe (Eckert, 1997), specifically in Germany (Pfister et al., 1993; Böttcher et al., 2013), France (Boucher et al., 2005), Poland (Karamon et al., 2012), Lithuania (Bružinskaitė et al., 2009), Hungary (Dán et al., 2018), Czech Republic (Kolářová et al., 2017), and Switzerland (Sydler et al., 1998). The study by Sydler et al. (1998) focused on outdoor pigs and found 9 (10%) of the 90 examined pigs to be infected with *E. multilocularis*. However, pigs kept indoors in large-scale husbandries can also become infected, as shown by Böttcher et al. (2013). In Switzerland, cases in pigs are officially very rarely reported: only two cases can be found in the database of the Swiss Food Safety and Veterinary Office (FSVO) since 1996. Although livers with any macroscopically visible lesion are condemned at slaughter, etiological diagnosis is not mandatory nor paid for, and therefore not performed. Thus, little is known about the prevalence of infectious hepatic diseases (such as *E. multilocularis* infection) in the Swiss pig population, and case-control studies to identify risk factors for infection are also lacking. We hypothesised that the infection in pigs is underreported in Switzerland.

Switzerland is considered endemic with a high environmental contamination of *E. multilocularis* eggs (Gottstein et al., 2015). In a recent meta-analysis of the EU and adjacent countries, the prevalence in foxes in Switzerland was considered as high, i.e. > 10% (Oksanen et al., 2016). The occurrence of *E. multilocularis* in Europe is considered as focal (reviewed in Conraths and Deplazes, 2015) and micro-local hotspots of parasite transmission have been described (Raoul et al., 2015). This could also apply to Switzerland, but data to support this hypothesis are scarce (Gottstein et al., 1996). The only way of infection for intermediate or aberrant hosts is through oral uptake of *E. multilocularis* eggs. Infected pigs indicate environmental contamination with viable eggs and may thus be a proxy for the infection risk for humans as well (Karamon et al., 2012). In pigs, large-scale monitoring at the slaughterhouses is easily achievable, as all livers are mandatorily controlled for various lesions in routine meat inspection (Anonymous, 2005).

The aim of this study was to estimate a minimal prevalence of *E. multilocularis* infection in slaughter pigs in Switzerland. Minimal prevalence was chosen for logistic reasons, as only one affected liver per batch of slaughter pigs was submitted. Spatial analysis was used to search for highly endemic regions where the human population might be investigated or informed by public health specialists. The design as a case-control study allowed the identification of factors associated with infection that will have implications for biosafety recommendations in pig husbandry.

2. Material & Methods

2.1. Sample collection and processing

The six largest abattoirs for pigs in Switzerland (Basel, Bazenheid, Courtepin, Langnau, Sursee, and Zürich) collaborated in this study. The sampling period was 12 consecutive months (April 2017 to March 2018), during which a total of 2'143'996 pigs (2'123'542 fattening pigs and 20'454 breeding pigs) were slaughtered at the six participating facilities. In 2018, a total of 2.57 million pigs were slaughtered in Switzerland (Proviande, 2018), therefore we assessed approximately 85% of the total annual production. Pigs were slaughtered in batches, and each batch represented a group of pigs originating from the same farm and slaughtered on the same day. Each batch of pigs that presented at least one liver with lesions suspicious for AE was recorded. A randomly collected sample (about 100 g) of one liver per affected batch (case farms) was collected. If only one lesion was present, the lesion plus adjacent healthy liver tissue was sampled, if numerous lesions were present, the sample was preferentially taken from the edges of the liver. Additionally, on the same day, a randomly selected liver of a macroscopically healthy batch of pigs (control farms) was sampled (about 100 g per liver). All samples were stored at 4 °C and sent with overnight express to the Institute of Parasitology in Bern within a week of slaughter. For each case and control batch, the following information was recorded by the slaughterhouse: date of slaughter, identification, and localisation of the farm, age group (fattening pigs: < 1 year old; breeding pigs: >=1 year old), single or multiple lesions per liver, and percentage of affected pigs per batch (< 10%, 10 - 60%, > 60%).

Each submitted lesion was first photographed and then split by dissection into different sub-samples used for processing for PCR, for storage at -20 °C as a backup, and fixation in formalin for histopathological analyses. Of the control livers, a piece of healthy liver was processed for PCR, and another one was stored at -20 °C.

2.2. DNA Extraction

Genomic DNA was extracted from approx. 20 mg of tissue using Qiagen DNeasy Blood & Tissue Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA was eluted in 200 µl elution buffer. Subsequently, 1 µl of DNA eluate was used for PCR.

