



HAL
open science

Meta-analysis of equine strongyle community structure

Michel Boisseau, Núria Mach, Marta Basiaga, Slawomir Kornas, Tetiana Kuzmina, Claire Laugier, Guillaume Sallé

► **To cite this version:**

Michel Boisseau, Núria Mach, Marta Basiaga, Slawomir Kornas, Tetiana Kuzmina, et al.. Meta-analysis of equine strongyle community structure. 2022. hal-03792762v2

HAL Id: hal-03792762

<https://hal.inrae.fr/hal-03792762v2>

Preprint submitted on 14 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

[Click here to view linked References](#)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 Meta-analysis of equine strongyle community structure

2 Michel Boisseau^a, Núria Mach^b, Marta Basiaga^c, Sławomir Kornaś^c, Tetiana Kuzmina^d, Claire Laugier^e, Guillaume Sallé^a

3
4 a, INRAE, Université de Tours, ISP, Nouzilly, France

5 b, UMR 1313 GABI, INRAE, AgroParisTech, Université Paris- Saclay, Jouy- en- Josas, France

6 c, Department of Zoology and Animal Welfare, Faculty of Animal Science, University of Agriculture in Kraków, 24/28

7 Mickiewicza Av., 30-059 Kraków, Poland

8 d, Department of Parasitology I. I. Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine

9 e, Conseil Général de l'Alimentation, de l'Agriculture et des Espaces Ruraux, Ministère de l'Agriculture et de l'Alimentation,

10 Paris, France

11
12 Corresponding author: michel.boisseau@inrae.fr

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

Purpose:

Grazing horses are infected by a wide range of strongylid species mostly located in the large intestine. Despite their impact on equine health and the emergence of drug resistant isolates, the phenology of these nematodes has been poorly characterized and factors structuring their communities are poorly studied.

Methods:

Using a meta-analysis, we compiled data on 45 equine strongylid species collected worldwide at the regional scale (upon deworming or after necropsy) to analyse their richness, diversity and associated factors of variation.

Results:

Worldwide, twelve species, *Cylicocycylus* ($n = 4$), *Cylicostephanus* ($n = 3$), *Coronocycylus* ($n = 2$) and *Cyathostomum* ($n = 2$), were found in at least 75% of datasets. We did not detect a significant difference in species richness across the different conditions examined but strongylid communities were significantly affected by these factors. *Cylicocycylus ashworthi*, *Tridontophorus serratus* and *Strongylus vulgaris* were more frequently found in continental Europe following deworming, whereas *Cylicocycylus brevicapsulatus* was more frequently found in Tropical America using necropsy. Finally, simulation were undertaken to quantify the impact of the analytical framework on the inferences made. The results indicates that study-wise presence/absence data are a better match of underpinning horse-wise data than study-wise species relative abundances.

Conclusion:

This study expands previous findings and underscores the challenges left to better understand how strongylid communities assemble in horses.

Keywords: nematode, strongyle, community, diversity, horse

1
2
38
4
39
6
40
7
41
9
42
10
11
43
12
44
13
45
15
46
16
47
18
48
19
49
21
50
22
51
24
52
25
53
27
54
28
55
30
56
31
57
33
58
35
59
36
60
38
61
39
62
41
63
42
64
44
65
45
66
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

Grazing horses are naturally infected by a diverse parasitic fauna including Strongylidae [1]. They encompass the subfamilies Cyathostominae and Strongylinae [2]. The use of anthelmintics has lowered the prevalence of *Strongylus* spp. that have the highest fatality rate among the Strongylinae [3], but has led to the emergence of resistant cyathostomin isolates worldwide [4]. Large and small strongylids affect horse growth rate and the massive emergence of encysted cyathostomin larvae can lead to fatal cases of cyathostomosis, the main cause of parasite mortality in young horses over the last three decades in Normandy, France [5]. Despite their importance in veterinary medicine, the phenology and the factors structuring their communities remain largely undefined due in part to the complexity of collecting data from large mammalian hosts under controlled conditions.

Inter-individual variation exists in host susceptibility to infection, with significant variation across age groups [6-9]. In addition, climatic conditions affect the development of the free-living stages on pastures [10] and species reappearance following anthelmintic drug treatment varies across species [11] suggesting that combinations of their phenology or genetic diversity increase their fitness. However, variations in community structure across contrasted geoclimatic conditions or using different recovery methods remain largely uncharacterised.

Better defining the patterns of variation in helminth communities could however greatly improve their management in the field, i.e. by preventing the emergence of subdominant species following elimination of more dominant ones [12] or anticipating consequences of infections by other pathogens [13-15]. Data on helminth community assemblages have been gathered across a wide range of host species including humans [16], rabbits [12], rodents [17, 18] and fish [19] with evidence of significant interactions between parasites. In horses, community structure has been determined from necropsy reports or after deworming [20-28]. However, these reports often remain descriptive and limited exploration of the relationship between community structure and host and environmental variables has been conducted. For instance, variation in species richness and diversity along the intestinal tract of horses has been reported [21, 23, 29]. A recent meta-analysis reported the impact of parasite recovery method and geographical region on cyathostomin abundance or prevalence [30]. However, this meta-analysis only focused on cyathostomin species, did not evaluate community dissimilarities and considered relative abundances derived from mean species counts.

The present study expands past work with additional strongylid species considered and with different analytical frameworks applied. In addition, simulations were performed using horse-wise strongylid community data to establish the impact of analytical frameworks on subsequent inferences.

1
2
3 **67 Materials and methods**

4
5
6 **68 Literature search and inclusion criteria**

7
8 **69** Studies were obtained from the Medline database (<https://pubmed.ncbi.nlm.nih.gov/>) using "strongyl* horse" as keywords,
9
10 **70** yielding a database of 1,032 articles. Article titles and abstracts were subsequently screened for particular terms including all
11
12 **71** possible combinations of the following: "intensity", "Intensity", "Abundance", "abundance", "Prevalence", "prevalence",
13
14 **72** "communit", "Communit", "Helminth" or "helminth". To identify studies reporting strongylid burdens, we also included the
15
16 **73** main genera ("Cylicocyclus", "Cyathostomum", "Cylicostephanus") as keywords. This filtering step retained 300 papers. For
17
18 **74** these, titles and abstracts were read to determine whether findings of strongylid species prevalence and abundance would be
19
20 **75** available.

21
22 **76** To broaden our database, we also ran a manual search on Google (<https://scholar.google.com/>) using the keywords
23
24 **77** "prevalence abundance Cyathostominae horses" and "prevalence abundance Strongylidae horse". This query identified six
25
26 **78** additional studies. Additional studies were identified after inspection of the references cited in three papers [21, 23] that
27
28 **79** yielded prevalence data from 13 additional studies.

29
30 **80** To be included in the meta-analysis, reported articles had to meet several criteria. First, studies were included in the dataset
31
32 **81** if they reported parasite prevalence (proportion of infected host), abundance (the number of parasite individuals per host,
33
34 **82** including uninfected individuals) or relative abundance. Second, published data were only included if the number of horses
35
36 **83** sampled was specified. Third, we removed studies before 1975 to avoid annotation mismatches due to different taxonomic
37
38 **84** identifications prior to that date.

39
40 **85** Finally, 34 papers met the aforementioned selection criteria (Supplementary Table 1). Among these, 25 papers presented both
41
42 **86** abundance and prevalence data, while others either only reported abundance ($n = 3$) or prevalence ($n = 6$). Papers from Africa
43
44 **87** ($n = 1$) and Australia ($n = 3$) were included to determine average relative abundance and prevalence across regional studies
45
46 **88** but were not considered in any further analyses as too few replicates were available to test for environmental effects. For
47
48 **89** analytical purposes, papers were further divided into studies to account for the distinct experimental groups (horse location
49
50 **90** or horse age) considered within each paper. This process yielded 54 community sets for 45 species encountered in at least one
51
52 **91** study.

