

Farm size and biosecurity measures associated with *Strongylus vulgaris* infection in horses

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Abstract

Background: Selective anthelmintic treatment, advocated due to evolving anthelmintic resistance, has been associated with an increase in *Strongylus vulgaris* prevalence. Reverting to routine interval anthelmintic treatments is not viable and therefore, identifying other management factors correlated with *S. vulgaris* infection is vital. **Objectives:** To investigate possible risk factors associated with the presence of *S. vulgaris* infection in residing horses on Swedish horse establishments. **Study design:** Internet-based questionnaire survey. **Methods:** A questionnaire, created using the internet-based survey platform Netigate, was distributed to owners of equine establishments throughout Sweden via established equine platforms and social media channels. The survey was available for response from May 21 until September 1 2022. Questions were closed ended with branching logic paths. **Results:** Four factors were significantly associated with *S. vulgaris* infection, with an increased odds of infection seen in livery yards (OR 1.67, 95% CI 1.18-2.36, $p = 0.004$) and premises with more than ten residing horses (OR 2.42, 95% CI 1.64-3.56, $p < 0.001$). A lower odds of infection was seen in establishments using quarantine routines (OR 0.69, 95% CI 0.50-0.96, $p = 0.03$) and anthelmintic treatment of new horses prior to arrival at the premise (OR 0.37, 95% CI 0.18-0.74, $p = 0.005$). **Main limitation:** Due to the presence of *S. vulgaris* infection in the present study being based on *S. vulgaris* diagnostics performed at the farm level, any association between faecal diagnostic use and risk of infection could not be investigated. **Conclusions:** Although the use of diagnostics for *S. vulgaris* can keep infection rates low, large farms or livery yards with many different horse owners, and those with low use of biosecurity measures as regards to new horses arriving at the premise, are associated with a higher risk of infection.

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residing horses (OR 2.42, 95% CI 1.64-3.56, $p < 0.001$). A lower odds of infection was seen in establishments using quarantine routines (OR 0.69, 95% CI 0.50-0.96, $p = 0.03$) and anthelmintic treatment of new horses prior to arrival at the premise (OR 0.37, 95% CI 0.18-0.74, $p = 0.005$).

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1. Introduction

As grazing animals, horses are inadvertently exposed to intestinal parasites, with the majority of horses infected to some degree with cyathostomins.¹⁻⁴ However, although considered ubiquitous in horses with pasture access, clinical disease associated with cyathostomins is fortunately rare.⁵ In contrast, *Strongylus vulgaris*, one of the large strongyles, has considerably greater pathogenicity, and thrombo-embolic disease with non-strangulating intestinal infarction caused by this parasite often has a fatal outcome.⁶⁻⁸ As a result of regular interval treatments with anthelmintic drugs, the prevalence of *S. vulgaris* in Sweden, as in most parts of the world, was radically reduced from 40-60% in 1979 to a mere 5% in the 1990s.^{9,10} However, due to the emergence of anthelmintic drug resistance, selective treatment, i.e. only treating certain horses based on individual faecal egg counts, often those excreting greater than 200 eggs per gram faeces (EPG), is recommended.¹¹⁻¹³ Such regimes can greatly reduce the amount of anthelmintic drugs used, without significantly increasing parasite pasture contamination caused by cyathostomins.¹⁴ As regards to *S. vulgaris*, however, specific diagnostics are required for detection, and both Denmark and Sweden, two countries that have strong adherence to anthelmintic treatment based on faecal diagnostics, have seen a recent increase in its prevalence.^{15,16} To this end, infection with *S. vulgaris* has been shown to be associated with the use of a selective anthelmintic treatment strategy, as opposed to regular treatment of all horses.^{15,17} Furthermore, Tydén et al. (2019) demonstrated that excluding specific diagnostics for *S. vulgaris* was associated with an increased risk of infection.¹⁶ However, other specific risk factors associated with *S. vulgaris* infection have yet to be determined. Since regression to regular interval treatment with anthelmintic drugs is not acceptable, alternative methods of reducing the risk of large strongyle infection in horses are crucial. Identifying specific risk factors for infection will assist in developing strategies other than regular anthelmintic treatment to protect horses from *S. vulgaris* infection. Using an internet-based questionnaire survey, the aim of the present study was therefore to investigate possible risk factors associated with the presence of *S. vulgaris* infection in residing horses on Swedish horse farms.