2.3. PCR

A multiplex PCR simultaneously detecting and differentiating *E. multilocularis*, *Echinococcus granulosus* s.l., and *Taenia* spp. DNA was performed with 861 liver samples. PCR was according to Trachsel et al. (2007) with the minor modification that it included the uracil-DNA-glycosylase system (use of dUTP instead of dTTP) (Sigma-Aldrich, Buchs, Switzerland) to prevent carry-over contamination (Longo et al., 1990). Cycling conditions were as follows: 1 cycle of 10 min at 20 °C; a Taq DNA polymerase activation step for 15 min at 95 °C; 40 cycles of 30 sec at 94 °C, 90 sec at 58 °C, 90 sec at 72 °C finished by 10 min at 72 °C and then cooled down to 4 °C. In cases where the multiplex PCR detected *Taenia* spp., the PCR was repeated with dNTPs containing dTTP, the amplicon was subsequently purified using Zymo Research DNA Clean & Concentrator-5 Kit (Zymo Research, Irvine, USA) according to the manufacturer's manual, and sent to a commercial sequencing service (Microsynth, Balgach, Switzerland). The assessment of the nucleotide sequence was performed by comparison to already known sequences using the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

2.4. Histology

Samples were fixed in 10% buffered formalin, embedded in paraffin wax and cut in 4 µm sections. The resulting slides were stained by

standard methods using haematoxylin-eosin staining (HE) and Periodic acid–Schiff reaction (PAS) at the Institute of Animal Pathology, Bern.

2.5. Questionnaire and geographical distribution

A total of 179 farms with a positive PCR result for *E. multilocularis* and the corresponding control farms were contacted. One hundred case farms (56%) and 110 control farms (63%) agreed to participate in a telephone interview. Questions asked pertained to the general farm structure and management of husbandry, housing, feeding, and hygiene (Appendix 1). In 26 case farms and 30 control farms, the pigs remained on the same premises throughout their lives.

To determine the geographical distribution of infection, farms were mapped with the software ArcGIS (www.esri.com). To test for differences between different geographical regions, farms were classified into Swiss bioregions. Data of Swiss bioregions were obtained from the Federal Office of the Environment (Good et al., 2001). Differences between prevalence of positive batches were tested with logistic regression analysis.

2.6. Statistical analyses

Data were analysed with the software NCSS 12 Statistical Software (2018) (NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss). Data from the cross-sectional sampling of pigs from batches with macroscopically suspicious lesions were analysed descriptively. The prevalence of PCR-positive samples among livers sent in as suspicious for *E. multilocularis* was compared between the different slaughterhouses by Fisher's exact test, correcting the level of significance for multiple testing with Bonferroni correction. A risk factor analysis was performed for the case and control farms included in the questionnaire study. All potential risk factors were screened for associations ($p < 0.2$) with the case farms by univariable logistic regression. If risk factors were correlated with each other (phi-coefficient > 0.7), they were summarised or the biologically more meaningful factor was selected. For example, two questions on observation of foxes in the pig shed and on the farm were summarised into one variable with the categories "observed in the pig shed", "observed only in other areas of the farm, and "not observed". Questions on presence and type of bedding materials were summarised into "straw bedding" and "no bedding material or sawdust". A multivariable logistic regression model was built by initial entry of all risk factors identified in univariable analysis, followed by stepwise backward selection on non-significant variables ($p >= 0.05$). Model fit was assessed by deviance and log-likelihood statistics, and visual assessment of residuals.

3. Results

3.1. Sample collection and PCR results

In total, 456 livers were sent as cases together with 417 control livers. In the first month, three abattoirs collected just one control per day rather than one control per case, and in the other 11 months, 12 cases were sent without control. Two cases and controls, respectively, were excluded due to missing information concerning their origin, and four cases plus their controls were not evaluated because of advanced autolysis.

All 411 control livers yielded negative PCR-tests for taeniid DNA. Of the 450 case-livers, 200 tested positive for *E. multilocularis* DNA; these 200 livers originated from 179 farms (Table 1). The proportion of suspicious lesions confirmed as *E. multilocularis* by PCR varied from 14 % to 81 % between the abattoirs, with a mean ratio of 44 % confirmed infections (Table 1). Five samples tested positive for *Taenia* spp.. Sequence analyses confirmed *Taenia taeniaeformis* (syn. *Hydatigera taeniaeformis*) (GenBank accession N°: EU219554.1) and *Taenia polyacantha* (DQ408419.1) in two cases each, and *Taenia martis* (JX415820.1) in the fifth sample (Supplementary Table 1).