53
54 **92 Parasite, environmental and host variables**

55
56 **93** When available, metadata related to environmental factors were collected and retained if at least four observations were
57
58 **94** available. Horse age was not considered as only limited and inaccurate data were available. The same applied for horse breed
59
60 **95** or management type (wild or managed horses) that were reported in three studies only. Following filtering, information
61
62 **96** regarding continent, country, sampling effort (number of horses sampled), the recovery method used (necropsy or deworming)
63
64 **97** were included. Because of the paucity of studies describing strongyle communities after critical tests ($n = 3$), this collection
65
66 **98** method was not considered further. Climatic conditions were determined using the broad Köppen-Geiger climate
67
68 **99** classification (<http://hanschen.org/koppen/#data>; continental, tropical, temperate, and arid) corresponding to the sample area.
69
70 **100** For studies without a precise location, the coordinates of the nearest major city (state or national capital) were used as a proxy
71
72 **101** (Supplementary Table 1).

1
2
102
4
103
104
6
7
8
105
10
106
12
13
107
15
108
16
109
18
110
19
111
21
112
22
113
24
114
25
115
27
116
28
117
29
30
118
31
32
119
33
120
35
121
36
122
38
123
39
40
124
41
125
42
43
44
126
45
46
127
47
128
48
129
50
130
51
131
53
132
54
133
56
134
58
135
59
136
60
137
62
63
64
65

Statistical analysis

Data were analysed with R [31] v. 3.6.3 and v. 4.0.2. Scripts have been made available under the GitHub (<https://github.com/MichelBoisseau37/metaAnalyses-Strongyles/>) repository.

Regional community sets

Prevalence and relative abundance matrices were merged together into a single study-wise presence/absence matrix (noted 0 and 1 for absence and presence respectively). Analyses were run on the study-wise species presence/absence matrix for two reasons. First, this approach makes use of every available study (no matter of the reported measure), thereby providing greater statistical power than an approach considering abundance or prevalence data only. Second, most studies ($n = 22$ out of the 32 considered) did not report any dispersion parameter of parasite abundance and prevalence, thereby preventing any implementation of meta-analysis frameworks.

Because of the data structure, geoclimatic conditions (aggregated continent and climate zones) and the recovery method (deworming or necropsy) were confounded. To overcome this issue, a synthetic variable combining the continent, the climatic zone and the recovery method was created (called “synthetic variable”). In addition, some conditions that were under-represented (less than four studies) were not considered further. This included the strongyle community sets from Asia ($n = 1$), Australia ($n = 3$), or from temperate Europe collected after deworming ($n = 3$). Within each study, the original experimental groups were kept independent, yielding 45 independent cohorts from 23 studies (**Supplementary Table 2**).

Data modeling

Generalized linear model (“glmer” function, binomial family, package lme4 v1.1-27.1) was applied on the presence/absence matrix of strongyle species with the number of horses, the year of publication (before and after 2000) and the synthetic variable as explanatory fixed effects. The study was set as a random factor. This analysis was done on a subset comprising the levels of the environmental variable having at least 5 observations and corresponding to 48 of the 58 starting communities (i.e. “Am-Temp-nec”, $n = 8$; “Am-Trop-nec”, $n = 4$; “Eu-Cont-dew”, $n = 28$ and “Eu-Temp-nec”, $n = 5$). Any p-value below 0.05 was deemed significant. In all cases, species with sufficient variance, i.e. observed in at least in 5 different studies and in no more than 40 out of the 45 studies, were further considered (23 species; Supplementary Table 3).

Diversity analyses

Diversity was tested on the level of the synthetic variable with more than 4 observation (i.e. Am-Temp-ne, Am-Trop-nec, Eu-Cont-dew and Eu-Temp-nec).

Species richness was taken as an α -diversity estimator. The effects of the synthetic environmental variable on species richness were estimated using a linear model (function “glm”, package stats v3.6.3), fitting the abovementioned explanatory variable, sample size and year of publication as fixed effects.

Second, the dissimilarity between study-wise strongylid communities (β -diversity) was estimated using the Jaccard distance as implemented in the vegan R package (v.5.7), and visualized using the Non-Metric Scaling (NMDS) ordination method through the “metaMDS” function of the same R package. This method generated fictitious axes maximizing the variance and allowing an optimal visualization of distances. NMDS is the most appropriate representation because it minimizes dissimilarities between similar communities and vice versa for more remote communities. NMDS was run on the whole set of communities to establish how the synthetic variable (aggregated factor of the geoclimatic zone and the recovery method),

1
2
138 the sampling effort (number of horses) and the year of publication (before or after 2000) were related to community structures.
139 A permutational multivariate analysis of variance (PerMANOVA) on inter-community Jaccard distance matrix (“adonis”
140 function of the vegan R package) was then applied to identify the factors that would influence most the community structure
141 and to obtain the percentage of variance explained by the factors of interested.

9
142 **Simulation to establish the effect of the analytical framework on subsequent inferences**

11
143 This study used study-wise presence/absence data, in contrast to past work that relied on relative abundances derived from
144 summary statistics estimated across horses within each study [30]. Both frameworks entail drawbacks, respectively owing to
145 the loss of variation when using binary presence/absence data or because species counts of rarer species tend to deviate from
146 normality, thereby making the mean a poor predictor of the underlying distribution.

147 To evaluate the impact of this approach and compare with the approach developed by others [30], we simulated strongyle
148 communities using horse-wise data collected upon necropsy [29, 32] or deworming [28, 33] as follows. This served as a
149 simple case example (variation across species or collection method) to evaluate how analytical frameworks affected inferences
150 made. At each of the 1,000 simulations, observed species count data were randomly sampled to create 60 studies (30 for each
151 collection method), of a random number of horses chosen from the published studies sample sizes. For each of these simulated
152 studies, a study-wise strongyle community matrix was derived using either average species relative abundances [30] or a
153 binary presence/absence matrix (current study) to compare the performance of both approaches.

154 First, the predictive ability of the modelling approaches of the study-wise presence/absence data on the underpinning horse-
155 wise data it was derived from was evaluated. Species presence/absence data were regressed upon the collection method,
156 strongyle species and the interaction between both variables, using a logistic framework and fitting a random effect accounting
157 for between-study variation. Study-wise models were trained on the whole dataset and tested against the horse-wise data to
158 estimate the Root-Mean-Square Errors (RMSE), i.e. the model predictive ability from study-wise data on horse-wise data. As
159 a control, horse-wise models were trained on 80% of the simulated data and tested against the remaining 20% of simulated
160 data.

161 Second, to evaluate how reliance on species count means was affecting subsequent estimations, log-transformed study-wise
162 or horse-wise species counts from the simulated sets were modelled using a similar modelling approach as above, i.e. fitting
163 the study as a random effect and considering the recovery method, species and their interactions as fixed effects.

164 Last, we determined the extent to which study-wise species relative abundances derived from their mean counts or the
165 dissimilarity between study-wise presence/absence data and the horse-wise matrices they were derived from. For this, the
166 mean Bray-Curtis (for relative abundances) or Jaccard (presence/absence data) distances were estimated between the study-
167 wise species matrices and every horse-wise species matrix they were derived from. For each distance type, a t-test was applied
168 to test for the difference between study-wise and horse-wise distances within each distance type.

