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

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## Meta-analysis of equine strongyle community structure

--Manuscript Draft--

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<b>Abstract:</b>	<p><b>Purpose</b></p> <p>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.</p> <p><b>Methods</b></p> <p>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.</p> <p><b>Results</b></p> <p>Worldwide, twelve species, <i>Cylicocycclus</i> ( n = 4), <i>Cylicostephanus</i> ( n = 3), <i>Coronocycclus</i> ( n = 2) and <i>Cyathostomum</i> ( 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. <i>Cylicocycclus ashworthi</i>, <i>Tridontophorus serratus</i> and <i>Strongylus vulgaris</i> were more frequently found in continental Europe following deworming, whereas <i>Cylicocycclus brevicapsulatus</i> 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.</p> <p><b>Conclusion</b></p> <p>This study expands previous findings and underscores the challenges left to better understand how strongylid communities assemble in horses.</p>	
<b>Corresponding Author:</b>	michel BOISSEAU, M.D INRAE Centre Val de Loire: Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement Centre Val de Loire nouzilly, FRANCE	
<b>Corresponding Author Secondary Information:</b>		
<b>Corresponding Author's Institution:</b>	INRAE Centre Val de Loire: Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement Centre Val de Loire	
<b>Corresponding Author's Secondary Institution:</b>		
<b>First Author:</b>	michel BOISSEAU, M.D	
<b>First Author Secondary Information:</b>		
<b>Order of Authors:</b>	michel BOISSEAU, M.D	
	Núria Mach	

	Marta Basiaga
	Sławomir Kornaś
	Tetiana Kuzmina
	Claire Laugier
	Guillaume Sallé
<b>Order of Authors Secondary Information:</b>	
<b>Author Comments:</b>	
<b>Suggested Reviewers:</b>	<p>Jakub Gawor gaworj@twarda.pan.pl interest in equine intestinal parasites</p> <p>Jacques Cabaret jcabaret37@gmail.com interest in equine intestinal parasites</p> <p>Adam Hayward adam.hayward@moredun.ac.uk work on statistics</p>

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## 1 **Meta-analysis of equine strongyle community structure**

2 Michel Boisseau<sup>a</sup>, Núria Mach<sup>b</sup>, Marta Basiaga<sup>c</sup>, Sławomir Kornas<sup>c</sup>, Tetiana Kuzmina<sup>d</sup>, Claire Laugier<sup>e</sup>, Guillaume Sallé<sup>a</sup>

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](mailto:michel.boisseau@inrae.fr)

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**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, *Cylicocyclus* ( $n = 4$ ), *Cylicostephanus* ( $n = 3$ ), *Coronocyclus* ( $n = 2$ ) 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. *Cylicocyclus ashworthi*, *Tridontophorus serratus* and *Strongylus vulgaris* were more frequently found in continental Europe following deworming, whereas *Cylicocyclus 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

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## 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.

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## Materials and methods

### Literature search and inclusion criteria

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

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

To be included in the meta-analysis, reported articles had to meet several criteria. First, studies were included in the dataset if they reported parasite prevalence (proportion of infected host), abundance (the number of parasite individuals per host, including uninfected individuals) or relative abundance. Second, published data were only included if the number of horses sampled was specified. Third, we removed studies before 1975 to avoid annotation mismatches due to different taxonomic identifications prior to that date.

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

### Parasite, environmental and host variables

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

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## 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  $\alpha$ -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 ( $\beta$ -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),

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

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

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

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

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

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

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

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## 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.* *Cylicocyclus 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*, *Cylicocyclus leptostomus*, *Cylicocyclus insigis*, *Cyathostomum pateratum*, *Coronocyclus 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, *Coronocyclus 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 *Cylicocyclus 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 ( $35.69 \pm 27.67$  and  $18.9 \pm 15.8$  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 *Cylicocyclus ashworthi*, *Tridontophorus serratus* and *Strongylus vulgaris*, that were more frequently recovered in continental Europe using deworming (Estimate equal to  $6.43 \pm 1.7$ ,  $P = 10^{-4}$ ;  $2.74 \pm 1.33$ ,  $P = 4 \times 10^{-2}$  and  $2.99 \pm 1.34$ ,  $P = 2.5 \times 10^{-2}$  respectively). Necropsies performed under tropical America had more likelihood of recovering *Cylicocyclus brevicapsulatus* relative to the temperate American conditions (Estimate equal

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to  $4.42 \pm 2.15$ ,  $P = 4 \times 10^{-2}$ ). Sampling effort had a small but significant effect on study-wise species presence/absence (Estimate =  $3 \times 10^{-2}$ ,  $P = 2 \times 10^{-3}$ ).