2. Materials and methods

A questionnaire designed on the internet-based survey platform Netigate (netigate.net) was distributed as an internet link made available for response from May 21 until September 1 2022 on specific nation-wide equine orientated websites (tidningenridsport.se, hastsverige.se, hippson.se) (Suppl. Table 1). In addition, awareness of and access to the questionnaire was achieved through social media channels, distributed by the authors directly, as well as by the proprietors of the equine websites named above, after contact with the authors. The target population was owners or managers of Swedish equine premises with adequate knowledge to be able to respond to the questions regarding all residing horses at their establishment, as opposed to individual horse owners. All questions apart from one, regarding the equine premises' postal code, were closed ended with pre-determined answer choices. Some questions were connected by branching logic, where certain answers opened up new questions, in order for the respondent to only face relevant queries. Prior to distribution, a test version of the questionnaire was sent to ten people with professional equine backgrounds, for control of time for completion and evaluation of the questions' clarity.

3. Statistical analyses

The questionnaire data were analysed using a generalized linear additive model in R v4.3.1 using *S. vulgaris* findings as response variable.¹⁸ Factors were removed stepwise until only significant remained and odds ratio was calculated using package autoReg v0.0.3 and visualized with ggplot2 v 3.4.4.^{19,20}

4. Results

4.1. Questionnaire data

4.1.1. *Strongylus vulgaris* diagnostics

The questionnaire was completed by a total of 1118 respondents, all of which were owners or persons responsible for the care of an entire equine premise and able to answer questions regarding their premise as a whole. Of these, 378 respondents did not use regular diagnostic tests for *S. vulgaris*, precluding knowledge of possible presence of infection on their establishment, and were therefore not included for further analysis. Another 23 respondents were excluded because, despite the use of regular diagnostics for *S. vulgaris*, they declared a lack of knowledge of the presence of infection on their premise. Out of the remaining 717 respondents, 335 reported to have had at least one horse infected with *S. vulgaris* over the past 24 months, with the remaining 382 respondents declaring no horse positive for *S. vulgaris* during that same time period, i.e. 47% of the farms using specific diagnostics for the parasite had detected the infection. Of the farms that reported to have positive horses for *S. vulgaris*, the number of positive horses detected over the previous two years varied as follows: 42% one horse, 29% two horses, 18% three to four horses and 6% more than four horses. In 5% of farms, the number of positive horses was unknown. The geographical distribution of all included farms is depicted in Figure 1. The majority of premises were located in the southern half of Sweden, which corresponds to the most horse-dense areas of Sweden.²¹

4.1.2. Anthelmintic routines

Although excluded from the risk assessment analysis, due to the presence of *S. vulgaris* infection over the past 24 months being unknown, there were some differences noted regarding which anthelmintic routines were employed on premises that used *S. vulgaris* diagnostics (n=717) and those that did not (n=378), as depicted in Figure 2. Notably, there was less veterinary involvement and a greater use of routine treatments on establishments that did not use regular extended diagnostic tests for *S. vulgaris*. Furthermore, it was more common for these premises to have no established unified treatment regime.

4.1.3. General description of included premises and management routines

A general description of all establishments using extended diagnostics for *S. vulgaris*, including premise type, farm-size and horse-turnover is shown in Table 1. For a description of anthelmintic routines and pasture management methods used on these premises, see Table 2.

4.2. Associations between questionnaire data and the presence of *S. vulgaris* infection

In total, four factors were significantly associated with the presence of *S. vulgaris* infection on the farm. Of these, two were farm-related, whereas the other two factors were related to management practices of new horses arriving at the premise. As such, farm size was significantly associated with the risk of having had at least one horse positive for *S. vulgaris* within the previous 24 months ($p < 0.001$), with 2.42 times (95% CI: 1.64-3.56) higher odds of infection on large premises (> 10 horses), compared with premises with ten or fewer horses (Figure 3). Furthermore, the presence of *S. vulgaris* infection was significantly associated with premise type, with 1.67 times (95% CI: 1.18-2.36) higher odds of infection in livery stables compared with other types of equestrian establishments ($p = 0.004$). Using quarantine of new horses arriving at the premise was associated with a significantly lower odds of infection (OR 0.69 (95% CI: 0.50-0.96), $p = 0.03$). In addition, anthelmintic treatment of new horses prior to arrival was associated with a decreased odds (OR 0.37 (95% CI 0.18-0.74)) of *S. vulgaris* positive horses being present on the farm ($p = 0.005$).