169

51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Results

Descriptive features of strongylid communities

The breakdown of publication counts for factor of interest is provided in Supplementary Table 4. As can be seen in Table 1, studies using deworming were mostly carried out in Eastern Europe. Our data showed an average number of horses per study of 28.65, ranging between 2 and 150 individuals.

We estimated for each strongyle species the average prevalence and abundance using data from the literature (Figure 1A, Supplementary Table 5) and prevalences at the scale of our study using presence and absence proportions (Figure 1B, Supplementary Table 5). Strongylid communities were dominated by a small number of species, *i.e.* *Cylicocycclus nassatus*, *Cylicostephanus longibursatus*, *Cyathostomum catinatum* (Figure 1A) with an average prevalence of $87.78\% \pm 19.06$. Alongside these three dominant species, seven additional species (*Cylicostephanus goldi*, *Cylicostephanus calicatus*, *Cylicostephanus minutus*, *Cylicocycclus leptostomus*, *Cylicocycclus insigis*, *Cyathostomum pateratum*, *Coronocycclus coronatus*) were found in more than 80% of the considered studies while infecting more than 25% of the sampled horses in these studies (Figure 1B). On the other hand, 26 rare species had an average prevalence of $8.13\% \pm 13.43$. These mean prevalence levels reported within horse populations were slightly corroborated when considering the presence-absence pattern at the regional scale (Figure 1B). Specifically, the dominant and subdominant species defined by an average prevalence of 25% or more at the horse population level were present in 75% of studies. Contrasting this broad pattern, *Coronocycclus labratus* was relatively frequent across studies (found in 85% of all studies) but with low prevalence within each study (average reported prevalence of 12.3%). On the contrary, the presence of *Cylicocycclus ashworthi* varied both across studies and horses (found in 54% of all studies, with an average prevalence of 33.9%; Figure 1B).

Altogether, ten species emerge as a core that is widely represented across equine strongylid communities worldwide, and with the ability to infect a large proportion of horses within each region considered.

Diversity analysis of worldwide strongylid communities

As a first step, an exploratory multivariate analysis was applied to our study-wise species incidence matrix (Figure 2). This analysis highlighted the outstanding contribution of the continent, the climate and the recovery method on strongyle community diversity (Figure 2, supplementary Fig 1). This was corroborated by the PERMANOVA ($R^2 = 0.16$, $P = 5 \times 10^{-4}$) and would suggest significant dissimilarity in communities across the levels of the synthetic variable considered. The second axis of the NMDS also closely matched a gradient defined by the temporal and the sampling effort scales. Significantly more horses were sampled in the necropsy-based studies (35.69 ± 27.67 and 18.9 ± 15.8 horses sampled on average respectively, $P < 2.2 \times 10^{-16}$). This sampling effort significantly contributed to the dissimilarity between study-wise strongylid communities ($R^2 = 0.08$, $P = 9 \times 10^{-4}$). On the contrary, the dissimilarity between study-wise communities was not associated with the year of publication ($R^2 = 0.01$, $P = 0.31$), despite a temporal disconnection between the deworming- and necropsy-based sets (published between 2003 and 2018 or between 1976 and 2016 respectively).

Generalized linear models revealed significant variation for *Cylicocycclus ashworthi*, *Tridontophorus serratus* and *Strongylus vulgaris*, that were more frequently recovered in continental Europe using deworming (Estimate equal to 6.43 ± 1.7 , $P = 10^{-4}$; 2.74 ± 1.33 , $P = 4 \times 10^{-2}$ and 2.99 ± 1.34 , $P = 2.5 \times 10^{-2}$ respectively). Necropsies performed under tropical America had more likelihood of recovering *Cylicocycclus brevicapsulatus* relative to the temperate American conditions (Estimate equal

1
2
206 to 4.42 ± 2.15 , $P = 4 \times 10^{-2}$). Sampling effort had a small but significant effect on study-wise species presence/absence
207 (Estimate = 3×10^{-2} , $P = 2 \times 10^{-3}$).
208 While significant variation was found between overall community structure, species richness was little affected by the
209 different conditions studied. Indeed, species richness was not statistically different between the levels of the synthetic
210 variable accounting for the geo-climatic area and the strongyle recovery method ($P = 0.41$). The same pattern was found for
211 the year of publication ($P = 0.27$). However, the difference in sampling effort was positively associated with species richness
212 (estimate = 0.15, $P = 3 \times 10^{-3}$).
213 To sum up, synthetic variable and sampling effort were driving dissimilarity between strongylid communities with discrete
214 variation found in species occurrences. Differences in sampling effort may underpin the observed dissimilarity.

215 **Impact of analytical frameworks on inferences made**

216 Past work has relied on reported species mean abundances to derive their respective relative abundances and regress the effect
217 of environmental effects upon these [30]. In contrast, this study chose to rely on binary presence/absence data aggregated at
218 the regional or study-wide level.

219 Inspection of horse-wise strongyle community data gathered after necropsy (Bucknell et al. 2015; Laugier et al. 2012) or
220 deworming (Kuzmina et al. 2012; Sallé et al. 2018) highlighted that strongyle species counts deviated from normality
221 (supplementary Figures 2A and 3A for deworming and necropsy data respectively). The Shapiro-Wilk's test values ranged
222 between 0.08 (*C. pateratum* counts after necropsy) and 0.64 (*C. catinatum* counts after deworming), far from the expected
223 value of one for normal distribution. However, simulated data showed that regression upon log-transformed observed horse-
224 wise counts or study-wise averaged species counts yielded well correlated estimates (Pearson's $r_{32000} = 0.9$, $P < 10^{-4}$).

225 This study relied on study-wise presence/absence data to avoid testing differences in relative abundances. Indeed, relative
226 abundance data are compositional, so that the proportion of a given species will be impacted by changes in abundance of the
227 others across conditions (supplementary Figure 4). Therefore, the relative abundance of a given species may encompass a
228 wide range of count values (supplementary Figure 4), thereby leading to moderate correlations between the two types of data,
229 estimated to be 0.53 and 0.54 for data collected upon necropsy or deworming respectively. Study-wise species relative
230 abundance also corresponds to a wide range of horse-wise relative abundance, especially for species like *C. nassatus* and *C.*
231 *longibursatus* (supplementary Figure 2B and 3B).

232 As a result, reliance on mean species counts to infer their study-wise relative abundance yielded strongyle community matrices
233 that departed more from the observed horse-wise communities than when relying on presence/absence data (Figure 3). The
234 respective differences were 1.84 and 1.39 standard deviation ($t = -109.71$, $df = 42120$, p-value $< 10^{-4}$) for study-wise species
235 relative abundance and presence/absence respectively.

236 Nonetheless, modelling study-wise presence/absence data was also suboptimal. While the modelling of horse-wise
237 community data performed well to predict independent observations (average RMSE of 0.11 ± 0.1 , Figure 4), the RMSE
238 coefficient of the study-wise model applied to horse-wise data had an average value of 0.53 ± 0.04 and never went below 0.39
239 (Figure 4).

240 Altogether, the framework applied in this study has a reduced divergence from the original horse-wise strongyle community
241 data, but inferences made using the presence/absence data at the study level are not a good fit for horse-wise data and rather
242 yield predictions at the regional scale.

