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Research paper

Apparent prevalence of and risk factors for infection with *Ostertagia ostertagi*, *Fasciola hepatica* and *Dictyocaulus viviparus* in Swiss dairy herds

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ABSTRACT

Infections with helminth parasites can negatively affect performance of dairy cows. Knowledge on infection intensity, spatial distributions and risk factors are key to develop targeted treatment strategies. Canada and most EU countries have conducted large investigations, but respective data for Switzerland were missing. We now performed a bulk tank milk serosurvey for *Ostertagia ostertagi*, *Fasciola hepatica*, and *Dictyocaulus viviparus* on a total of 1036 voluntarily participating dairy herds that were sampled at confinement periods, i.e. in winter 2014/15 or 2015/16, respectively. All samples were analyzed with commercial ELISAs for antibodies (AB) against *O. ostertagi* and *F. hepatica*, and those of the first sampling period additionally with an in-house ELISA for AB against *D. viviparus*. Testing for the latter parasite was not done in the second year of the study, as the sampling period might have missed infections due to the short lived nature of specific antibodies. The possible influence of geographic, climatic, and farm management variables on AB levels were assessed for each parasite using scanning cluster and multiple regression analysis.

Overall seroprevalence for *O. ostertagi* was 95.5% (95% C.I.: 94.0–96.6), with a mean optical density ratio (ODR) of 0.83, for *F. hepatica* 41.3% (95% C.I.: 38.3–44.4), and for *D. viviparus* 2.9% (95% C.I.: 1.6–4.7). There were no significant differences between the two sampling periods. For all parasites, significant geographic clusters of higher AB levels could be established. Furthermore, AB levels against all three parasites were positively correlated with each other, indicating either cross-reactions or co-infections. For *O. ostertagi*, herd size and percentage of pasture in the ration were positively correlated with AB levels. For *F. hepatica*, altitude above sea level (a.s.l.) positively, and milk production per cow and year was negatively correlated with AB levels.

This work provides baseline data for further studies performing in-depth risk factor analysis and investigating management as well as targeted treatment options to control the parasites.

1. Introduction

In Switzerland, as generally in Western Europe, dairy production is an important part of agriculture representing about 20% of the total revenue of Swiss agriculture (Leuenberger, 2016). In the last years, however, the number of dairy herds and dairy cows declined because producers had to face a drop in the milk price (Leuenberger, 2016). In this tough economic environment, producers aim at optimizing their systems. Thus, subclinical infections with parasites that have negative effects on productivity, such as *Ostertagia ostertagi* (reviewed in Charlier et al., 2009), *Fasciola hepatica* (Schweizer et al., 2005; Charlier et al., 2012), and *Dictyocaulus viviparus* (Dank et al., 2015; Charlier et al., 2016) gain more attention from both producers and veterinarians. The availability of anthelmintic drugs with zero day withdrawal for milk makes deworming of dairy cows attractive. However, there is a risk of anthelmintic resistance development, if drug pressure increases, as shown by emerging resistant gastrointestinal (GI)-nematodes in cattle (Demeler et al., 2009; Sutherland and Leathwick, 2011; Geurden et al., 2015), and, as a consequence of repeated treatments due to poor immunological response of cattle against liver fluke, triclabendazole-resistant *F. hepatica* (Brennan et al., 2007). Anthelmintic drugs therefore should only be used upon indication.

The availability of ELISAs that measure antibodies in bulk tank milk (BTM) against *O. ostertagi*, *F. hepatica*, and *D. viviparus* allow timely and cost reduced diagnostics on herd level and large seroepidemiological surveys. Such surveys have been conducted in e.g. Canada and many

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Table 1
Summary descriptive statistics of farm characteristics for numeric variables.

Variable	Year 1			Year 2		
	No. of Response	Mean (median)	SD (interquartile range)	No. of Responses	Mean (median)	SD (interquartile range)
Altitude (m.a.s.l)	518	748.2 (634.3)	391.2 (518.1–810.2)	509	720 (668.8)	262.8 (546.5–849.3)
Pasture size (ha)	480	10.9 (6.0)	25.9 (4.0–11.3)	482	10.6 (7.1)	12.1 (4.5–12.4)
Number of cows per farm	491	33.7 (28.0)	19.1 (20.5–40.0)	493	32.1 (27.0)	19.1 (20.0–38.0)

European countries in the last decade (among others: Forbes et al., 2008; Bennema et al., 2010; Höglund et al., 2010; Vanderstichel et al., 2012). They have evidenced marked differences in respective seroprevalences between different countries (Forbes et al., 2008; Bennema et al., 2010). Information about spatial distribution of the parasites and risk factors for infection helps in designing targeted treatment protocols, ideally reducing the number of anthelmintic treatments. For Switzerland, such studies have been lacking so far. The aims of the present study therefore were to (1) determine apparent seroprevalences for *O. ostertagi*, *F. hepatica*, and *D. viviparus* in Swiss dairy herds, and (2) to identify risk factors for infection as well as geographic clusters of high prevalence for each of the three parasites.

