



Exposure to *Ostertagia ostertagi* in Swedish dairy cattle: Insights from a 20-year comparison based on BTM-ELISA and questionnaire analysis

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ABSTRACT

Gastrointestinal parasites, especially *Ostertagia ostertagi* – the stomach worm – are known to affect the health and productivity of cattle. This study investigates the level of *O. ostertagi* antibodies on Swedish dairy farms and aims to investigate differences in the parasitic exposure over 20 years. Bulk-tank milk (BTM) samples were taken from 509 farms across Sweden, and the results were analysed along a north-south gradient in relation to three main country regions. Antibody levels against the stomach worm were quantified using a standardised and validated enzyme linked immunosorbent assay (ELISA) kit for the detection of antibodies in milk samples and compared with those obtained from similar samples from 2005/6. A questionnaire was also sent to farmers to update information on location, herd size, management practices including anthelmintic control measures. The results of the two studies (i.e. 2024 and 2005/6) were visualised for comparison. Statistical analysis of BTM-antibodies revealed significantly higher levels in the two northernmost regions (Norrland and Svealand) in 2024, while the increase in the southernmost region (Götaland) was not significant. There were also differences in husbandry practices: in 2024 herds were larger, the use of pour-on solutions was more common, and the grazing season was slightly longer, while in 2005/6 the heifers were housed later and dewormed less frequently. In this study, the BTM-ELISA test is used as a non-invasive and cost-effective method for mapping parasite hotspots and provides insight into the dynamics of *O. ostertagi* exposure over the years.

1. Introduction

Gastrointestinal nematodes (GIN) are a major problem for cattle health, welfare, and profitability of pasture-based dairy herds worldwide (Charlier et al., 2012; Perri et al., 2011). In temperate climates, *Ostertagia ostertagi* is the most important GIN of concern to farmers and veterinarians. It is assumed that all grazed ruminants in temperate regions are infected at some point (Van Dijk et al., 2010). Studies in North America and Europe have shown that GIN infection is widespread in grazing adult cows, with prevalence rates between 80 % and 100 % (Charlier et al., 2020a). This is to be expected as all pastures are likely to be contaminated and thus all grazing animals are exposed to the infection. Accordingly, efficient management and control of helminth infections is crucial for increasing livestock production in the face of shrinking natural resources and for feeding a growing and increasingly insecure population (Vercruyse et al., 2018).

Ostertagia eggs or infective larvae can survive for weeks or months

even in winter or drought conditions, while larval development depends on temperature, rainfall and UV light (Dijk et al., 2009). Many *O. ostertagi* worms can exist in an inhibited (hypobiotic) intramucosal fourth larval stage. Most animals harbour up to 10,000 worms, while a smaller proportion have a high burden (>10,000 worms). *Ostertagia* eggs or infective larvae can survive for weeks or months even in winter or drought conditions, while larval development depends on temperature, rainfall and UV light (Dijk et al., 2009). Farms that rely heavily on pasture may therefore have higher levels of infection. Calves are more susceptible to the effects of parasitism than heifers, who are in turn more susceptible than adults (Charlier et al., 2020a). A significant decrease in weight gain has been observed in both first and second season grazing cattle in Sweden (Larsson et al., 2006). Although subclinical infections are common, production in dairy herds can be affected (Charlier et al., 2009a; Höglund, 2010). Primary effects include reduced milk yield and impaired reproductive performance (Charlier et al., 2015; Morgan et al., 2013; Almería et al., 2009; Blanco-Penedo et al., 2012). In practise,

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parasite-related damage is often diagnosed by a positive production response following an anthelmintic treatment. This type of GIN infections in cattle is referred to as "infections of economic importance". The challenge is to find the optimal allocation of pasture, roughage and concentrates to maximise economic performance, while accounting for farm-specific constraints and input costs (Charlier et al., 2015).

It is argued that global warming in temperate regions, combined with treatment failures due to anthelmintic resistance poses a major threat to the sustainability and efficiency of livestock production (Morgan et al., 2013). In addition to ecoclimatic factors affecting pasture contamination, on farm management practices, such as pasture mowing and length of grazing season, are also critical in accurately predicting infection risk (Bennema et al., 2011). For example, significant differences in Optical Density Ratio values (reflecting the exposure to the parasite) were found between herds kept indoors and those kept outdoors, while other influential factors such as the type of paddocks, the timing of turnout and housing influence the risk of infection (Forbes et al., 2008). As a result, grazing conditions, in addition to regional climatic and weather differences lead to varying degrees of exposure to the parasite (Kowalczyk et al., 2018), which can be determined by quantifying antibodies against *O. ostertagi* (Bennema et al., 2010).

To mitigate the effects of GIN in livestock, highly effective and relatively inexpensive anthelmintics have been developed to treat ongoing infections and prevent associated production losses. As a result, worm control has become highly dependent on these drugs (Kaplan, 2004), and the availability of anthelmintics (i.e. eprinomectin) with a zero-day withdrawal in milk makes their frequent use in dairy cows attractive (Demeler et al., 2009; Sutherland & Leathwick, 2011; Geurden et al., 2015). Unfortunately, the intensive use of anthelmintics has also led to the emergence of resistance in bovine GIN (e.g. Edmonds et al., 2010; Gasbarre, 2014; Sutherland & Leathwick, 2011; Knox et al., 2012; Waghorn et al., 2006; Areskog et al., 2013; Kaplan et al., 2023). Although resistance has been confirmed in most European countries (Rose et al., 2015), data on its prevalence and impact on production remains limited and inconsistent (Charlier et al., 2020b).