5. Discussion

Sweden, as well as Denmark, are unique countries in that the majority of equine owners perform anthelmintic

treatment of their adult horses based exclusively on faecal sample results.^{15,16,22} This extensive use of faecal diagnostics and consequent knowledge of current and historic presence of parasite infection on equine premises allows for the use of survey-based studies. As such, using an internet-based questionnaire, we demonstrated farm-related risk factors associated with the presence of *S. vulgaris* infection in horse farms in Sweden. Specifically, large premises and livery stables were correlated with a greater risk of infection. Furthermore, management practices concerning new horses arriving at the premise influenced the presence of infection, with the use of quarantine of new horses after arrival and treating new horses with an anthelmintic drug prior to arrival at the farm associated with a lowered risk.

To date, most studies investigating risk factors associated with intestinal parasite infection in horses have focused on cyathostomins and *Parascaris* spp. Infection rates in these parasites have shown a strong association with age, with young individuals more prone to infection and high parasite burdens.²³⁻²⁶ In addition, pasture access is significantly correlated with cyathostomin infection.²⁶⁻²⁸ In this context, one study showed that both young age and increase in pasture access were associated with an increase in both strongyle egg excretion and the presence of *S. vulgaris* antibodies.¹⁷ In agreement, Stoughton et al. (2023) found that racehorses had significantly lower odds of having a positive titer to *S. vulgaris*, speculated to be due to more limited grazing time compared with non-racehorses.²⁹ However, purposefully restricting access to grazing is not an appropriate measure to lower infection risk, whereby studies exploring other management or farm related factors associated with the risk of *S. vulgaris* infection are needed.

The present results showed an association between the presence of *S. vulgaris* infection and large equine establishments and livery stables. Similarly, *S. vulgaris* infection in donkeys was shown to be significantly associated with herd size, with increasing infection rates in herds with more than 50 animals.³⁰ Large farm size has also been linked to an increased prevalence of *Parascaris* infection in young horses, thought to be a result of higher infection pressures and a greater risk of anthelmintic resistance.³¹ Although anthelmintic resistance in *S. vulgaris* as yet has not been reported, a greater infection pressure associated with a larger number of residing horses is a probable explanation for the increased risk of *S. vulgaris* infection observed in the present study. Furthermore, most livery stables in Sweden are so-called DIY yards, where each owner cares for their own horse, with shared pastures and other facilities. Thus, speculatively, despite veterinary involvement and regular faecal testing, the increased odds risk of *S. vulgaris* infection demonstrated on such yards may be related to a lack of consensus regarding anthelmintic routines, such as timing of faecal samples and anthelmintic treatments.

In the present study, treating horses with an anthelmintic drug before arrival was associated with a lower odds of *S. vulgaris* infected horses at the establishment. Treating horses prior to arrival could involve a risk of horses being re-infected at the existing premises, and therefore it is somewhat surprising that this strategy, as opposed to treating at arrival, appeared to be the most favourable strategy. However, it is appreciated that the questionnaire did not allow for further specification as to how horses receiving an anthelmintic drug prior to arrival were managed after treatment; for example, the use of separate gravel paddocks or similar could reduce the risk of re-infection. Further, usage of quarantine practices for new arrivals lowered infection risk. By not introducing new horses to a shared pasture immediately at arrival, time is given to treat the horses with an anthelmintic drug without the risk of prior pasture contamination. Furthermore, previous studies have shown that applying biosecurity measures when introducing new horses also decreases the risk of introducing resistant parasites.³²⁻³⁴

Somewhat surprisingly, no pasture management method in the present study was found to be significantly associated with the presence of *S. vulgaris* infection. Thus, the present study suggests that, at low infection levels and with regular *S. vulgaris* diagnostics, pasture management does not appear to have a major influence on the risk of *S. vulgaris* infection. However, the results nonetheless point to further potential for reducing parasite infection pressures. For example, similarly to what has been shown in previous surveys, only a minority of premises in the present study declared to use regular faecal removal in the summer.^{22,35-38} This is regrettable, given that faecal removal twice weekly has been shown to be highly effective in reducing parasite infection pressures.^{39,40} Furthermore, other management practices to reduce parasite burdens, such

as resting pastures, ploughing or rotational grazing with another species, were only employed by a minority of the included farms.