Table 1
Samples submitted and percentage of *Echinococcus multilocularis* PCR-positive per participating slaughterhouse.

SH*	livers analysed		PCR pos (%)			
	cases	controls	cases	controls	case farms **	Significantly different from SH ***
1	124	113	20 (23%)	0	20	3, 4
2	37	33	10 (27%)	0	10	3
3	148	138	120 (81%)	0	108	1, 2, 4, 5, 6
4	40	33	23 (58%)	0	21	1, 3, 6
5	21	20	7 (33%)	0	7	3
6	80	74	11 (14%)	0	9	3, 4
Total	450	411	200 (44%)	0	179	

* SH: slaughterhouse.
** as sometimes more than one liver from a positive farm was obtained, the number of positive farms is also given.
*** A significance level of $p < 0.0033$ was used (Bonferroni correction for 15 individual comparisons).

3.2. Minimal prevalence for E. multilocularis infection in pigs

Of the 200 confirmed *E. multilocularis*-positive livers, 177 originated from fattening pigs, 22 from breeding sows, and in one case the information was missing. The minimal prevalence in all age groups was 0.009% (200 of 2'143'996 animals). For the 2'123'542 fattening pigs and 20'454 breeding animals that were slaughtered at the six participating facilities in the study period, the minimal prevalences were 0.008% and 0.11%, respectively.

Batch sizes of the 200 positive herds varied between one and 201 animals (median = 24; mean = 32; 0.25 quantile = 13; 0.75 quantile = 44). Prevalence of affected livers per batch of pigs was as follows: In 99 batches, less than 10 % of the pigs were affected, in 79 between 10 % and 60 %, and in 22 more than 60 % were affected.

As only one affected liver per batch was submitted, the true prevalence of *E. multilocularis* infection in the pigs of the study group must have been higher than what we have reported.

3.3. Macroscopy of PCR-confirmed E. multilocularis lesions

Eighty-one livers had single lesions ranging in size from 4 mm to 21 mm, 119 livers had multiple lesions between 1 mm and 20 mm in diameter (Fig. 1). Fattening pigs had lesions between 1 mm and 21 mm in diameter, the livers of breeding sows had lesions with a maximum of 10 mm in diameter. The shape of the lesions varied from round, to oval, to irregular, coloured whitish to yellowish. Some lesions were clearly demarcated and elevated over the liver tissue, whereas others had blurry borders, and some were covered with a thin layer of intact liver tissue. When cut open all lesions had a fibrous capsule of varying thickness, except when they were completely calcified (n = 7), then there was no visible capsule. All seven calcified lesions were smaller than 5 mm; six occurred in breeding sows, one in a fattening pig. Seventy-five per cent of all lesions were filled with a crumbly to creamy to liquid yellow necrotic mass (Fig. 1B); the other 25% were mostly lesions smaller than 10 mm and consisting of fibrous tissue only.

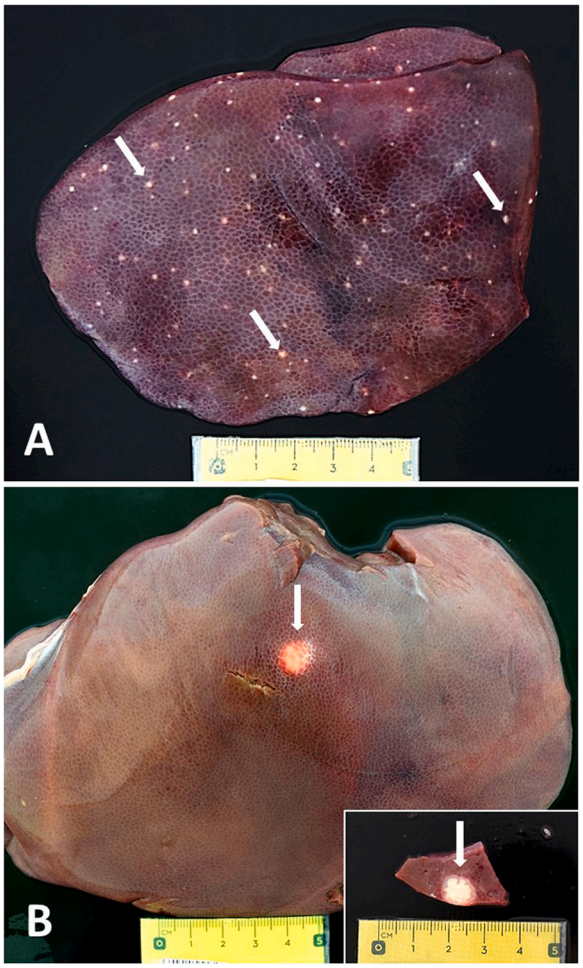

Fig. 1. Macroscopic presentation of natural hepatic *Echinococcus multilocularis* lesions in domestic pigs.
A: multifocal, 1 – 2 mm, well-demarcated whitish lesions (arrows) in the liver parenchyma. B: single, approx. 10 mm in diameter nodule, protruding from the liver parenchyma. Insert: cross-section of lesion B, displaying a whitish core mass surrounded by a light tan, 1 – 2 mm thick capsule.