Despite the lack of data, the need for sustainable control that reduces reliance on anthelmintics should be emphasised (Charlier et al., 2014; Vande Velde et al., 2015). Given this challenge, anthelmintics should be used only when necessary (Frey et al., 2018). At the same time, there is a conflict between limiting the use of anthelmintics to avoid resistance and optimising productivity (Charlier et al., 2005a). Current control measures rely heavily on treatments in which entire herds are blanket treated with anthelmintics. However, this approach needs to be replaced by more rational and sustainable treatment strategies (Ravinet et al., 2016).

Cattle are the economically most important livestock in Sweden. Milk and beef production is based on around 1.6 million cattle, including 355,000 dairy cows. These contribute to 64 % of the total economic value of domestic livestock production, with the majority (93 %) being kept in conventional herds (Anon, 2009). Meanwhile, policy aims to increase organic agricultural production to 20 %, which will lead to a steady increase in organic livestock production in Sweden (Höglund, 2010). Furthermore, the National Animal Welfare Act in Sweden stipulates that cattle older than six months must have outdoor access during the grazing season, regardless of whether they are kept in organic or conventional systems. This requirement inevitably exposes dairy herds to pasture borne parasitic infections (Anon, 2007). Surprisingly, infection rates with parasites in organic herds have not increased dramatically despite the limited use of anthelmintics, possibly due to the implementation of alternative preventive measures (Svensson et al., 2000; Höglund et al., 2001).

To monitor the spatial distribution of *O. ostertagi* infection, specific antibody levels as measured by the ELISA ODR in combination with a geographic information system (GIS) enables the identification of high-risk areas, which facilitates the development of regionally customised control strategies. In this study, 509 bulk-tank milk (BTM) samples from

herds throughout Sweden were processed and analysed using a commercially available enzyme linked immunosorbent assay (ELISA) kit. The results were visualised in maps created with QGIS and compared on a regional basis with similar data collected more than 20 years ago. In addition, a questionnaire was sent to the participating farms to collect data on their agricultural practices. It was hypothesised that infection rates may have changed in relation to climate and recent structural changes in the Swedish dairy sector.

2. Materials and methods

2.1. Sample analysis

BTM samples were randomly selected from Swedish dairy farms ($n = 509$) and taken in September 2024 by a technician from the Eurofins laboratory in Jönköping (Sweden) as part of a test milking on randomly selected dairy farms in Sweden. The milk was filled into specially prepared tubes that had been pre-treated with Bronopol, a preservative with no known effects on antibodies. Upon arrival at the Swedish University of Agricultural Sciences (SLU), they were stored in a refrigerator at 4°C and centrifuged at 3000 × g for 10 min at 4°C. The lipid layer was then carefully removed. The samples (1 ml each of the liquid phase) were aliquoted into two sets (duplicates), A (1 ml) and B (1 ml) and stored in Micronic® tubes at -20 °C until further analysis. The tubes were removed from the freezer the day before analysis and placed in the refrigerator overnight to thaw. A portion of the data of 2005/6 has been previously published as national-level results by Bennema et al. (2010).

2.2. ELISA

A standardised ELISA kit (SVANOVIR® Ostertagia-Ab ELISA kit, INDICAL BIOSCIENCE, Leipzig, Germany) validated for individual and BTM milk samples was used to detect specific antibodies against the stomach worm *O. ostertagi* based on a crude adult worm capture antigen, according to the manufacturer's instructions. The substrate was measured at 405 nm using a spectrophotometer and the results were expressed as optical density ratio (ODR) calculated as follows: $ODR = (OD \text{ sample} - OD \text{ neg-control}) / (OD \text{ pos-control} - OD \text{ neg-control})$, where controls are the ODs of a negative and positive test control sample on each plate. The herds were divided into three categories, (i) ODR values of 0.7 or higher were associated with a high exposure to the parasite, (ii) values below 0.4 signified low exposure and (iii) values between 0.4 and 0.7 corresponded to medium exposure.

2.3. Data analysis

Data from the ELISA and saved data from similar experiments conducted in 2005/6 were collected. A two-way ANOVA and a post hoc test were performed in RStudio version 2025.05.1 + 513 "Mariposa Orchid" Release to assess statistical significance when comparing ODR values by region and year. All figures were created using the Microsoft Power BI tool version 2.144.878.0.

2.4. GIS

The sampled farms included were first georeferenced by address (exact location with coordinates) and grouped by region (Norrland in northern Sweden, Svealand in central Sweden and Götaland in southern Sweden) and year (2005/6, 2024). Two maps were created to compare the data from the two studies. The points on the maps followed the same categorisation as the ODR values, namely high, medium and low shown in different colours: the farms are shown with blue (high ODR), orange (medium ODR) or green (low ODR) dots. The visualisation of the geographical distribution was created in QGIS version 3.34.12-Prizren.