While significant variation was found between overall community structure, species richness was little affected by the different conditions studied. Indeed, species richness was not statistically different between the levels of the synthetic variable accounting for the geo-climatic area and the strongyle recovery method ( $P = 0.41$ ). The same pattern was found for the year of publication ( $P = 0.27$ ). However, the difference in sampling effort was positively associated with species richness (estimate = 0.15,  $P = 3 \times 10^{-3}$ ).

To sum up, synthetic variable and sampling effort were driving dissimilarity between strongyle communities with discrete variation found in species occurrences. Differences in sampling effort may underpin the observed dissimilarity.

### Impact of analytical frameworks on inferences made

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

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

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

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

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

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

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## 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].

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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.

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### **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.

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## 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

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A: Species count distribution (on the log<sub>10</sub> scale) is represented for 12 species consistently found in strongyle communities collected upon deworming in horses from Poland (Sallé et al. 2018) and Ukraine (Kuzmina et al. 2016). Vertical dashed line materializes the mean.

B: Species relative abundances estimated from the same data. Each faded dot corresponds to an observation made in one horse, with the species average across the two studies is materialized with a plain dot.

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

A: Species count distribution (on the log<sub>10</sub> scale) is represented for 12 species consistently found in strongyle communities collected upon deworming in horses from Australia (Bucknell et al., 1995) and France (Collobert-Laugier et al. 2002). Vertical dashed line materializes the mean.

B: Species relative abundances estimated from the same data. Each faded dot corresponds to an observation made in one horse, with the species average across the two studies is materialized with a plain dot.