2. Materials and methods

2.1. Study population

The dairy herds sampled in our study (n = 1036) represented approx. 4.8% of all Swiss dairy herds (Leuenberger, 2016). They were sampled by local veterinarians during the winter confinement period between November 2014 and March 2015, or November 2015 and March 2016, respectively. This period was chosen because during winter months, a period of less intense work load, farmers are generally more willing to participate in studies. Most of the samples (77%) were collected in November and December. Veterinarians were recruited through informative meetings given by some of the authors (CF, RE, BH) and a mailing campaign of Biokema S.A., and they then approached the farmers. The serological tests were free of charge for participants. Results of the test were communicated to the veterinarians. A short questionnaire asking for demographic and management data (milk production, number of cows, cow density on pasture, percentage of pasture in the ration, and duration of pasture season) was completed at the time of sample collection. In the first study period (2014/15), the questionnaire also included questions related to anthelmintic treatment in heifers and dairy cows, and vaccination against *D. viviparus*.

2.2. Meteorological data for 2014 and 2015

Data on average temperatures, sunshine, and precipitation were obtained from the Swiss Federal Office of Meteorology and Climatology MeteoSwiss (MeteoSwiss 2015 & 2016).

2.3. ELISAs to detect antibodies against O. ostertagi, F. hepatica, and D. viviparus in bulk tank milk

Milk samples were centrifuged (2000 × *g*, 15 min) upon arrival at the laboratory and the skimmed milk was stored at −20 °C until further processing. Commercial SVANOVIR® *O. ostertagi* Ab ELISA (SVANOVIR, Uppsala, Sweden) was performed as recommended by the manufacturer to detect antibodies against adult *O. ostertagi*. Results were expressed as optical density ratio (ODR) at 405 nm calculated as follows: ODR = (ODsample–ODnegControl)/(ODposControl–ODnegControl) (Charlier et al., 2009). ODR values < 0.5 were interpreted as negative, values ≥0.5 and < 0.8 as low level of infection, levels ≥0.8 and < 1.1 as medium, and values ≥1.1 as high level of infection, as recommended by the manufacturer.

Commercial IDEXX Fasciolosis Verification test kit (IDEXX, Maine, USA) was used according to the manufacturers' instructions to detect antibodies against the f2 antigen of *F. hepatica*. Results were expressed as the percentage of the OD value of the positive control at 450 nm (S/P %). Values < 30% were interpreted as negative, values ≥30 and < 80% as low infection, values ≥80 and < 150% as medium infection, and values of ≥150% as strong infection, as recommended by the manufacturer.

An in-house ELISA based on major sperm protein (MSP) of adult male *D. viviparus* worms was carried out as described by Fiedor et al. (2009). The cut-off value of 0.41 ODR was used as validated for BTM testing by Schunn et al. (2012) to reliably detect herds with more than 20% infection rate. Analyses for *D. viviparus* were performed with milk samples from the first study season only (winter 2014/15), as the sampling period was not ideal for this parasite, since MSP antibodies have been shown to persist only for two to six months after initial infection (Fiedor et al., 2009).

2.4. Data analysis

The relationship between the level of infection as measured by the ELISA and risk factors was assessed for each parasite. Considered risk factors were: year of sampling (accounting for different weather conditions), pasture size, level of milk production, number of cows, stocking density on pasture, duration of pasture season, proportion of pasture in the ration, bioregion, altitude above sea level (a.s.l.), deworming of heifers, deworming of cows, vaccination against *D. viviparus* (Tables 1 & 2). The location, bioregion, and altitude of each farm corresponded to the coordinates of the centroid of the postal code where the farm was located. The following variables were log-transformed to allow normality: number of cows, pasture size, and results for *F. hepatica* and *D. viviparus* ELISAs. Stocking density was calculated as the number of cows divided by the pasture size and then log-transformed. Since this variable did not improve any result (data not shown) it was excluded from the analyses. To avoid any bias due to participation to the study (i.e. altered treatment protocols or other management factors) for farms that participated in both years, only the data of the first year was used for the respective farms.

In a first step, the relationship between each explanatory and response variable was assessed separately by univariate linear regression. Since lungworm serology was only done during the first year of sampling, the year variable was excluded from the analysis for this parasite. Explanatory variables with a significance level of < 0.1 were selected to be included in multivariable linear regression models for each parasite. Multicollinearity was checked using the variance inflation factor. The selection of the best model was performed in a stepwise backward selection process. Each model obtained by dropping one variable of the full model, was compared to the full model by a likelihood ratio test. The variable with the least significance level was removed from the full model and the process was continued until all explanatory variables became significant.

Presence of geographic clusters of higher parasite infection was assessed with the SaTScan Software (version 9.4.2, www.satscan.org) using a purely spatial normal model. The purely spatial model was preferred to the spatio-temporal model because no significant effect of