2.5. Questionnaire

A questionnaire was created on the Netigate platform (www.netigate.net) and sent by email to 450 farmers who participated in the 2024 study. It contained essentially the same content as the questionnaire used in a previous study (2005/6) and consisted of 34 closed and 6 open questions (Supplementary material 1). Information was collected on location, herd size, management practices and anthelmintic control measures in calves, heifers and adult cows. Calves were defined as animals younger than one year of age, heifers as animals aged one year to first calving, and post first calving animals were defined as cows (adults). Finally, dry animals were the non-milking adult animals.

2.6. Statistical analyses

2.6.1. Questionnaire data

For each management variable (a–y), the distribution of categorical responses was analysed using chi-square (χ^2) tests of independence. To evaluate temporal differences, responses from 2005 and 2024 were pooled across regions (Götaland = G, Svealand = S, Norrland = N) and compared using a $2 \times K$ contingency table, where K is the number of valid response categories. Regional variation was assessed by combining the results for all years and then using $3 \times K$ contingency tables, corresponding to the three regions (G, S, N). Missing or empty categories were excluded from the analysis. A significance level of $p < 0.05$ was applied. All analyses were conducted in GraphPad Prism 10 (version 10.6.1) for macOS.

2.6.2. Multiple linear regression

The association between ODR and spatial, host- and management-related variables was analysed using multiple linear regression, with ODR treated as a continuous response variable. ODR was modelled according to the linear model

$$\text{ODR} = \beta_0 + \beta_1X + \beta_2Y + \beta_3\text{Herd size} + \beta_4\text{Grazing time} + \beta_5\text{Cow density} + \beta_6\text{Mowing} + \epsilon,$$

where X and Y denote longitude and latitude, categorical predictors were included as factors and interpreted relative to their reference categories, and ϵ represents the residual error term. Models were fitted using ordinary least squares in GraphPad Prism.

Analyses were conducted on 459 complete observations collected in 2005/06; records with missing values were excluded before model fitting. Overall model significance was assessed using analysis of variance (ANOVA), and the contribution of individual predictors was evaluated using partial F-tests. Model assumptions were assessed using standard diagnostic procedures: linearity and homoscedasticity were evaluated by inspection of residual plots, residual normality was examined using Q–Q plots, and multicollinearity was assessed using variance inflation factors (VIFs).

3. Results

3.1. ELISA

Of the 509 farms analysed in 2024, 233 produced BTMs with high, 238 with medium and 38 with low antibody levels (Table 1). These results correspond to an increase in high IgG-levels from 24 % to 45 % compared to the data from 1089 farms in 2005/6 (Table 2, partly published in Bennema et al., 2010), when 264 farms produced milk with high, 619 with medium and 206 with low antibody levels. At the same time, the medium and low Ig levels decreased, from 56 % to 46 % and from 18 % to 7 %, respectively. The results indicate a trend towards higher exposure to the parasite over the years. This pattern also appears to apply to the individual regions. As can be seen in Fig. 1, both Norrland and Svealand showed a highly significant increase in ODR levels

Table 1

ODR values and their percentages of the total population in 2024.

ODR	Norrland 2024	Svealand 2024	Götaland 2024	SUM
> =0.7	18 (7.7 %)	54 (23.2 %)	161 (69.1 %)	233 (45.8 %)
0.4–0.7	18 (7.6 %)	35 (14.7 %)	185 (77.7 %)	238 (46.8 %)
= <0.4	0 (0.0 %)	1 (2.6 %)	37 (97.4 %)	38 (7.5 %)
SUM	36 (7.1 %)	90 (17.7 %)	383 (75.3 %)	509 (100.0 %)

Table 2

ODR values and their percentages of the total population in 2005/6.

ODR	Norrland 2005/6	Svealand 2005/6	Götaland 2005/6	SUM
> =0.7	8 (3.0 %)	50 (18.9 %)	206 (78.0 %)	264 (24.2 %)
0.4–0.7	69 (11.1 %)	211 (34.1 %)	339 (54.7 %)	619 (56.8 %)
= <0.4	40 (19.4 %)	108 (52.4 %)	58 (28.16 %)	206 (18.9 %)
SUM	117 (10.7 %)	369 (33.9 %)	603 (55.4 %)	1089 (100.0 %)

($p < 0.0001$), while the difference between years in Götaland was not significant ($p = 0.42$).

To compare the mean values and the data distribution by region and year, a violin plot was created. Fig. 2 contains the maximum, minimum, median, mean and standard deviation, which can be found analytically in Table 3. The mean has increased in all regions, namely by a factor of 0.24 in Norrland ($p < 0.0001$), 0.25 in Svealand ($p < 0.0001$) and 0.03 in Götaland ($p = 0.06$).

As can be seen in Fig. 3, the number of farms with high ODR (blue dots) is proportionally higher in 2024 than in 2005/6, while the number of medium and low ODR (orange and green dots, respectively) is lower.