Although it is a major concern that one third of all responders declared not to make use of regular *S. vulgaris* diagnostics, previous studies conducted in Sweden showed an even greater lack of specific diagnostic usage, suggesting that diagnostics for *S. vulgaris*, although not universal, are becoming increasingly more commonplace.^{16,22} To this end, countries where national legislation enforces prescription only restrictions on anthelmintic drugs, which includes Sweden as well as Denmark and the United Kingdom, appear to be experiencing pronounced changes in anthelmintic treatment strategies, with a clear increase in adherence to current recommendations.^{22,41-43}

Overall, the number of *S. vulgaris* positive horses over the past two year period was low, with only one to two positive horses detected on the majority of farms that had the infection. A recently published study, presenting data from the Swedish Veterinary Institute’s parasite monitoring program during the years 2008-2017, showed between 4-11% of horses to be positive for *S. vulgaris*.¹⁴ A substantially higher occurrence was found in the study performed by Tydén et al. (2019), where 28% of all tested horses were positive for *S. vulgaris*.¹⁶ A major difference between our study and that of Tydén et al. (2019) was that the present study was based purely on questionnaire data. Thus, the infection rate on the farms that did not use regular diagnostics for *S. vulgaris* (34%) was unknown and these farms had to be excluded from further analyses. Considering that Tydén et al. (2019) demonstrated a 2.9 higher odds of infection in farms not using diagnostic testing for *S. vulgaris*, the actual number of farms with positive horses in the present study is likely to have been much greater.¹⁶ Moreover, in the present study, premises that did not use *S. vulgaris* diagnostics were less likely to base anthelmintic treatments on faecal samples and/or veterinary advice, with 27% declaring to routinely treat their horses 1-4 times per year and 20% reporting either no knowledge of which anthelmintic routines were used or a lack of consensus in a defined anthelmintic routine. In contrast, all farms using extended diagnostics, both with and without positive horses, declared high veterinary involvement and low use of routine treatment. Thus, further studies combining questionnaire data with faecal samples and serology for detecting *S. vulgaris* infection are needed to fully elucidate risk factors for infection, including diagnostics and treatment routines.

In conclusion, the present study appears to support the use of diagnostics for *S. vulgaris* to keep infection rates low. However, infection can still occur, despite regular faecal diagnostic tests for the parasite, primarily on large farms or livery yards with many different horse owners, and those with low use of biosecurity measures as regards to new horses arriving at the premise.

Table 1. Response to questions regarding establishment type and size, including number of new arrivals, type of housing and pasture access, expressed as a percentage of the total number of responses for premises with *S. vulgaris* positive horses (in bold) (n = 335) and those without positive horses (n = 382).

Question	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response	Response
Question	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives	alternatives
Type of premises	Livery stable	Racing stable	Racing stable	Competition stable	Competition stable	Competition stable	Stable	Stable	Stable	Stable	Riding School	Riding School	Riding School	Horse sale	Horse sale	Horse sale	Horse trek
Number of residing horses	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85
	72.5%	24.2%	12.4%	11.0%	11.0%	11.0%	10.4%	10.4%	10.4%	10.4%	6.1%	3.7%	3.7%	3.7%	1.8%	1.8%	1.8%
	39.7%	39.7%	22.4%	22.2%	22.2%	22.2%	23.1%	14.9%	14.9%	14.9%	14.9%	9.0%	0.3%	0.3%	0.3%	6.3%	3.1%

Question	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives						
Number of new arrivals past 12 month	0	0	0	0	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	6-10	6-10	6-10	6-10
Type of housing	Loose box with daily pasture access	Active stable														
Separation summer/winter pastures/paddocks?	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No						
Type of summer paddock/pasture	Grazing meadow	Forest land	Sand/Grave	Sand/Grave	Sand/Grave											
Type of winter paddock/pasture	Grazing meadow	Forest land	Sand/Grave	Sand/Grave	Sand/Grave											

