1   **Risk factor analysis of equine strongyle resistance to anthelmintics**

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21

22   **Abstract**

23   Intestinal strongyles are the most problematic endoparasites of equids as a result of their wide

24   distribution and the spread of resistant isolates throughout the world. While abundant

25   literature can be found on the extent of anthelmintic resistance across continents, empirical

26   knowledge about associated risk factors is missing. This study brought together results from

27   anthelmintic efficacy testing and risk factor analysis to provide evidence-based guidelines in

28   the field. It involved 688 horses from 39 French horse farms and riding schools to both

29   estimate Faecal Egg Count Reduction (FECR) after anthelmintic treatment and to interview

30   farm and riding school managers about their practices. Risk factors associated with reduced

31   anthelmintic efficacy in equine strongyles were estimated across drugs using a marginal

32   modelling approach. Results demonstrated ivermectin efficacy (96.3% FECR), the inefficacy

33   of fenbendazole (42.8% FECR) and an intermediate profile for pyrantel (90.3% FECR). Risk

34   factor analysis provided support to advocate for FEC-based treatment regimens combined

35   with individual anthelmintic dosage and the enforcement of tighter biosecurity around horse

36   introduction that contributed to lower drug resistance risk by 1.75. Premises falling under this

37   typology also relied more on their veterinarians suggesting they play an important role in the

38   sustainability of anthelmintic usage. Similarly, drug resistance risk was halved in premises

39   with frequent pasture rotation and with stocking rate below five horses/ha. This is the first

40   empirical risk factor analysis for anthelmintic resistance in equids, whose findings should

41   guide the implementation of more sustained strongyle management in the field.

42

43   **Keywords**: horse, nematode, anthelmintic resistance, strongyle, cyathostomin

44

45   **1. Introduction**

46   The diversity of helminth species infecting horses is large, and differences in life cycles,

47   epidemiology, pathogenicity and drug susceptibility make it increasingly challenging to

48   define good sustainable parasite control programs. Strongyles remain a major concern. They

49   can be classified into two sub-families, namely Strongylinae (large strongyles) and

50   Cyathostominae known as small strongyles or cyathostomins (Lichtenfels et al., 2008). The

51   large strongyle *Strongylus vulgaris* is associated with a high mortality rate resulting from

52   parasite associated verminous arteritis (Nielsen et al., 2016). This species has been

53   successfully controlled by anthelmintics, despite recent reports of putative re-emergence

54   associated with reduced frequency of anthelmintic treatments (Nielsen et al., 2012; Nielsen et

55   al., 2016). On the contrary, cyathostomins have become a growing concern in the field

56   (Matthews, 2014; Peregrine et al., 2014). This group of nematodes encompasses 50 known

57   species (Lichtenfels et al., 2008) that infect both young and adult horses (Corning, 2009) and

58   have a ubiquitous distribution throughout geo-climatic conditions (Sallé and Cabaret, 2015a).

59   Their life cycle is direct and usually involves encystment of infective larvae into the caeco-

60   colic mucosa of their hosts (Corning, 2009). In heavily infected horses, *en-mass* emergence

61   of these encysted larvae can cause severe clinical pathology characterized by a loss of weight,

62   colic, diarrhoea, protein-losing enteropathy and the eventually death of the animal (Murphy

63   and Love, 1997; Love et al., 1999).

64   The use of anti-infectious drugs puts pathogen populations under selection pressure that can

65   ultimately lead to the emergence of resistant or multi-drug resistant populations (Kennedy

66   and Read, 2017). Over the past decades, small strongyle populations, like other livestock-

67   infecting parasitic nematodes (Kaplan and Vidyashankar, 2012), have demonstrated a gradual

68   increase in their resistance to available anthelmintics in every part of the world (Matthews,

69   2014; Peregrine et al., 2014). Under French settings (Traversa et al., 2012) like in other

70   European (Traversa et al., 2007; Traversa et al., 2009; Relf et al., 2014) or American

71   countries (Slocombe and de Gannes, 2006; Lyons et al., 2008; Molento et al., 2008; Canever

72   et al., 2013), resistant strongyle populations have been reported for every available class of

73   anthelmintics, namely benzimidazoles, tetrahydropyrimidines or macrocyclic lactones.