3.4. Histology

3.4.1. Lesions PCR-positive for E. multilocularis

The lesions showed various signs of periparasitic inflammation and other histopathological changes. The main inflammatory characteristics were abscesses (n = 174), follicle-like accumulations of inflammatory cells (n = 136) (Fig. 2A), granulomas (n = 16), clusters of eosinophilic granulocytes (n = 6) or degenerated neutrophilic granulocytes (n = 2). Fibrosis (n = 7) and partial calcification (n = 130) were also additional pathological changes found in the lesions. In 105 cases, a thin non-cellular and irregularly folded PAS-positive laminated layer was detected in the centre of the lesion (Fig. 2B). The presence of the laminated layer allowed the aetiological diagnosis of *E. multilocularis* infection. Thus, histology had a diagnostic sensitivity of 53% when compared with PCR. The distribution of the previously described characteristics occurred in all possible combinations. In the case of one breeding sow, the laminated layer was lined loosely on one side with a few cells presumably of parasitic origin that was interpreted as a germinal layer (Fig. 2C). However, there were no indications of the formation of protoscoleces in any of the cases.

3.4.2. Lesions PCR-negative for E. multilocularis

None of the 245 livers submitted as case livers that tested negative for *E. multilocularis* or other *Taenia* spp. in PCR showed a laminated or

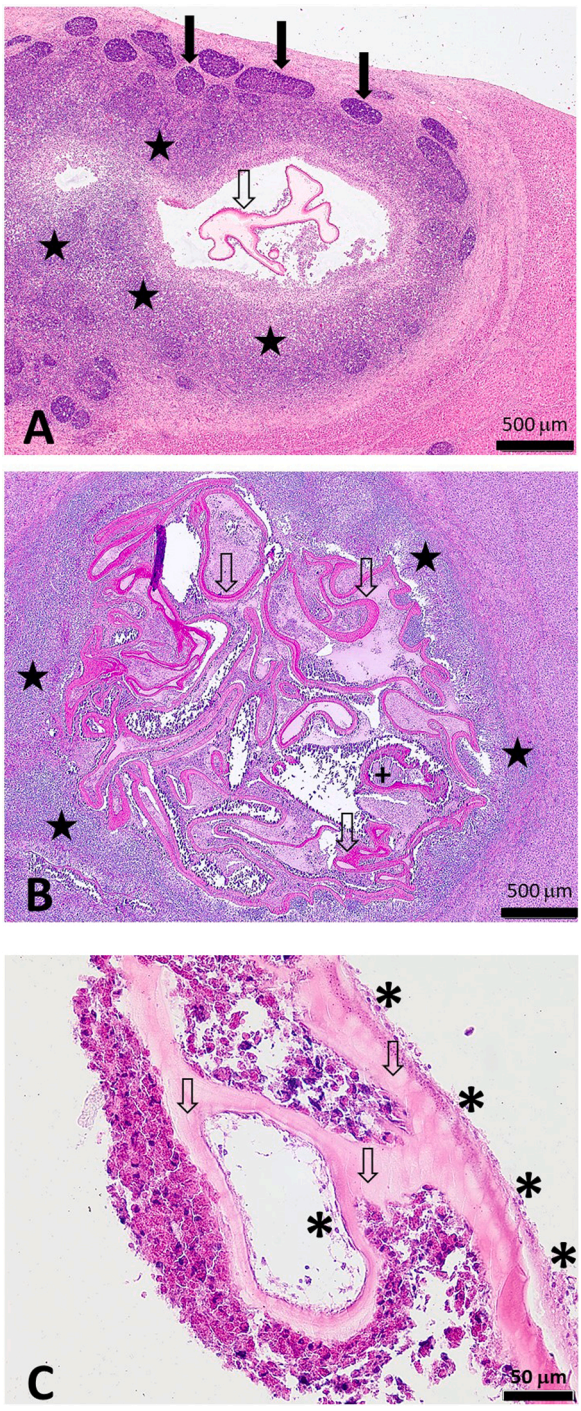

Fig. 2. Histopathological presentations of natural hepatic *Echinococcus multilocularis* lesions in domestic pigs.
A: HE staining, 40 x: abscess (stars) with additional follicle-like accumulations of inflammatory cells (solid arrows) surrounding laminated layer (open arrow). B: PAS staining, 40 x: PAS-positive laminated layer (open arrows) in an abscess (stars) with calcification (+). C: HE staining, 400 x: laminated layer (open arrows) with the associated germinal layer (*).