3.2. Questionnaire

A total of 139 out of 509 farmers surveyed in 2024 (27 %) provided information on their farming practices. Most of them were located in Götaland (77 %), followed by 18 % in Svealand and 5 % in Norrland. Most of the farms that responded (91 %) consisted mainly of dairy cows only and their size varied between less than 60 (26 %), 60–150 (48 %) and more than 150 (25 %) animals, with 23 % of them being organic in 2024 compared to only 7 % in 2010 (Höglund et al., 2010).

In 2006, a similar questionnaire was sent out, to which 494 farmers responded, of which 54 % were in Götaland, 35 % in Svealand and 11 % in Norrland. The herds consisted mainly of pure dairy cows (88 %) with a size of less than 30 (36 %), 30–60 (40 %) and more than 60 (24 %) cows.

A summary of the questionnaires' responses and the outcome of chi-square analyses evaluating differences in management practices and herd characteristics across regions (Götaland, Svealand, Norrland) and between years (2005/6 vs 2024) are provided as Supplementary Tables S1 and S2, respectively.

3.2.1. Farm type and herd size

The distribution of farm types did not differ significantly between regions ($p = 0.26$). However, a strong temporal effect was observed, with a significant decline in mixed herds between 2005/6 and 2024 ($p < 0.001$). Herd size showed no regional effect ($p = 0.11$) but increased markedly over time, with a greater proportion of large herds (>150 cows) in 2024 ($p < 0.0001$).

ODR by Region/Year

ODR Category ● <0.4 ● >=0.4, <0.7 ● >=0.7

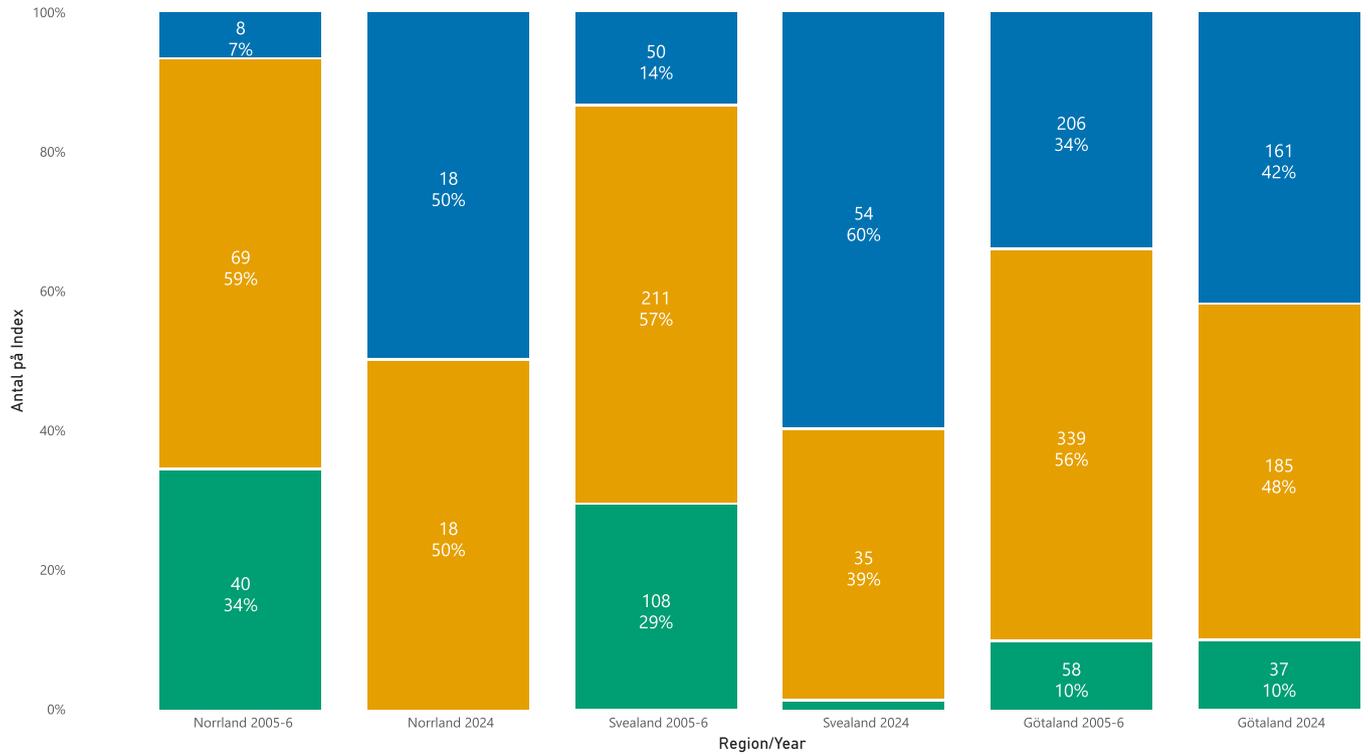


Fig. 1. Bar chart of optical density ratios (ODRs) of *Ostertagia*-antibody levels showing temporal and spatial differences by year and region. The low ODR values are shown in green, the medium in orange and the high in blue colour.