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

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

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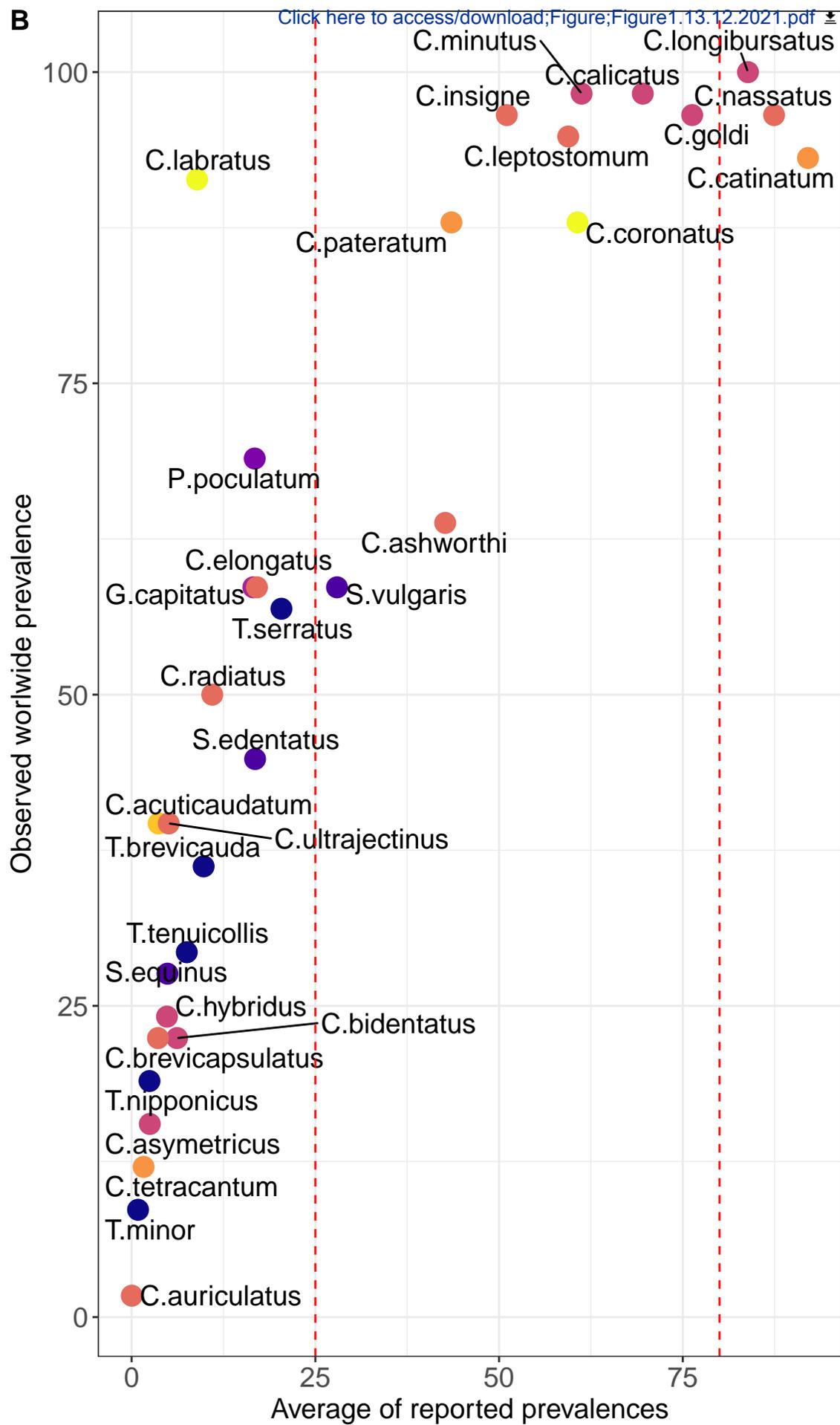
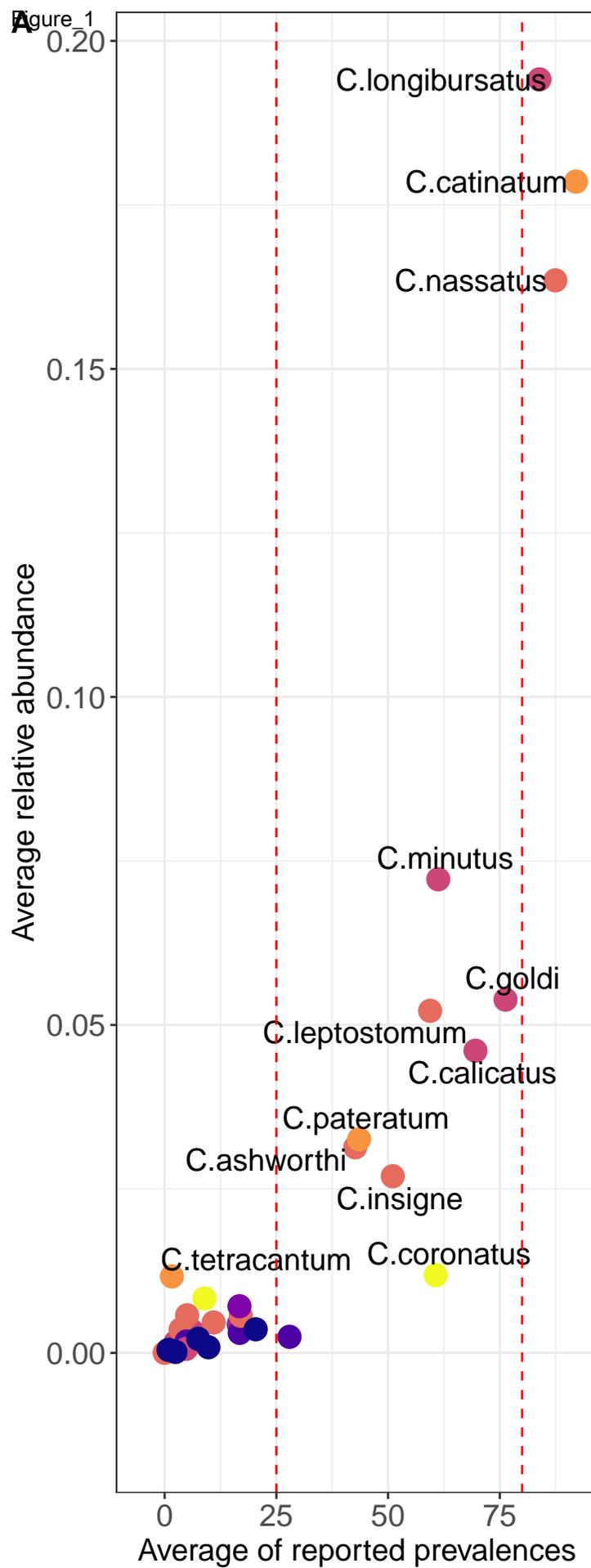