Question	Response alternatives																		
Type of paddock/pasture on farms with permanent year round pastures	Grazing meadow	Forest wood-land	Sand/Grave	Sand/Grave	Sand/Grave	Sand/Grave													
Number of horses/paddock	One	One	One	One	Two	Two	Two	Two	Two	Two	3-5	3-5	3-5	3-5	3-5	3-5	>5	>5	
summer season	5.0%	5.3%	5.3%	5.3%	12.7%	12.7%	12.7%	12.7%	12.7%	12.7%	16.5%	16.5%	16.5%	16.5%	16.5%	16.5%	16.5%	18.1%	18.1%
winter season	12.3%	12.9%	12.9%	12.9%	14.6%	14.6%	14.6%	14.6%	14.6%	14.6%	17.2%	17.2%	17.2%	17.2%	17.2%	17.2%	17.2%	18.6%	18.6%
permanent pasture	10.7%	10.8%	10.8%	10.8%	15.8%	15.3%	15.3%	15.3%	15.3%	15.3%	28.0%	28.0%	28.0%	28.0%	28.0%	28.0%	28.0%	30.7%	30.7%
Stocking density	>1	>1	>1	>1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
summer season	26.9%	26.9%	26.9%	26.9%	26.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%	31.9%
winter season	8.8%	8.8%	8.8%	8.8%	8.8%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%	35.4%

Question	Response alternatives																
- permanent pasture	17.3%	27.8%	27.8%	27.8%	27.8%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%	50.7%

+more than one alternative possible

Table 2. Response, expressed as a percentage of the total number of responses, to questions regarding anthelmintic routines and pasture management for premises with *S. vulgaris* positive horses (in bold) and those without positive horses.

Question	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives
Anthelmintic treatment routine	Routine 1-4/year	Routine 1-4/year	Routine 1-4/year	Selective based on faecal samples or as directed by veterinarian	Selective based on faecal samples or as directed by veterinarian	Selective based on faecal samples or as directed by veterinarian	Selective based on faecal samples or as directed by veterinarian	Selective based on faecal samples or as directed by veterinarian	Selective based on faecal samples or as directed by veterinarian	No consensus or unknown							
Treatment when <i>S. vulgaris</i> positive horses are detected (only farms with positive horses)⁺	All horses sharing same pasture	All horses at the establishment	All horses at the establishment	All horses at the establishment	Only positive horse(s)	Only positive horse(s)	Treated once										
	39.1%	38.2%	38.2%	38.2%	22.7%	22.7%	22.7%	22.7%	22.7%	22.7%	25.4%	25.4%	25.4%	25.4%	25.4%	25.4%	25.4%

Question	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives	Response alternatives
Treatment of new arrivals	if indicated by faecal sample	Always	Always	Always	Never	Never	Never	Never	Sometimes	Sometimes
Quarantine of new arrivals	Yes, for 1-2 weeks	Yes, for 1-2 weeks	Yes, for 1-2 weeks	Yes, for 1-2 weeks	Yes, for 1-2 weeks	Yes, for 1-2 weeks	No	No	No	No
Herd composition and use of separate pastures by season	Fixed herd composition	Fixed herd composition	Movement of horses between herds	Movement of horses between herds	Movement of horses between herds	Separate winter/summer paddocks	Separate winter/summer paddocks	Separate winter/summer paddocks	Separate winter/summer paddocks	Permanent pastures
Pasture management	Rotational grazing with other species	Rotational grazing with other species	Ploughing of pastures	Ploughing of pastures	Ploughing of pastures	Rest of pasture	Rest of pasture	Rest of pasture	Rest of pasture	Rest of pasture
Faecal removal - summer season	Daily	Daily	1-2 times/week	1-2 times/week	1-2 times/week	1-2 times/month	1-2 times/month	1-2 times/month	1-2 times/month	Irregular

Question	Response alternatives																					
- winter season	19.4%	20.7%	19.4%	20.7%	23.0%	27.5%	23.0%	27.5%	23.0%	27.5%	14.3%	12.8%	14.3%	12.8%	14.3%	12.8%	14.3%	12.8%	14.3%	12.8%	14.3%	39.4%

⁺only premises with positive *S. vulgaris* horses during the past 24 months, ⁺⁺only premises with separate summer/winter paddocks

Figure Legends

Figure 1. Map depicting the included establishments' geographical location in Sweden. Made with Natural Earth vector data.