germinal layer or fragments of it associated with the lesions examined in histology. Thus, histology had a diagnostic specificity of 100% when compared with PCR. Three lesions were identified to be of neoplastic origin (lipogranuloma n = 2; carcinoid n = 1), while the other 242 lesions contained variations of inflammatory and degenerative changes. These findings included (multiple finding per lesion possible): granulomas (n = 141), infiltration with eosinophilic granulocytes (n = 125),

fibrosis (n = 104), abscesses (n = 9), clusters of degenerated neutrophilic granulocytes (n = 29), follicle-like accumulations of inflammatory cells (n = 5), haemorrhages (n = 2), as well as mononuclear (n = 1) and lymphocytic (n = 1) infiltrations. In eight cases the inflammation was accompanied by partial calcification. Overall, all histopathological changes in PCR-negative lesions were too unspecific to reach an aetiological diagnosis, except for the three cases with neoplastic alterations.

3.5. Factors associated with E. multilocularis infection in pigs

In univariable screening, 12 risk factors were significantly associated with increased odds for *E. multilocularis* infection, and 6 risk factors were non-significant, but had a p-value <0.2 (suppl. Table 2). Nine of these risk factors remained significant in the multivariable model after stepwise backward variable selection (Table 2). Reportedly "having seen foxes in the pig shed" (OR = 26.9; 95% CI: 1.5 - 474.4) had the strongest association with *E. multilocularis* infection, although this risk factor also had a large confidence interval because only seven farms had observed foxes in the pig shed. "Foxes that were seen on the farm premises" (OR = 7.5; 95% CI: 2.1 - 26.5) was also associated with an increased risk. "Keeping other animals in the pig shed" (OR = 9; 95% CI: 1.2 - 67.3) increased the risk for infection significantly. "Having one (OR = 4.3; 95% CI: 1.3 - 14.1) or two (OR = 9.2; 95% CI: 2.6 - 33) pig sheds compared to three" represented a higher risk for *E. multilocularis* infection. This seemed related to the tendency to have less infection in larger pig herds, although this was only significant in univariable analysis. "Outdoor feeding of the pigs" (OR = 4.3; 95% CI: 1.8 - 10.3) and "feeding of grass" (OR = 3.7; 95% CI: 1.2 - 10.9) were other risk factors. In univariable analysis, straw bedding increased the risk of infection with *E. multilocularis*, but this was no longer significant after correcting for other risk factors in multivariable analysis.

Management factors were identified as main protective factors such as "operating a hygiene barrier" (OR = 0.2; 95% CI: 0.1 - 0.5), which was closely correlated to disinfection of boots and having clothes especially assigned to the pig shed. Additionally, having some measures in place for "rodent control" (OR = 0.4; 95% CI: 0.2 - 1), either poison, traps or a pest controller, also reduced the risk of infection. "Deworming of sows two times per year" (OR = 0.1; 95% CI: 0 - 0.5) also decreased the probability of an infection significantly. Finally, the "presence of a farm dog" (OR = 0.4; 95% CI: 0.2 - 0.9) acted as a protective factor independently of the deworming status of the dog. All factors included in the risk factor analysis can be found in Supplementary Table 2.

3.6. Geographical distribution

The geographic distribution of case and control farms was similar, and corresponded well to the main pig producing areas of Switzerland (Fig. 3). The majority of study farms was located in the regions with the highest pig density (Fig. 3), with no clusters of higher infection identified. Case and control farms were quite evenly distributed among the biogeographical regions (data not shown). Depending on the farming structure the pigs could be housed on up to four different farms during their lives. Of the 200 animals positive for *E. multilocularis*, 52 remained on the same farm, 66 had lived on different farms in the same region, and 81 were moved to different regions within Switzerland. In one case this information was missing. Pigs on the farm that tested positive in the canton of Ticino, a canton with very low *E. multilocularis* prevalence in foxes (Guerra et al., 2014), were born and fattened on the same farm.

Table 2
Factors significant in a multivariable logistic regression model for infection with *Echinococcus multilocularis* in domestic pigs.