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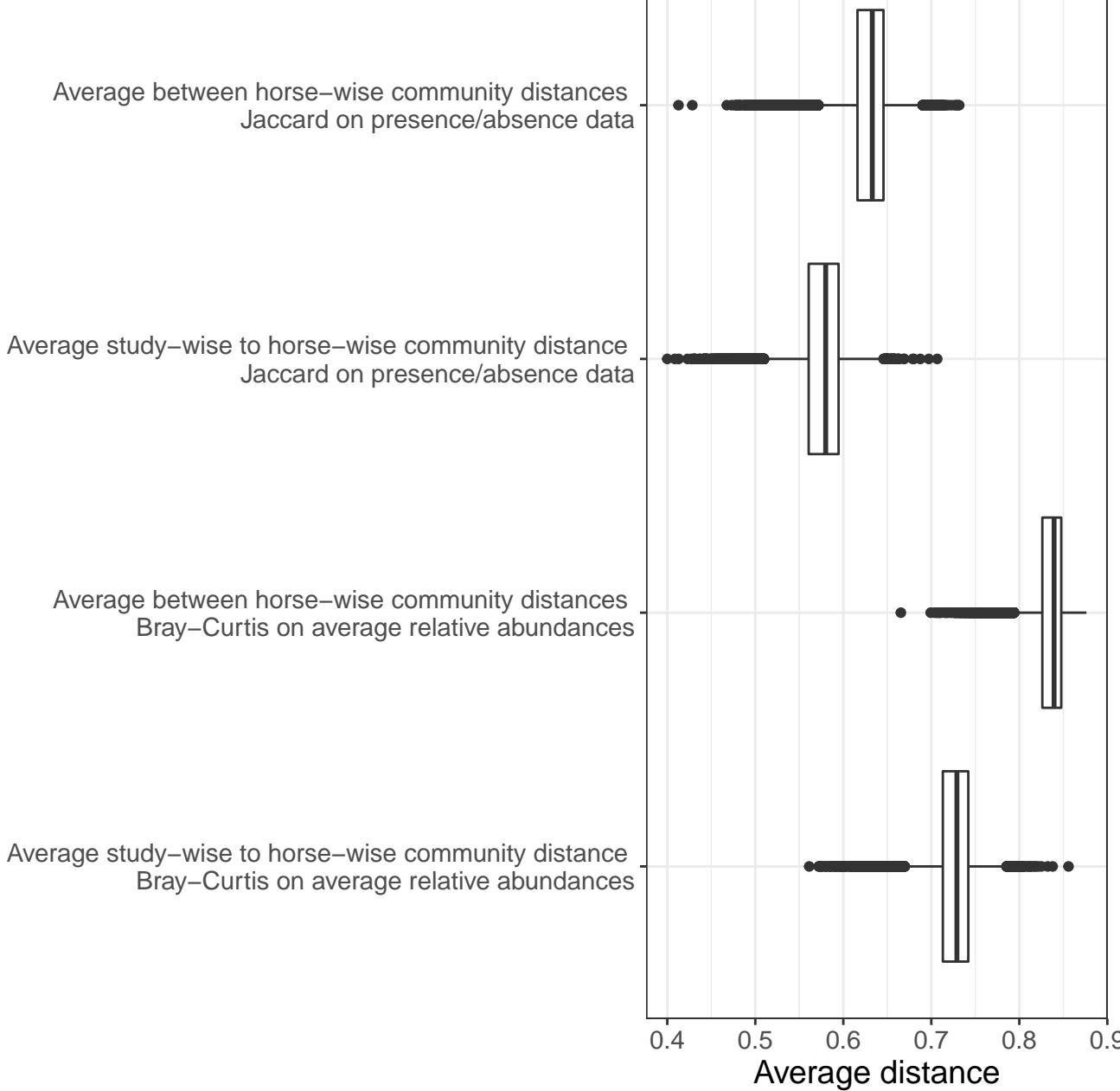
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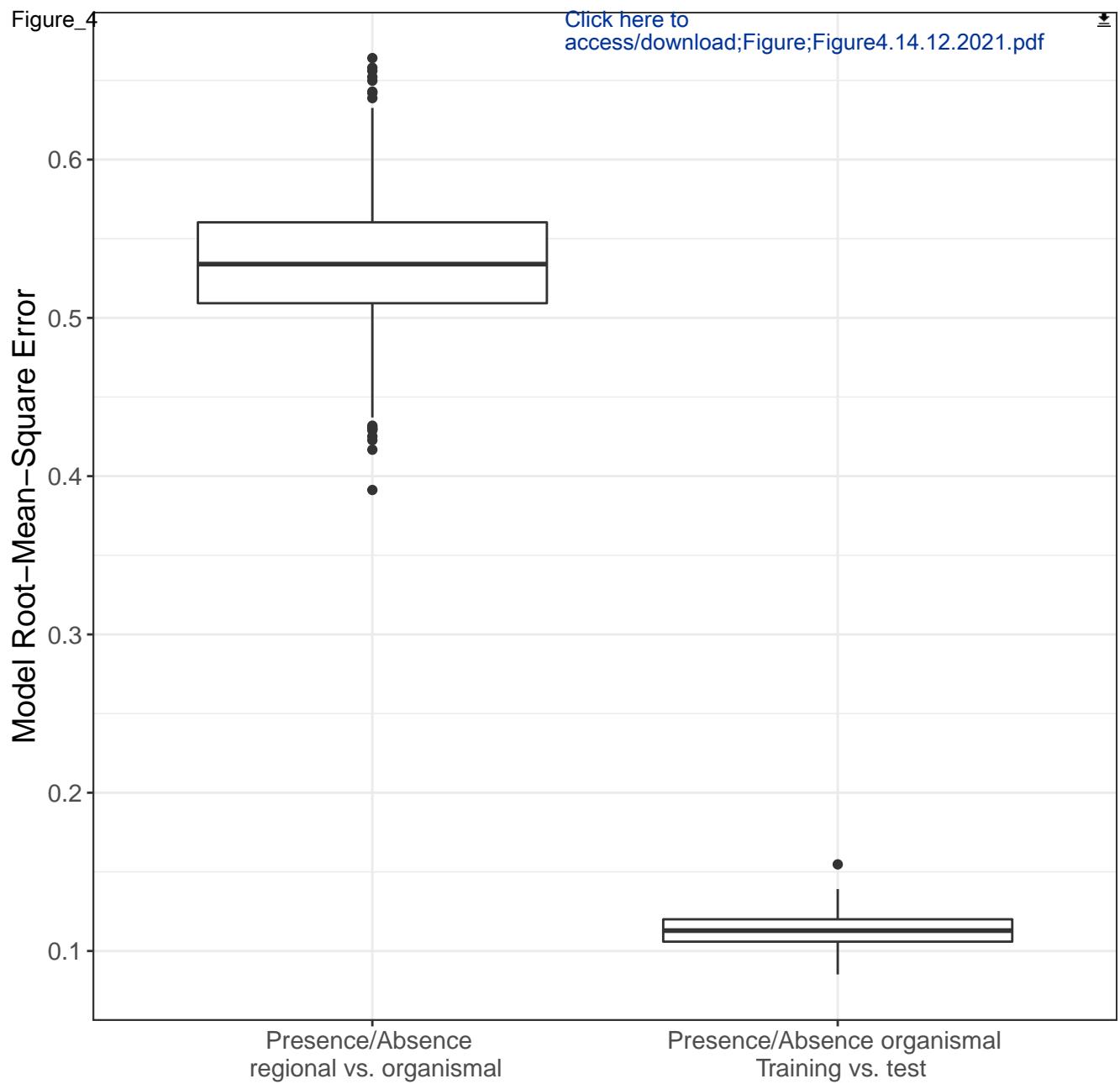
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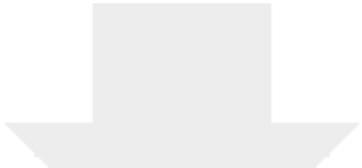
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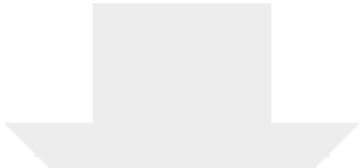
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