1
2
243
~~3~~
244
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
245
4
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
62
63
64
65

Discussion

This meta-analysis has explored how the equine strongyle community structure was affected by geoclimatic data, strongyle recovery methods and the sampling effort using study-wise presence/absence data. The findings highlighted little variation of strongyle communities across contrasted levels of this variable, with most of the variation being underpinned by differences between the strongyle recovery methods. Simulations performed in this study found that study-wise species presence/absence was a better descriptor of underpinning horse-wise strongyle communities than the species average relative abundance. As such, our results and the undertaken simulation study complement and expand the past work by Bellaw and Nielsen [30] with different approaches and interpretations of the findings.

First, our study focused on strongylid species, thereby accounting for more species than the only cyathostominae covered in [30]. This unravelled significant variation for strongylinae species including *S. vulgaris* and *T. serratus* that were more often recovered in continental Europe using deworming.

Second, reliance on presence/absence data restricted the analysis to the only subdominant species, such as *C. ashworthi* and *C. brevicapsulatus*, whose occurrences were also higher in tropical America relative to temperate America conditions. As a result, our results expand past conclusions on species variation across conditions.

The presence-absence framework was originally decided upon to overcome the lack of appropriate summary statistics (standard deviation or confidence interval) in the papers selected for this meta-analysis. However, the simulation approach on horse-wise community data found that modelling on log-transformed species mean counts yielded well correlated estimates to the horse-wise counts. This would validate the use of study-wise mean counts to describe the underlying distribution.

The presence/absence framework considered in this study certainly overlooked finer grain patterns found with abundance data [34], and the simulations performed underscored the limited predictive ability of study-wise inferences on horse-wise species occurrences. This is mostly the result of the existing asymmetry between presence of the parasite - a single infected individual is enough - and absence - that requires the species to be entirely absent [35]. Reliance on this binary matrix defined at the regional level also prevented the use of other statistics such as the effective number of species [36] or individual based rarefaction [37] to better account for differences in scales (geographical and temporal) across the species sets considered.

On the contrary, our simulations found that study-wise species presence/absence was a better match than study-wise relative abundances to the underpinning horse-wise strongylid communities. This is in agreement with the compositional nature of relative abundance data that obscure the variation in the respective species counts across conditions [38].

Overall and as in Bellaw and Nielsen [30], our results highlighted a core of ten species that are consistently found across studies with sustained prevalence levels across horses, irrespective of the recovery method. Of note, *C. nassatus* – one of the most dominant species - was consistently associated with drug resistance reports suggesting their phenology may have favoured higher fitness towards the use of modern anthelmintics [39]. It is unclear however what phenotypic trait underpins their higher abundance and prevalence in horse populations. Their respective fecundities estimated from the number of eggs found in the uteri of female worms was among the lowest suggesting their dispersal is unlikely to be defined by a better ability to colonize their hosts [33].

Similar to Bellaw and Nielsen [30], the analytical framework applied herein highlighted a significant effect of the recovery method that overwhelmed the contribution of geoclimatic factors. However, this clear distinction between the recovery methods was also associated with significant variation in scales. Worm collection upon deworming was performed more recently, and more horses were sampled for the necropsy-based studies. This is likely driving the observed dissimilarity between communities, as the more communities are sampled, the more likely species recovery will vary [35].

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

The variation found between the different collection methods might relate to the anthelmintic treatment efficacy in the studies applying deworming. However, none of these studies [25-28, 33, 40-43] reported drug resistance. They also relied on ivermectin, whose efficacy remains largely sustained despite reports of reduced faecal egg count reduction or shorter egg reappearance periods after treatment [6, 44-47].

Altogether, this study recalls the consensus that strongylid species are structured around a core of ten species. Disentangling the effect of environmental factors on strongylid community structure and the rules underpinning their assembly remains however challenging because of the limited data available. Application of the latest barcoding approaches [48] should provide valuable contributions to describe and analyse the patterns of strongylid species assemblages across various conditions.

Acknowledgements

MBo and this work was supported by the Institut Français du Cheval et de l'Équitation (IFCE) and Fonds Éperon grant. The authors are greatly indebted to Pr. Ian Beveridge who kindly shared the data produced by Bucknell and collaborators and reviewed this manuscript.

Author's contributions

MBo, NM and GS conceived the ideas and designed methodology; MBa, SK, TK and CL collected the data; MBo, NM and GS analysed the data; MBo, NM and GS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The python and R scripts used to collect and analyse the data have been deposited at: <https://github.com/MichelBoisseau37/metaAnalyses-Strongyles/>. The data will be made available upon manuscript acceptance.

Conflict of interest

The authors declare that they have no conflicts of interest.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure and table legends

Figure 1. Descriptive features of worldwide equine strongylid communities

A: Strongylid species (colored by their respective genera) relative abundances and prevalences averaged across published mean estimates. Dotted lines distinguish between rare, subdominant and dominant species in the left, middle and right quadrants respectively.

B: Comparison of observed species prevalence across studies (from the presence-absence matrix) against the average published prevalence estimates. Colour and dotted lines match that used in B.

Figure 2. Ordination applied to strongylid meta-community with fitted environmental variables

Figure shows the first two axes from a NonMetric Dimensional Scaling (NMDS) ordination applied to published metacommunity data. The colours correspond to the different types of environment considered, coded as Continent – Climate – Recovery method. For visualization, species names were abbreviated with correspondence given in Supplementary Table 5.

Figure 3. Divergence between study-wise strongyle community matrices and their respective horse-wise sub-communities for two dissimilarity measures

The mean Jaccard (presence/absence) or Bray-Curtis (relative abundances) distances between study-wise strongyle community and the horse-wise sub-communities they were derived from is presented. Data were generated using 1000 simulated studies of 60 cohorts of n horses, n ranging between 8 and 150. The figure suggests a reduced divergence is found using study-wise species presence/absence rather than relying on study-wise averaged relative abundances.

Figure 4. Predictive ability of species presence/absence modelling using study-wise or horse-wise data

The Root-Mean-Square Error distribution of logistic modelling applied to a 1,000 simulated strongyle communities are represented for a model built on study-wise species presence/absence data (left) or on horse-wise data only. The figure suggests that study-wise species presence/absence data are poor predictor of underlying horse-wise community structure.

Table 1: Summary table of the studies considered.

Supplementary material

Supplementary Figure 1. Principal Coordinates Analysis on the study-wise presence/absence incidence matrix

The ordination plot shows the coordinates of the considered study-wise strongyle communities. Each community is each materialized by a dot whose shape relates to the corresponding environmental factors, i.e. Eu-Temp-nec: Europe – Temperate – Necropsy (blue), Eu-Cont-dew: Europe- Continental – deworming (green), Am-Trop-nec: America – Tropical – necropsy (red); Am-Temp-nec: America – Temperate – necropsy (black). This plot underscores the outstanding contribution of the collection method on the dissimilarity between strongyle communities.

Supplementary Figure 2. Strongyle species counts or relative abundance distribution and respective mean values as estimated from 245 dewormed horses

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

347 A: Species count distribution (on the log10 scale) is represented for 12 species consistently found in strongyle communities
348 collected upon deworming in horses from Poland (Sallé et al. 2018) and Ukraine (Kuzmina et al. 2016). Vertical dashed line
349 materializes the mean.
350 B: Species relative abundances estimated from the same data. Each faded dot corresponds to an observation made in one
351 horse, with the species average across the two studies is materialized with a plain dot.

352
353 **Supplementary Figure 3. Strongyle species counts or relative abundance distribution and respective mean values as**
354 **estimated from 177 necropsied horses**

355 A: Species count distribution (on the log10 scale) is represented for 12 species consistently found in strongyle communities
356 collected upon deworming in horses from Australia (Bucknell et al., 1995) and France (Collobert-Laugier et al. 2002). Vertical
357 dashed line materializes the mean.
358 B: Species relative abundances estimated from the same data. Each faded dot corresponds to an observation made in one
359 horse, with the species average across the two studies is materialized with a plain dot.