Factor	OR*	95% CI**	p-value
Foxes in pig shed	26.9	1.5 - 474.4	0.024
Foxes on farm	7.5	2.1 - 26.5	0.002
N° of pig sheds (1 vs. 3)	4.3	1.3 - 14.1	0.016
N° of pig sheds (2 vs. 3)	9.2	2.6 - 33.0	0.001
Other animals in pig shed	9.0	1.2 - 67.3	0.032
Outdoor feeding	4.3	1.8 - 10.3	0.001
Grass feeding	3.7	1.2 - 10.9	0.020
Hygienic barrier	0.2	0.1 - 0.5	0.002
Deworming sows 2x/year	0.1	0.0 - 0.5	0.014
Rodent control	0.4	0.2 - 1.0	0.034
Dog on farm	0.4	0.2 - 0.9	0.024

* OR odds ratio; OR > 1 = risk factor, OR < 1 = protecting factor.
** CI confidence interval.

4. Discussion

The present study aimed to establish a minimal prevalence of *E. multilocularis* infection in slaughter pigs in Switzerland. This cross-sectional approach was facilitated by the mandatory meat inspection that requires close inspection of the liver and rejection in cases with visible alterations (Anonymous, 2005). The collaborating slaughterhouses processed about 85% of the annual pig production; thus this study was highly representative of the general pig population in Switzerland. Nevertheless, some regions with less intensive pig production and some production forms (outdoor pigs) are probably underrepresented by our sampling strategy, as those pigs are very likely to be processed in smaller, local facilities.

Our study revealed a calculated minimal prevalence of 0.009% overall, and 0.008% in fattening pigs and 0.11% in breeding pigs, respectively. Other comparable prevalence data on the slaughter pig population is also available from studies in Hokkaido, Japan, and from Sweden, Finland, and mainland Norway. In Hokkaido, a mean prevalence of 0.1% was recorded after meat inspection of over 18 million pigs between 1983 and 1999 (Ito et al., 2003). In our study, only one animal per positive batch was sampled, but in most batches, more than one pig displayed typical lesions, therefore the true prevalence in Switzerland is higher than what we determined. The prevalence in Switzerland might well be comparable to the numbers found in Hokkaido, with both regions being highly endemic for *E. multilocularis* (Deplazes et al., 2017). In contrast, data collected in Sweden, Finland, and mainland Norway between 2000 and 2009, revealed no positive pigs in approx. 125'000 pigs with access to pasture and 213'000 wild boars (Wahlström et al., 2011) at meat inspection. These three European regions were considered free from *E. multilocularis* until 2011 when the first infections in foxes in Sweden were reported (Osterman Lind et al., 2011). Thus, the epidemiologic situation in these regions is quite different from Switzerland and the absence of *E. multilocularis* infection in pigs and wild boars mirrors this difference.

The minimal prevalence of *E. multilocularis* infection in Swiss breeding pigs was about ten times higher than in fatteners. This difference can be explained by the longer lives and therefore an extended potential time of exposure of breeding pigs, and possibly also by different housing conditions. A similar finding has been reported by Bružinskaitė et al. (2009) for infections with *E. granulosus* in pigs. These authors found a significantly higher prevalence in pigs older than one year compared to the younger animals (Bružinskaitė et al., 2009).

Macroscopy and histology of the lesions of 200 pigs did not provide any evidence of fertile lesions, i.e. formation of brood capsules or protoscoleces. A germinal layer was identified in a single breeding pig, but no further indications of fertile lesions were seen in this individual. These findings are in accordance with published studies of natural and experimental infection of pigs and wild boars with *E. multilocularis* (Pfister et al., 1993; Ito et al., 2003; Boucher et al., 2005; Deplazes et al., 2005; Bružinskaitė et al., 2009; Karamon et al., 2012; Böttcher et al., 2013; Dán et al., 2018). Our study therefore supports the hypothesis that the pig is very efficient at controlling this infection, resulting in a dead-end situation for the parasite.

The sampling in this study was done by meat inspectors at the slaughterhouse. We offered visual training and pictures of confirmed