74   Although previous studies have demonstrated how widespread anthelmintics resistance is,

75   critical assessment of associated risk factors and species composition of resistant parasitic

76   populations is still lacking (Nielsen, 2012), thereby preventing the implementation of clear

77   clear field guidelines. There have been a limited number of reports focusing on factors

78   associated with prevalence of strongyle infection in horses in Germany (Fritzen et al., 2010;

79   Hinney et al., 2011a) or the impact of faeces removal on prevalence in the UK (Corbett et al.,

80   2014). Available studies have considered drenching practices (Lendal et al., 1998; O'Meara

81   and Mulcahy, 2002; Lind et al., 2007; Hinney et al., 2011b; Relf et al., 2012) or estimation of

82   anthelmintic efficacy (Relf et al., 2014; Tzelos et al., 2017). But no attempt has been made to

83   reconcile drenching practices and drug efficacy data. As a consequence, a knowledge gap

84   about putative risk factors and their impacts remains (Nielsen, 2012).

85   The results reported herein have been gathered as part of a large-scale survey involving 688

86   horses from 39 French horse farms and riding schools. At each location, an anthelmintic

87   efficacy test and a questionnaire interview about their practices were performed. From this

88   data, risk factors associated with reduced anthelmintic efficacy in equine strongyles have

89   been estimated across drugs. The objective of this study was to bring together anthelmintic

90   efficacy testing and risk factor analysis to provide evidence-based guidelines to the field.

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92

93   **2. Materials and methods**

94   **2.1. Farm and riding school sampling**

95   Our study aimed to evaluate drug efficacy for three drug classes and if possible, to have a

96   control group. Therefore, we aimed to build four groups of at least five horses with a minimal

97   faecal egg count (**FEC**) of 150 eggs/g as recommended by the World Association for the

98   Advancement of Veterinary Parasitology, WAAVP guidelines (Coles et al., 1992). To reach

99   this number of infected individuals, bigger stud farms, i.e. with at least 20 producing mares,

100   were pre-selected from the French Horse and Riding Institute (IFCE) database, in Normandy,

101   Loire Valley, Aquitaine and Burgundy.

102   Two additional criteria were defined to increase the chance of finding horses with sufficient

103   excretion load to undertake FEC reduction test. First, premises with less than 40 horses were

104   discarded as FEC is usually over-dispersed and focusing on fewer individuals would have

105   reduced our chance to build treatment groups. In Aquitaine however, two farms with slightly

106   smaller herd sizes (25 and 31 horses) were enrolled. Second, last anthelmintic treatment

107   should have been performed three months earlier as this corresponds to the minimal post-

108   moxidectin treatment egg reappearance period (Boersema et al., 1998) advertised on product

109   information (Tzelos et al., 2017). Flyers explaining the purpose of the project were then sent

110   to pre-selected farms before a phone call was made to each manager to make sure that their

111   premises fulfilled requested criteria (at least 40 horses not drenched in the last three months)

112   and to confirm their willingness to participate. Nineteen stud farms were enrolled, *i.e.* five in

113   Normandy, four in Loire Valley, four in Burgundy and six in Aquitaine. Approximately half

114   of these farms (n=11) were involved in horse racing (Thorougbreds and Anglo-Arabians,

115   French trotters or Thorougbreds and other than Thoroughbreds), while the remainder

116   produced leisure ponies (n=4) or leisure horses (n=3) or reared dairy mares (n=2). For each of

117   these, matching riding schools located within each stud farm area were subsequently

118   identified and enrolled for anthelmintic efficacy test, with an additional riding school enrolled

119   in Aquitaine, providing a set of 20 riding schools. This set of matched riding schools was

120   used to investigate putative differences between stud farms and riding schools.

121

122   **2.2. Horse sampling and anthelmintic resistance tests**

123   A first round of faecal sampling was made one week before drenching, to select for

124   individuals with a minimum excretion level of 150 eggs per g (epg) as recommended by the

125   WAAVP guidelines (Coles et al., 1992). Faecal material was stored at 4°C before being

126   processed for faecal egg counting within 24h. Based on individual FEC measured, three

127   treatment groups, *i.e.* fenbendazole (Panacur Equine Guard®, Intervet, France), pyrantel

128   (Strongid®, Zoetis, France), or ivermectin (Eqvalan pâte, Merial, France), balanced for FEC

129   were built. To do so, individuals were sorted according to their FEC and sequentially

130   allocated to each treatment group. A control group was built in every farm where additional

131   horses with minimal excretion level were available. On day 0, each horse was weighed using

132   a girth tape and orally administered an anthelmintic dose following manufacturer's

133   requirements. Treatment was administered by the veterinarians enrolled as part of this

134   project.

135   Faecal material was subsequently taken from each horse 14 days after treatment. Every

136   ivermectin-treated individual still present on the premise 30 days after drenching (n=157 out

137   of 159) was sampled again to identify cases of shortened egg-reappearance period. This short

138   time interval was chosen to minimise disturbance with activities on the premises (horse sales

139   or movements).