360
361 **Supplementary Figure 4. Relationship between strongyle species relative abundance and observed species count across**
362 **422 horses following necropsy (A) or deworming (B).**

363 Strongyle species counts from 245 dewormed horses from Poland (Sallé et al. 2018) or Ukraine (Kuzmina et al. 2016), and
364 177 necropsied horses from Australia (Bucknell et al., 1995) and France (Collobert-Laugier et al. 2002) are plotted along the
365 x-axis. Its corresponding relative abundance within a given host is given on the y-axis. Plots indicate that a given species
366 count corresponds to a wide range of relative abundance values.

1
2
369
4
370
5
371
6
372
7
373
8
374
9
375
10
376
11
377
12
378
13
379
14
380
15
381
16
382
17
383
18
384
19
385
20
386
21
387
22
388
23
389
24
390
25
391
26
392
27
393
28
394
29
395
30
396
31
397
32
398
33
399
34
400
35
401
36
402
37
403
38
404
39
405
40
406
41
407
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

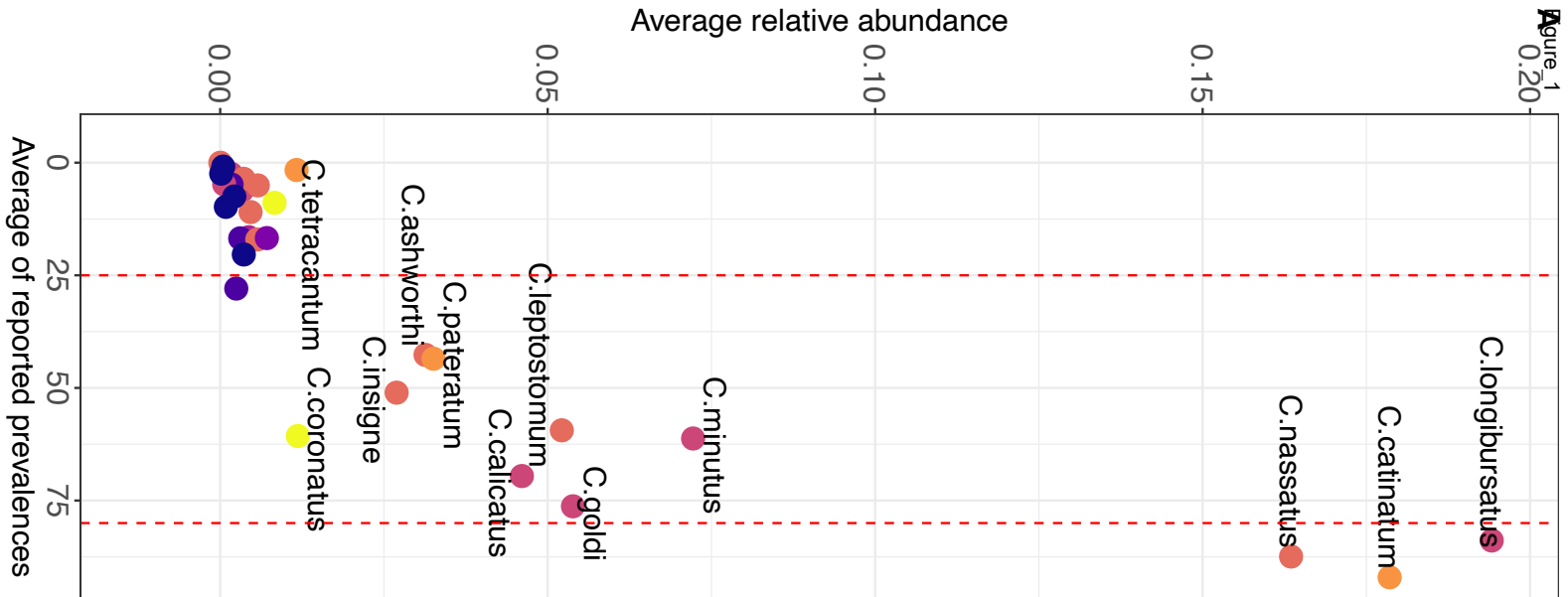
1. Taylor, M.A., R.L. Coop, and R.L. Wall, *Veterinary parasitology*. Third edition ed. 2012.
2. Lichtenfels, J.R., V.A. Kharchenko, and G.M. Dvojnjos, *Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae)*. *Veterinary parasitology*, 2008. **156**(1-2): p. 4-161.
3. Herd, R., *The changing world of worms: The rise of the cyathostomes and the decline of Strongylus vulgaris*. *Compendium on Continuing Education for the Practicing Veterinarian*, 1990. **12**(5): p. 732-736.
4. Matthews, J.B., *Anthelmintic resistance in equine nematodes*. *International Journal for Parasitology: Drugs and Drug Resistance*, 2014. **4**(3): p. 310-315.
5. Sallé, G., et al., *Compilation of 29 years of postmortem examinations identifies major shifts in equine parasite prevalence from 2000 onwards*. *International Journal for Parasitology*, 2020.
6. Relf, V., et al., *Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs*. *Parasitology*, 2013. **140**(5): p. 641.
7. Wood, E., et al., *Variation in fecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation*. *Parasitology*, 2013. **140**(1): p. 115-128.
8. Kornaś, S., et al., *Estimation of genetic parameters for resistance to gastro-intestinal nematodes in pure blood Arabian horses*. *International journal for parasitology*, 2015. **45**(4): p. 237-242.
9. Debeffe, L., et al., *Negative covariance between parasite load and body condition in a population of feral horses*. *Parasitology*, 2016. **143**(8): p. 983-997.
10. Nielsen, M.K., et al., *Climatic influences on development and survival of free-living stages of equine strongyles: implications for worm control strategies and managing anthelmintic resistance*. *The Veterinary Journal*, 2007. **174**(1): p. 23-32.
11. Kooyman, F., et al., *Species composition of larvae cultured after anthelmintic treatment indicates reduced moxidectin susceptibility of immature Cylicocyclus species in horses*. *Veterinary parasitology*, 2016. **227**: p. 77-84.
12. Lello, J., et al., *Competition and mutualism among the gut helminths of a mammalian host*. *Nature*, 2004. **428**(6985): p. 840-844.
13. Telfer, S., et al., *Species interactions in a parasite community drive infection risk in a wildlife population*. *Science*, 2010. **330**(6001): p. 243-246.
14. Sweeny, A.R., et al., *Experimental parasite community perturbation reveals associations between Sin Nombre virus and gastrointestinal nematodes in a rodent reservoir host*. *Biology Letters*, 2020. **16**(12): p. 20200604.
15. Brosschot, T.P., et al., *Impaired host resistance to Salmonella during helminth co-infection is restored by anthelmintic treatment prior to bacterial challenge*. *PLOS Neglected Tropical Diseases*, 2021. **15**(1): p. e0009052.
16. Tchuem Tchuente, L.A., et al., *Polyparasitism with Schistosoma haematobium and soil-transmitted helminth infections among school children in Loum, Cameroon*. *Tropical medicine & international health*, 2003. **8**(11): p. 975-986.
17. Behnke, J., *Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions or pure randomness?* *Parasitology*, 2008. **135**(7).
18. Dallas, T.A., A.-L. Laine, and O. Ovaskainen, *Detecting parasite associations within multi-species host and parasite communities*. *Proceedings of the Royal Society B*, 2019. **286**(1912): p. 20191109.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
19. Poulin, R. and E.T. Valtonen, *The predictability of helminth community structure in space: a comparison of fish populations from adjacent lakes*. International Journal for Parasitology, 2002. **32**(10): p. 1235-1243.
 20. Reinemeyer, C., et al., *The prevalence and intensity of internal parasites of horses in the USA*. Veterinary Parasitology, 1984. **15**(1): p. 75-83.
 21. Ogbourne, C.P., *The prevalence, relative abundance and site distribution of nematodes of the subfamily Cyathostominae in horses killed in Britain*. Journal of Helminthology, 1976. **50**(3): p. 203-214.
 22. Mfitilodze, M. and G. Hutchinson, *Prevalence and abundance of equine strongyles (Nematoda: Strongyloidea) in tropical Australia*. The Journal of parasitology, 1990: p. 487-494.
 23. Gawor, J., *The prevalence and abundance of internal parasites in working horses autopsied in Poland*. Veterinary Parasitology, 1995. **58**(1-2): p. 99-108.
 24. Lind, E.O., et al., *Expulsion of small strongyle nematodes (cyathostomin spp) following deworming of horses on a stud farm in Sweden*. Veterinary parasitology, 2003. **115**(4): p. 289-299.
 25. Kornas, S., M. Basiaga, and V. Kharchenko, *Composition of the cyathostomin species in horses with a special focus on Cylicocyclus brevicapsulatus*. Medycyna Weterynaryjna, 2011. **67**(01).
 26. Kuzmina, T., et al., *Analysis of the strongylid nematodes (Nematoda: Strongylidae) community after deworming of brood horses in Ukraine*. Veterinary Parasitology, 2005. **131**(3-4): p. 283-290.
 27. Kuzmina, T., et al., *Biodiversity of strongylids (Nematoda: Strongylidae) communities in domestic horses from Poland and Ukraine*. Helminthologia, 2011. **48**(2): p. 77-84.
 28. Kuzmina, T.A., I. Dzeverin, and V.A. Kharchenko, *Strongylids in domestic horses: Influence of horse age, breed and deworming programs on the strongyle parasite community*. Veterinary parasitology, 2016. **227**: p. 56-63.
 29. Collobert-Laugier, C., et al., *Prevalence, abundance and site distribution of equine small strongyles in Normandy, France*. Veterinary Parasitology, 2002. **110**(1-2): p. 77-83.
 30. Bellaw, J.L. and M.K. Nielsen, *Meta-analysis of cyathostomin species-specific prevalence and relative abundance in domestic horses from 1975–2020: emphasis on geographical region and specimen collection method*. Parasites & Vectors, 2020. **13**(1): p. 1-15.
 31. Team, R.C., *R: A language and environment for statistical computing*. 2013.
 32. Bucknell, D., R. Gasser, and I. Beveridge, *The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia*. International journal for parasitology, 1995. **25**(6): p. 711-724.
 33. Sallé, G., S. Kornaś, and M. Basiaga, *Equine strongyle communities are constrained by horse sex and species dispersal-fecundity trade-off*. Parasites & vectors, 2018. **11**(1): p. 279.
 34. Brian, J. and D. Aldridge, *Abundance data applied to a novel model invertebrate host sheds new light on parasite community assembly in nature*. bioRxiv, 2020.
 35. Chase, J.M., et al., *Species richness change across spatial scales*. Oikos, 2019. **128**(8): p. 1079-1091.
 36. Chase, J.M. and T.M. Knight, *Scale- dependent effect sizes of ecological drivers on biodiversity: why standardised sampling is not enough*. Ecology letters, 2013. **16**: p. 17-26.
 37. Chase, J.M., et al., *Embracing scale- dependence to achieve a deeper understanding of biodiversity and its change across communities*. Ecology letters, 2018. **21**(11): p. 1737-1751.
 38. Chong, F. and M. Spencer, *Analysis of relative abundances with zeros on environmental gradients: a multinomial regression model*. PeerJ, 2018. **6**: p. e5643.