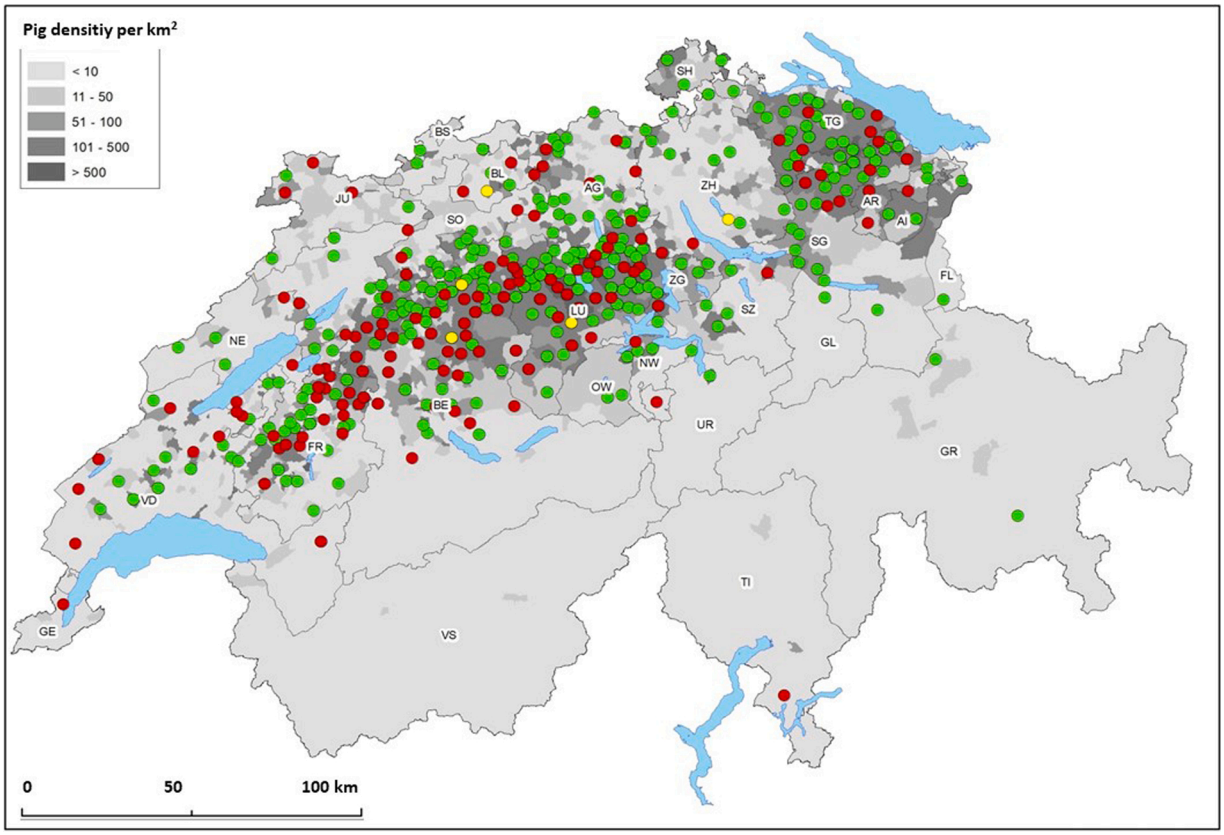

Fig. 3. Geographical distribution of case and control farms.
Map of Switzerland with participating farms included as dots and overall pig densities per km^2 in grey scales.
Red dots: FarmsC PCR-positive for *Echinococcus multilocularis* infection in slaughter pigs (n = 179). Yellow dots: Farms PCR-positive for *Taenia* spp. infection in slaughter pigs (n = 5). Green dots: PCR-negative farms (n = 534).

E. multilocularis lesions in pigs before the beginning of the sampling period. Success rates for recognition of lesions varied significantly across the six slaughterhouses, this may be due to the time restraints that the meat inspectors work under or a consequence of lack of experience in the context of recognition of *E. multilocularis* lesions, resulting in a low sensitivity. A recent study from Hungary also stated that without appropriate training it was difficult for meat inspectors to recognize *Echinococcus* spp. lesions in pigs (Dán et al., 2018). A study by Wahlström et al. (2011) assumed a very low sensitivity of only 10% for meat inspection under non-endemic settings. However, in our study, the success rate improved over time and with our feedback. The training leans towards the typical presentation of *E. multilocularis* infection in pigs, i.e. whitish nodules, therefore the risk arises that atypical lesions, e.g. fertile metacestodes, would probably be missed. The only way to circumvent this risk would have been to assign a professional familiar with all presentations of *E. multilocularis* metacestodes to this task. This was not feasible in the context of this study.

In the present study, no geographic clusters of *E. multilocularis* infection in pigs were identified. This confirms the almost ubiquitous contamination with *E. multilocularis* eggs in the assessed regions of Switzerland. However, we would have expected some regional differences in infection prevalence, as prevalence in the two most important definitive hosts (fox and domestic dog; Hegglin and Deplazes, 2013) was shown to vary greatly between areas and sampling seasons: In foxes, prevalences ranging from 13 % to 57 % were found in an early study in Canton Zurich (Ewald et al., 1992) and a similar range was also encountered in more recent studies (e.g. Hegglin and Deplazes, 2013). An interesting finding from our study was the case farm in southern Ticino, a region that is generally considered as lower risk for *E. multilocularis* infection (Guerra et al., 2014). The pigs spent their whole lives on this particular farm, therefore, it seems very probable that this finding reflects a local egg contamination.