ODR by Region/Year

Region/Year ● Norrland 2005-6 ● Norrland 2024 ● Svealand 2005-6 ● Svealand 2024 ● Götaland 2005-6 ● Götaland 2024 ■ Median Value □ Mean Value

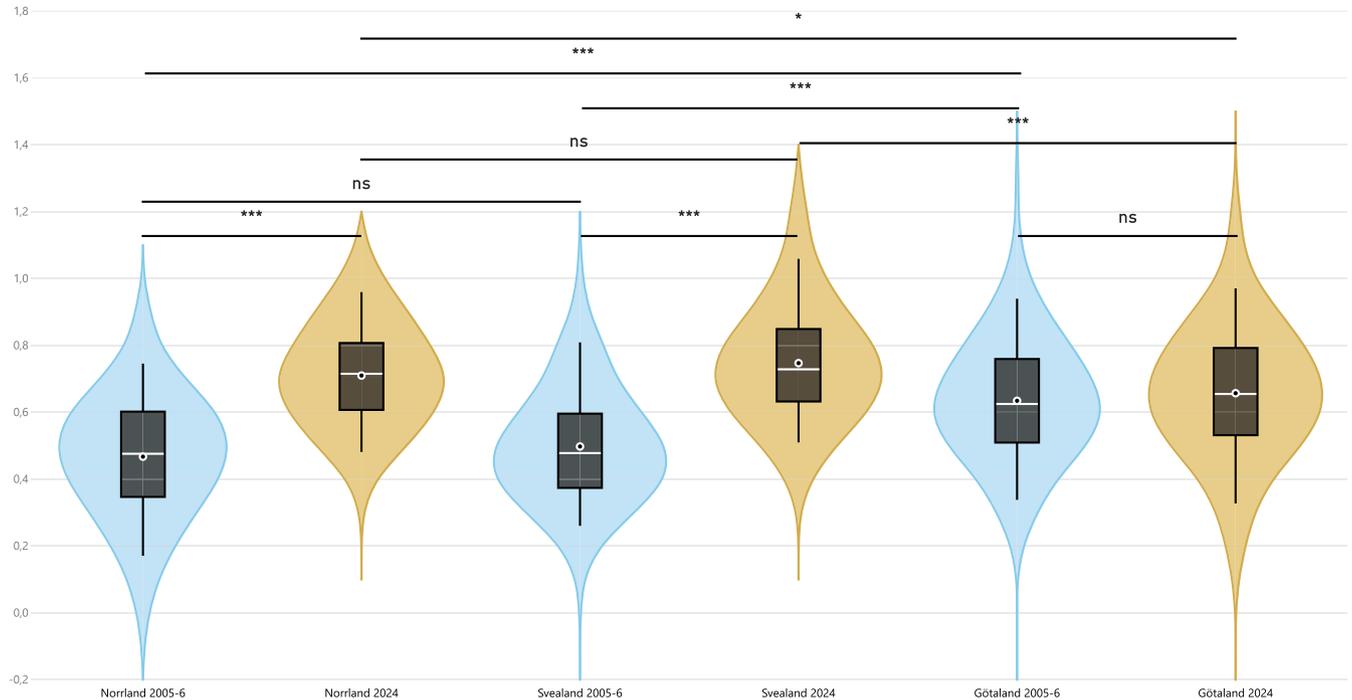


Fig. 2. Violin plot of optical density ratios (ODRs) of *Ostertagia*-antibody levels highlighting temporal and spatial differences by year and region, and their significance (ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Table 3
Analysis of ODR values.

ODR	Norrland 2005/6	Norrland 2024	Svealand 2005/6	Svealand 2024	Götaland 2005/6	Götaland 2024
Maximum	0.87	1.00	1.08	1.21	1.31	1.27
Minimum	0.00	0.44	0.03	0.40	0.21	0.12
Median	0.48	0.72	0.48	0.73	0.63	0.65
Mean	0.47	0.71	0.50	0.75	0.63	0.66
Standard deviation	0.18	0.14	0.17	0.17	0.18	0.20