Figure 2. Anthelmintic routines (i.e. routine treatment, treatment based on veterinary advice and/or positive faecal samples or no unified consensus/unknown treatment routine) used by premises using regular diagnostics for *S. vulgaris* (n=717) and premises not using *S. vulgaris* diagnostics (n=378).

Figure 3. Odds ratio of the four variables significantly associated with *S. vulgaris* infection based on 717 responses. For question regarding anthelmintic treatment of new horses, the response "never", answered by 58 responders, was set as the reference value. Significant response alternatives for each variable are depicted in blue and the response used as a reference in grey.

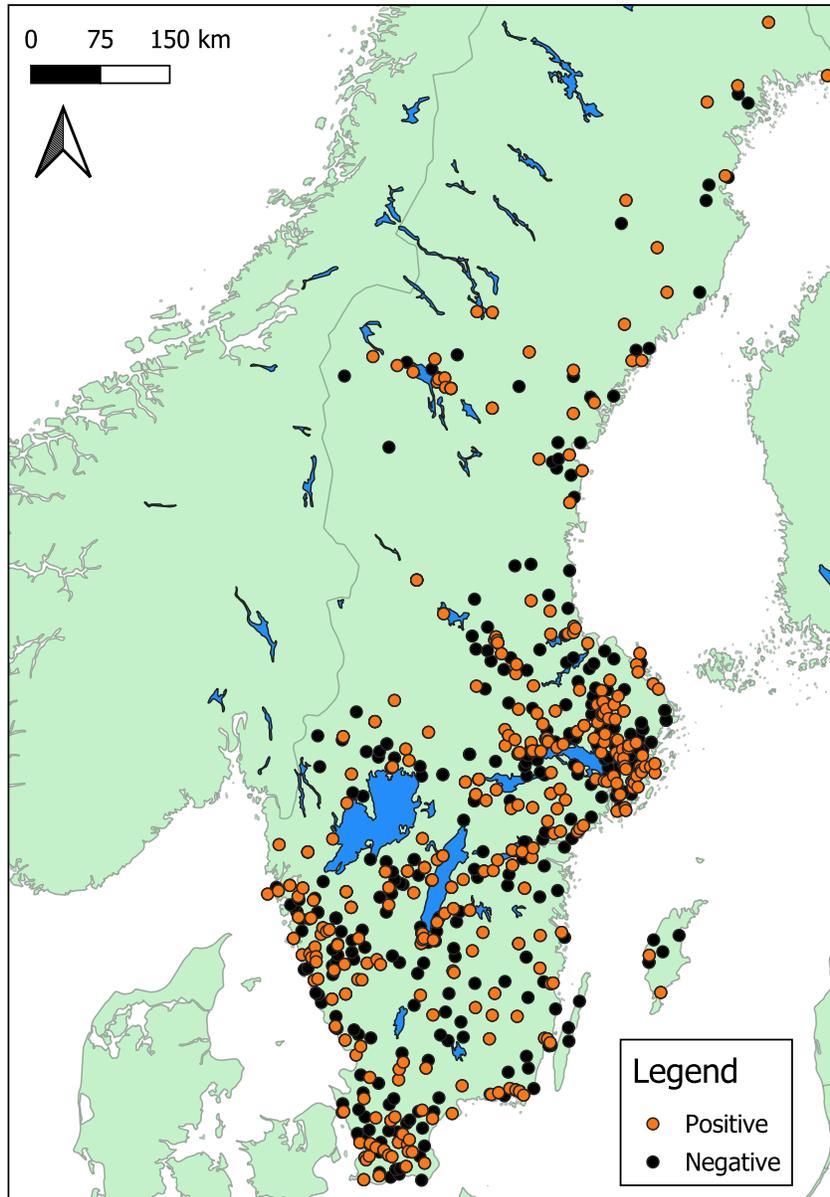
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