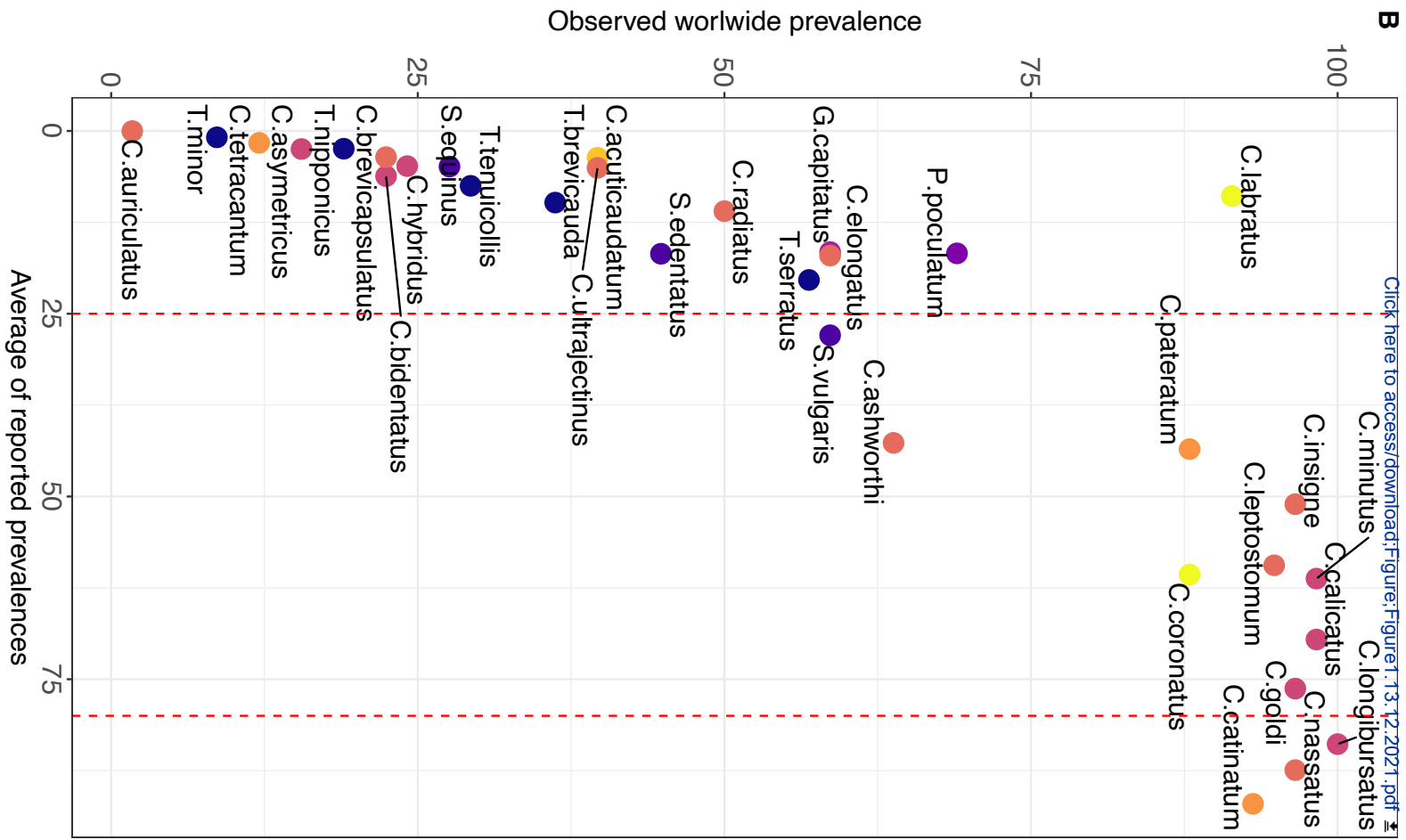
1
2
3 447 39. van Doorn, D., et al., *Cylicocycclus species predominate during shortened egg reappearance period in horses after*
4 448 *treatment with ivermectin and moxidectin*. Veterinary parasitology, 2014. **206**(3-4): p. 246-252.
5
6 449 40. Kuzmina, T.A. and V.O. Kharchenko, *Anthelmintic resistance in cyathostomins of brood horses in Ukraine and*
7 450 *influence of anthelmintic treatments on strongylid community structure*. Veterinary parasitology, 2008. **154**(3-4): p.
8 451 277-288.
9
10 452 41. Slivinska, K., G. Dvojnos, and G. Kopij, *Helminth fauna of sympatric Przewalski's Equus przewalskii Poljakov,*
11 453 *1881 and domestic horses E. caballus L. in the Chernobyl exclusion zone, Ukraine*. Helminthologia, 2006. **43**(1): p.
12 454 27-32.
13
14 455 42. Slivinska, K., J. Gawor, and Z. Jaworski, *Gastro-intestinal parasites in yearlings of wild Polish primitive horses*
15 456 *from the Popielno Forest Reserve, Poland*. Helminthologia, 2009. **46**(1): p. 9.
16
17 457 43. Slivinska, K., Z. Wróblewski, and J. Gawor, *Occurrence of gastro-intestinal parasites in Polish primitive horses from*
18 458 *the Roztocze National Park, Poland*. Vestnik zoologii, 2013. **47**(6): p. 53-61.
19
20 459 44. Traversa, D., et al. *Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United Kingdom*
21 460 *and Germany*. in *Parasites & Vectors*. 2009. BioMed Central.
22
23 461 45. Milillo, P., et al. *Faecal Cyathostomin Egg Count distribution and efficacy of anthelmintics against cyathostomins*
24 462 *in Italy: a matter of geography?* in *Parasites & Vectors*. 2009. Springer.
25
26 463 46. Näreaho, A., K. Vainio, and A. Oksanen, *Impaired efficacy of ivermectin against Parascaris equorum, and both*
27 464 *ivermectin and pyrantel against strongyle infections in trotter foals in Finland*. Veterinary parasitology, 2011. **182**(2-
28 465 4): p. 372-377.
29
30 466 47. Canever, R.J., et al., *Lack of Cyathostomin sp. reduction after anthelmintic treatment in horses in Brazil*. Veterinary
31 467 *Parasitology*, 2013. **194**(1): p. 35-39.
32
33 468 48. Poissant, J., et al., *A repeatable and quantitative DNA metabarcoding assay to characterize mixed strongyle*
34 469 *infections in horses*. International Journal for Parasitology, 2020.
35
36
37
38 470 z