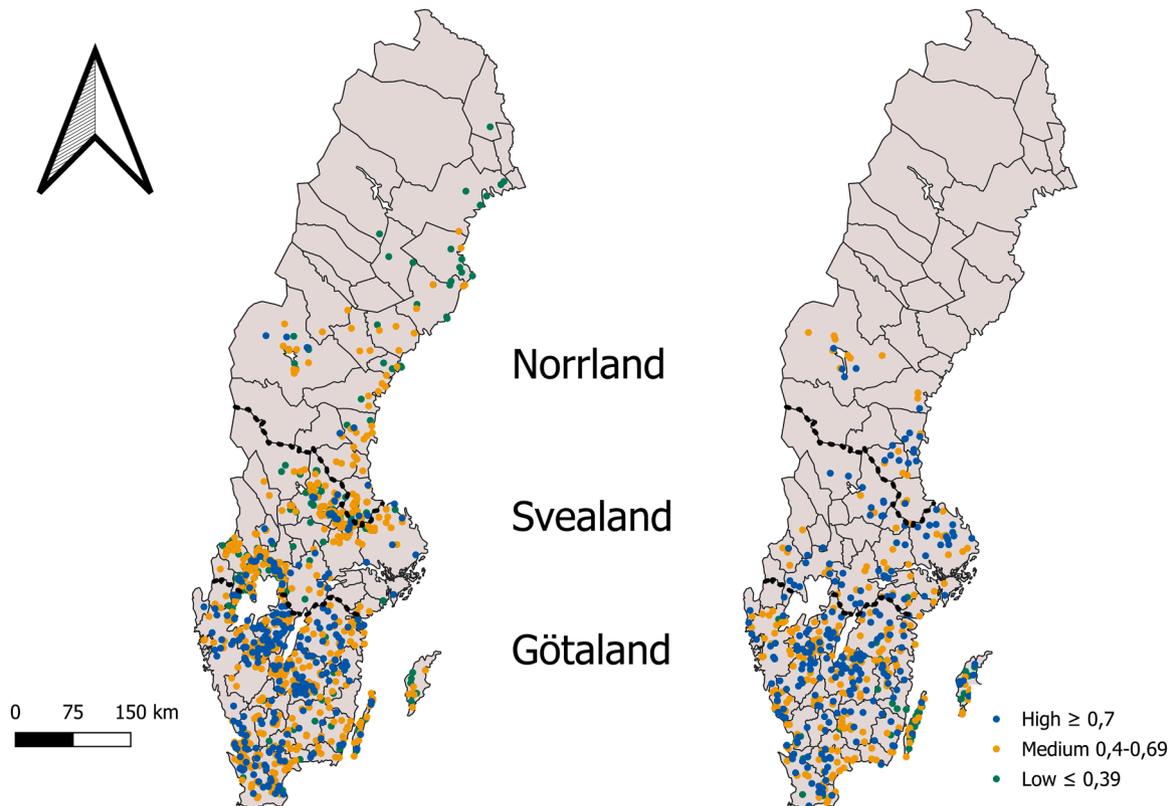


Fig. 3. Map illustration of optical density ratios (ODRs) of *Ostertagia*-antibody levels according to the farm coordinates. Left: Data collected in 2005/6. Right: Data collected in 2024.

3.2.2. Calf management

Calves were younger at release on pasture in 2024 than in 2005/6 ($p = 0.002$). Both calf deworming and calf treatment showed regional variation (both $p < 0.0001$) and significant temporal increase in prophylactic treatment (both $p < 0.0001$). Calf treatment frequency differed significantly between regions ($p < 0.0001$) but not between years ($p = 0.23$).

3.2.3. Grazing and outdoor access

Cow grazing changed substantially, with many herds reporting limited access to pasture in 2024 ($p < 0.02$). This reduction was not observed across regions ($p = 0.06$). Cow outdoor access also increased significantly ($p < 0.0001$), shifting from < 6 h/day to > 6 h/day access. In contrast, heifer grazing and outdoor access remained consistently high, showing no significant temporal or regional variation.

3.2.4. Turn-out and housing

Both cow and heifer turn-out differed significantly by region and year (both $p < 0.0001$). Earlier turn-out dates were more frequent in 2024, especially for heifers, with a predominance of May turn-out. Housing practices varied significantly between regions ($p < 0.0001$). A temporal effect was detected for heifers ($p < 0.0001$), with earlier housing observed in 2024, whereas cow housing showed no temporal

change ($p = 0.27$).

3.2.5. Pasture management, stocking density and diet

Pasture use in the previous year showed both regional ($p = 0.025$) and temporal variation ($p < 0.0001$), with more herds reporting only cows on pasture in 2024. The presence of other stock with cows also varied regionally ($p = 0.003$) and temporally ($p < 0.0001$). Stocking density declined for cows and increased for heifers significantly over time ($p = 0.01$ and $p = 0.005$, respectively) but showed no regional effect ($p = 0.98$; $p = 0.15$).

The proportion of grass in the diet changed significantly, with less herds feeding only grass in 2024; regional differences were observed as well (cows: $p = 0.02$; heifers: $p = 0.003$; both temporal $p < 0.0001$). Mowing of grass on pastures showed clear temporal reductions for both cows and heifers. For cows, mowing frequency declined strongly over time ($p < 0.0001$) with no regional effect ($p = 0.06$). For heifers, both regional ($p = 0.001$) and temporal ($p = 0.0005$) differences were observed, with more herds cutting 50–100% of pastures in 2024.

3.2.6. Deworming practices

Cow deworming showed regional variation ($p = 0.026$) but no temporal change ($p = 0.78$). Heifer deworming exhibited a significant ($p = 0.025$) and highly significant ($p < 0.0001$) regional and temporal

effect, respectively, i.e. in 2024 the curative treatments were more frequent. The type of heifer deworming also varied significantly (region: $p = 0.0013$; year: $p < 0.0001$), with pour-on treatments becoming more common in 2024.