One potential reason why we were unable to see spatial differences is because the control sample, although randomly selected, was often conveniently taken from the next healthy batch of pigs. As pig batches from neighbouring farms tend to arrive at the abattoir in the same transporters, samples and controls were unintentionally geographically matched. While this limited the ability to recognise geographic clusters, it helped to identify risk factors.

Risk factors were assessed by a structured telephone interview. The time effort for the farmer was smaller than for a farm visit, which probably helped us to achieve a good response rate. On the other hand, this might have led to some misclassification bias, because the answers could not be checked with personal observation. We thus cannot exclude that for questions such as cleaning and disinfection or deworming routines, socially desirable answers might have been given. Several risk factors for infection with *E. multilocularis* in pigs were identified in this study. The interviews were conducted with farmers from the premises where the pigs were last housed before slaughtering. Thus, it is possible, that some infections had occurred on previous farms where the animals had lived. However, when the analyses were repeated with only those pigs that were housed on a single farm their entire life, the risk factor of feeding grass and the protective factors of maintaining a hygienic barrier and rodent control remained significant (data not shown).

The reasons outlined above can be backed up by parasite biology. The presence of foxes on the premises or even in the pig shed is very likely to increase environmental contamination with *E. multilocularis* eggs and therefore the infection risk. Consequently, the presence of other animals in the pig shed was a risk factor, while having a hygiene barrier on entry into the pig facility and rodent control would decrease

the risk of infection. Factors associated with feeding were also significant, such as outdoor feeding and feeding grass as risk factors. The high prevalence of 10% in outdoor pigs reported by Sydler et al. (1998), and other studies finding infection mainly in family farmed pigs (Bružinskaitė et al., 2009; Dán et al., 2018) indirectly confirm this risk factors. Other protective factors may reflect general good management, e.g. the regular deworming of sows. Interestingly, some risk factors were reminiscent of risk factors for humans, like chewing grass, or hunting/handling foxes (Conraths et al., 2017). Owning a dog was demonstrated to be a protective factor in the present study. This is contradictory to the meta-analysis in humans, where dog ownership was a risk factor for infection (Conraths et al., 2017). We interpreted this finding to suggest that the farm dogs probably chased away the foxes. As the latter have a higher prevalence of intestinal infection than dogs (Oksanen et al., 2016), the net effect was therefore protective. Many of the identified risk factors can be mitigated by management solutions, like the installation of hygiene barriers, the control of pig feed and rodent control.

5. Conclusions

The systematic sampling of all batches of slaughter pigs with suspicious liver lesions in a period of 12 months out of a population representing approx. 85% of the annually slaughtered pigs in Switzerland allowed for a good overview of prevalence in the Swiss pig population. Prevalence was established as 0.009% over all age classes, with 0.008% in finishing pigs, and 0.11% in breeding pigs, respectively. However, these numbers are an underestimation of the actual prevalence as just one pig per affected batch was sampled, but most batches were reported to have multiple animals with altered livers. This description of 200 pigs with *E. multilocularis* infection represents one of the largest sample sets for natural infection of pigs with this parasite. All metacestodes were found to be without protoscoleces, thus adding weight to the hypothesis that the pig is a dead-end host for *E. multilocularis*. No geographical clusters could be identified, with infections originating from all parts of Switzerland where pig production takes place. Identified risk factors for *E. multilocularis* infection in pigs could be explained by parasite biology and the infection risk lowered by increasing hygiene and management standards.

Declaration of Competing Interest

The authors report no declarations of interest.

CRediT authorship contribution statement

Anika Meyer: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Philipp Olias:** Data curation, Formal analysis, Methodology, Visualization, Writing - review & editing. **Gertraud Schüpbach:** Data curation, Formal analysis, Methodology, Validation, Visualization, Software, Writing - review & editing. **Martin Henzi:** Conceptualization, Data curation, Investigation, Resources, Writing - review & editing. **Thomas Barmettler:** Investigation, Writing - review & editing. **Brigitte Hentrich:** Investigation, Methodology, Writing - review & editing. **Bruno Gottstein:** Project administration, Supervision, Resources, Writing - review & editing. **Caroline F. Frey:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vpoa.2020.100031.

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