39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

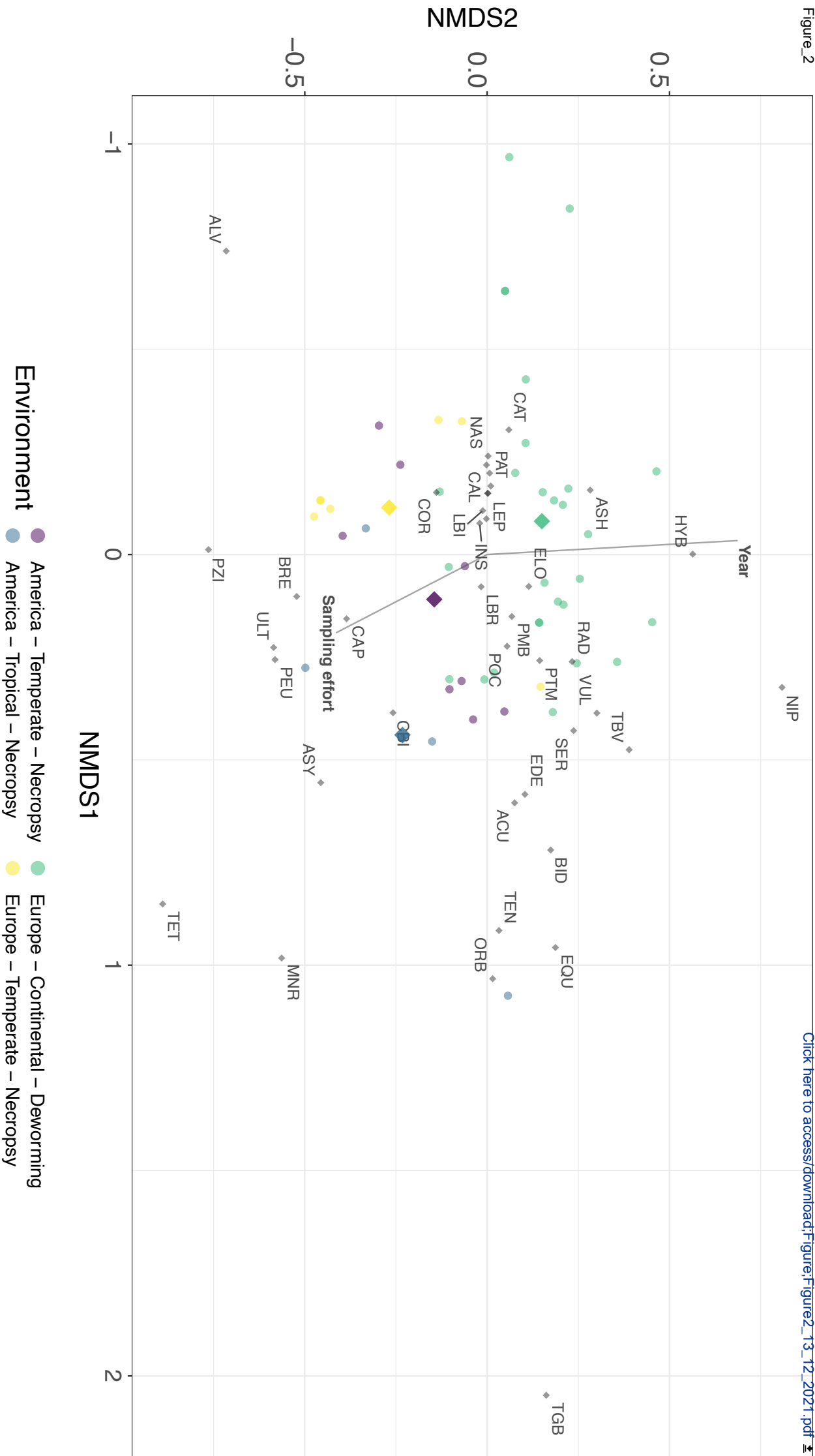
Figure 1



B

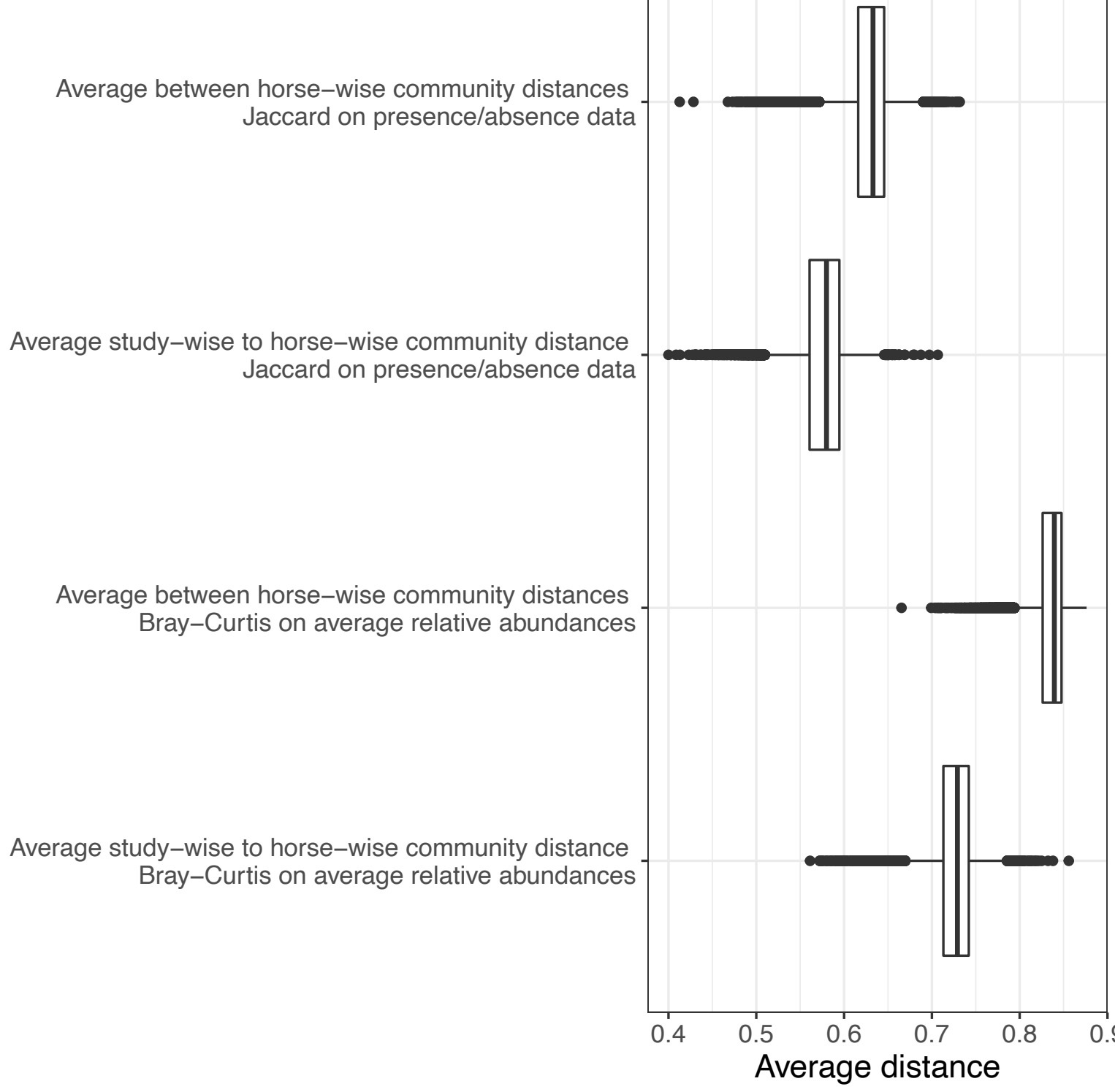


Click here to access/download:Figure:Figure1:13_12_2021.pdf



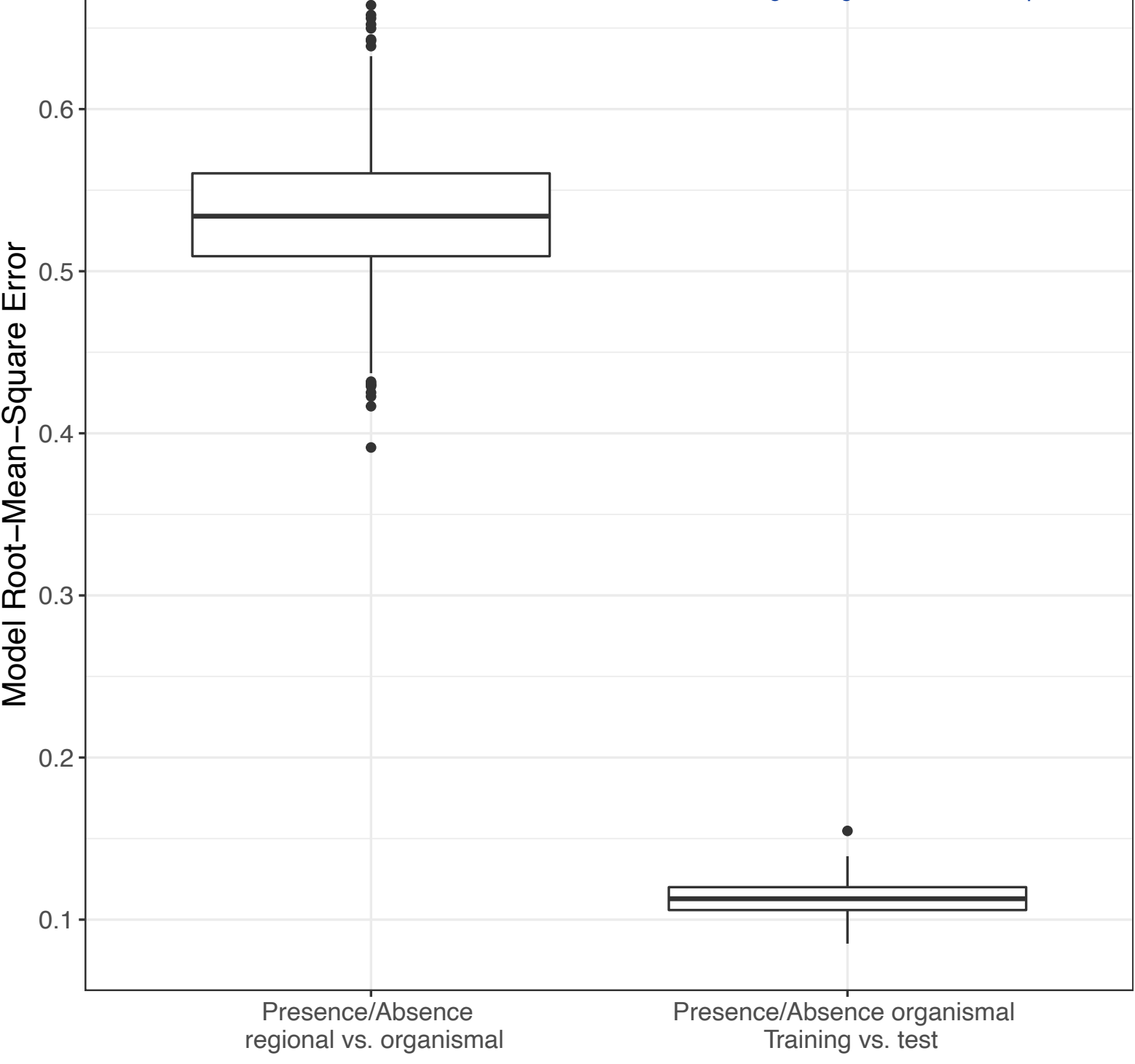
Figure_3

[Click here to access/download;Figure;Figure3.14.12.2021.pdf](#)



Figure_4

[Click here to access/download;Figure;Figure4.14.12.2021.pdf](#)





Click here to access/download
Supplementary Material
Supplementary_material.docx





Click here to access/download
Supplementary Material
Supplementary_figure_1_13_12.pdf





Click here to access/download
Supplementary Material
Supplementary_Figure2.pdf



Click here to access/download
Supplementary Material
Supplementary_Figure3.pdf