3.3. Multiple linear regression

The multiple linear regression model based on 2005/06 data was significant overall ($F_{11,447} = 9.14$, $p < 0.0001$; $n = 459$) and explained 18.4 % of the variation in ODR (Supplementary Table S3). Latitude (Y) was negatively associated with ODR ($\beta = -2.90 \times 10^{-7}$, $F_{1,447} = 40.7$, $p < 0.0001$), whereas longitude (X) was not ($p = 0.375$). Cow grazing time was also significantly associated with ODR ($F_{2,447} = 9.99$, $p < 0.0001$). Compared with the reference grazing category, grazing for < 6 h/day ($\beta = -0.097$, $p = 0.0009$) and > 6 h/day ($\beta = -0.084$, $p = 0.0002$) were associated with lower ODR values. Cow density was also significant ($F_{3,447} = 3.31$, $p = 0.020$). Sites with > 5 ha per animal had lower ODR than the reference category ($\beta = -0.063$, $p = 0.0021$), while other density categories were not significant. Herd size was significant overall ($F_{2,447} = 4.55$, $p = 0.011$); however, individual category contrasts did not reach statistical significance. Mowing of pastures used by cows was not significantly associated with ODR (all $p > 0.05$). Model diagnostics indicated no major violations of regression assumptions. Residuals were approximately normally distributed, with only minor deviations, and multicollinearity was low across predictors (all VIFs < 1.5), supporting the robustness of the model estimates (Supplementary Table S4).

4. Discussion

In this study, bulk tank milk (BTM) antibodies against *Ostertagia ostertagi* were analysed to assess parasite exposure in Swedish dairy herds. Antibody levels, expressed as optical density ratio (ODR) levels, were used to categorise herds into high, medium and low antibody groups. The results from 2024 were compared with those from a similar study conducted in 2005/6 to evaluate changes in parasite exposure over time. Unlike earlier analyses (Bennema et al. 2010), the historical Swedish data were re-analysed to capture spatial variation.

The results indicate an overall higher exposure to *O. ostertagi* in 2024 compared with 2005/6, driven mainly by a significant increase in the two northernmost regions, Norrland and Svealand. Unlike in Svealand, the observed difference between study periods in Norrland may be partly explained by the absence of samples from the northernmost locations in the later period. However, when farms within the same restricted area in Norrland are compared between study periods, *O. ostertagi* antibody levels have clearly increased (Fig. 3). No such increase was observed in the southernmost region, Götaland. The stability of antibody levels in Götaland over time suggests comparable test performance between study periods, as the same commercial ELISA kit was used in both surveys. This is consistent with previous evaluations of test stability (Charlier et al., 2009b). Taken together, these findings indicate that changes in *O. ostertagi* exposure are not uniform across Sweden but follow distinct regional patterns that have varied over time.

Such regional variation is expected, as climatic conditions strongly influence the development and survival of the free-living pre-parasitic stages. Development from egg to third larval stage (L3) occurs outside the host (Krishnamoorthy et al., 2024). Increased rainfall leads to higher pasture moisture, which facilitates larval migration by softening the protective surface crust (Rose et al., 2015). Consequently, warm temperatures combined with sufficient moisture are particularly favourable for larval development (Gettinby and Paton, 1981). Although weather data were not included in the present analysis, climatic differences is likely to have contributed to the observed regional variation in parasite exposure.

The information obtained from the questionnaire complements the serological results and indicates structural intensification of Swedish

dairy farming between 2005/6 and 2024. One of the most notable changes is the increase in herd size, reflecting a transition from several smaller farms to fewer larger units. This finding is in line with Frey et al. (2018), who identified larger herd size as an important risk factor, although it contrasts with results from other studies (Forbes et al., 2008; Bennema et al., 2010; Höglund et al., 2010; Pablos-Tanarro et al., 2013; Almería et al., 2009). Nevertheless, the present findings suggest that Swedish dairy cows in general may now be at a higher risk of exposure to pasture borne nematodes than in the past. This risk is likely influenced by a dynamic interaction between management practices and environmental conditions (Bennema et al., 2009).

Several studies have reported a seasonal pattern in ODR values, with an increase during the grazing period corresponding to greater exposure to infective L3 larvae, followed by a decrease during the housing period when contact with pasture is restricted (Sanchez et al., 2002; Charlier et al., 2005b; Bennema et al., 2010; Forbes et al., 2008; Sekiya et al., 2013; Bloemhoff et al., 2015; Bosco et al., 2018). This seasonality likely varies by region, reflecting differences in climate and epidemiology (Charlier et al., 2007). In the present study, no data on seasonality were collected, as samples were obtained at a single time point (September 2024). However, an earlier Swedish study found no clear seasonal pattern in ODR values (Höglund, 2010), possibly due to the year-round calving system commonly used in Sweden. Under such conditions, BTM samples collected shortly after turnout may be useful to determine whether antiparasitic measures are needed later in the grazing season.

The primary factor influencing the ODR values in this study is regional variation along the north-south gradient. This association remained evident, even though all Swedish dairy cows must be outdoors during the grazing season under the Swedish Animal Welfare Act. Multivariable regression analysis based on the 2005/06 data confirmed that latitude was the strongest predictor of ODR values, while longitude had no effect, indicating limited east-west spatial variation in exposure. Additionally, several management-related variables, including larger herd size, longer grazing periods, and higher cow density, were independently associated with increased ODR values. These findings support the role of intensified production systems in parasite transmission. The absence of an effect from mowing suggests that this practice alone has limited impact under current grazing conditions.

Grazing management has been consistently linked to *O. ostertagi* exposure. Animals without access to pasture had low antibody levels (Sekiya et al., 2013; Bosco et al., 2018). Longer daily grazing time, earlier turnout and delayed housing have also been identified as risk factors for higher ODRs (Bennema et al., 2010; Forbes et al., 2008; Bellet et al., 2018). Similarly, a longer grazing period and a higher proportion of grass in the diet have been associated with increased ODR values (Płoneczka-Janeczko et al., 2019; Forbes et al., 2008). In the present study, farmers reported a slightly longer grazing period in 2024 compared with 2005/06. However, more limited access to production pastures and more restricted grazing opportunities for cows were paradoxically associated with higher ODR values. This may reflect increased stocking pressure and reduced pasture rotation, leading to accumulation of infective L3 on grazed areas.

The importance of overwintered larvae has been demonstrated previously in both Norway (Helle and Tharaldsen, 1976) and Sweden (Dimander et al., 2003), suggesting that pasture contamination early in the grazing season contributes substantially to parasite exposure. In contrast to our findings, mowing pasture has been shown to reduce the number of overwintered infectious larvae (Morley and Donald, 1980; Nansen et al., 1987). Anthelmintic treatment has also been associated with lower ODR values in several studies (Forbes et al., 2008; Bennema et al., 2010; Guitián et al., 2000). However, cows in the present study were rarely treated, consistent with Swedish control strategies that focus on developing immunity during the first grazing season. In contrast, a higher proportion of heifers were dewormed in 2024 than in 2005/6, possibly reflecting increased use of pour-on macrocyclic lactones.

Macrocyclic lactones are currently the preferred anthelmintics

against *O. ostertagi*, as they provide sustained efficacy over several weeks and are effective against all parasitic life stages of the nematodes, including the fourth larval stage (L4). Administration practices have evolved, with pour-on solutions increasingly used in Sweden, often replacing rumen capsules and injectable preparations. The most recent compound, eprinomectin, has no milk withdrawal period, allowing its use in lactating cows. This facilitates targeted treatment strategies, particularly for high producing animals in negative energy balance. Control through pasture management can act synergistically with anthelmintic treatment by reducing host–parasite contact.

The effect of anthelmintic treatment on ODR values appears to be variable. Some studies have reported no significant change after treatment (Frey et al., 2018; Charlier et al., 2005a), while others have observed reduced values (Vanderstichel et al., 2012). In certain cases, milk production has increased without compromising milk quality, thus economically justifying treatment (Gross et al., 1999; Sithole et al., 2005). However, the development of anthelmintic resistance poses a threat to sustainable parasite control. Resistance is promoted by inappropriate treatment practices (Rojo-Vázquez and Hosking, 2013), highlighting the need to reduce overall anthelmintic use (Morgan et al., 2013).

In Sweden, control strategies primarily target young stock, while adult cows are generally left untreated. Decisions to treat adult animals are also influenced by factors beyond BTM results, such as the risk of other parasitic infections (Forbes et al., 2008). Treatment recommendations should be farm-specific and ideally focus on particular grazing groups or individuals to achieve sustainable helminth control (Morgan et al., 2013). Blanket treatments, although inexpensive and easy to apply, are unsuitable due to variable treatment responses, strong selection pressure favouring drug resistance, and concerns about drug residues (Ravinet et al., 2014). Targeted and targeted-selective treatment strategies offer more sustainable alternatives by limiting treatment to animals that benefit most and by maintaining a susceptible population in refugia (Ravinet et al., 2014).

Antibody-based methods have inherent limitations, including cross-reactivity, an inability to distinguish between past and current infections, and reduced sensitivity to newly acquired infections. The *O. ostertagi* ELISA used in the present study may cross-react with other helminths, such as *Cooperia oncophora* and *F. hepatica* (Bennema et al., 2009). Additionally, cows with acute mastitis may show elevated ODR due to non-specific antibodies (Charlier et al., 2006). Although these limitations may reduce the precision in identifying highly exposed areas, the method remains useful for assessing infection pressure at the herd level. Coprological examinations are not practical in cows, as patent infections are rare in adult cattle (Höglund, 2010). Instead, combining ELISA results with information on husbandry practices provides a more comprehensive understanding of the herd infection status and may support economically justified treatment decisions. Repeated sampling, rather than reliance on a single time point, could further improve surveillance and decision-making (Opsal et al., 2023).

In summary, *O. ostertagi* infection rates are a multifactorial challenge, that cannot be adequately assessed using ELISA-derived ODR values alone. Regional differences, such as climate and husbandry practices, should be considered. Repeated sampling combined with detailed analysis of management practices may provide more accurate guidance for veterinarians and farmers when designing farm-specific control programmes. Increased focus on individual animals and tailored treatment strategies may also help address the growing problem of anthelmintic resistance, while maintaining farm profitability.

CRedit authorship contribution statement

Giulio Grandi: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization. **Marios Lazaros Michailidis:** Writing – review & editing, Writing – original draft, Visualization,

Methodology, Investigation, Formal analysis, Data curation. **Johan Höglund:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.vetpar.2026.110726](https://doi.org/10.1016/j.vetpar.2026.110726).

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