140

141   **2.3. Processing of faecal material**

142   FEC were measured by sampling 5g of faecal material for each individual horse,

143   subsequently diluted and thoroughly mixed into 75 mL of a NaCl solution (density of 1.18).

144   Prepared solution was loaded on a McMaster slide and strongyle eggs were counted with a

145   sensitivity of 15 epg.

146

147   **2.4. Questionnaire survey and variable definition**

148   A questionnaire, built upon previous published surveys (Fritzen et al., 2010; Maddox et al.,

149   2012), was used to interview each manager as part of a larger survey on antibiotic and

150   anthelmintic resistance. The anthelmintic-associated questions fell into four categories that

151   addressed global premise overview, available pasture and management, horse health

152   management, and drenching strategy.

153   For statistical inference, a few variable levels were redefined to avoid redundancy and to

154   provide the analysis with more statistical power. Therefore, one farm that did not apply

155   systematic drenching upon horse arrival was considered as not drenching any horse upon

156   arrival. Rotation between pastures was recoded as occurring either never, or more (frequent)

157   or less (rare) than every 3 months.

158   In addition, pasture strategies either involved own private pastures dedicated to horses or

159   alternative strategies that included co- grazing with cattle, or access to pastures shared

160   between several breeders. Stocking density was also computed as the herd size divided by

161   available pasture surface, and binned into three categories (more than 5 horses/ha, between 2

162   to 5 horses/ha or less than 1 horse/ha). A three-level workload variable was defined as the

163   number of horses per worker, falling in either less than 10 horses/employee, between 10 and

164   20 horses/employee or more than 20 horses/employee.

165   Two types of managers were defined; those who tried to manage health problems themselves

166   before calling their practitioner and those who called as soon as possible.

167   Anthelmintic provider was considered as a two-level factor contrasting cases where

168   veterinarians delivered the drug or not. The reasons guiding drenching programs were the

169   same across farms, *i.e.* driven by horse well-being and growth, and were not included further

170   in the risk factor analysis. In the end a set of 21 variables was used (supplementary table 1).

171

172   **2.5. Statistical analyses**

173   **2.5.1. Egg Reduction Rate (FECR) and bootstrapping procedure**

174   Sample size to estimate reduced FECR prevalence was determined using EpiTools web-

175   server (Humphry et al., 2004).

176   When a control group was available, FECR values was computed at farm level by averaging

177   treatment group FEC following (Dash et al., 1988):

178    $ERR = 1 - \left(\frac{\text{mean FEC control, before treatment}}{\text{mean FEC control, after treatment}} \times \frac{\text{mean FEC treated, after treatment}}{\text{mean FEC treated, before treatment}} \right) \times 100,$

179   hereafter referred to as **Method 1**.

180   Otherwise, FECR has been computed following (Coles et al., 1992):

181    $ERR = \left(1 - \frac{\text{mean FEC treated, after treatment}}{\text{mean FEC treated, before treatment}} \right) \times 100,$

182   hereafter denoted **Method 2**.

183   Drug class FECR confidence intervals at the farm level were not estimated as too few

184   individuals were available within each treatment group preventing robust inference of

185   estimate variability (Chernick, 1999). However, for both methods, associated 95% percentile

186   confidence intervals was determined for the region, premise type and drug class and their

187   respective intersections following a block bootstrap approach. This approach takes into

188   account the correlation among observations from the same individual (before and after

189   treatment). For both FECR computation methods, blocks of FECs from the same horse were

190   sampled with replacement from the observed data collected before and after treatment (within

191   region, premise type and drug class), and were used to compute an FECR estimate using

192   equation (1) or (2) accordingly. In that case of method 1, the time-matched control group is

193   used to account for variation in FEC between the two sampling time-points independent of

194   treatment. Therefore, blocks of individual FEC before and after treatment were sampled with

195   replacement from horses belonging to the treated or the control group within a farm.

196   In both cases, computation was performed 10,000 times to yield the empirical distribution of

197   the FECR from which 2.5 and 97.5% percentiles were sampled to derive the 95% confidence

198   interval.

199   Pearson's correlations between FECR of the two methods were estimated using observations

200   from the 24 premises where a control group was available using the *rcorr()* function of the

201   Hmisc package v4.0-3 (Harrell and Dupont, 2017).

202

203   **2.5.2. Variable selection procedure**

204   To overcome model convergence issues, variables showing too little or no variation across

205   sites (control serology or coproscopy upon horse arrival, faeces removal, access to pasture,

206   horse weight estimation, veterinarian specialization, number of veterinary practices

207   considered for diagnostic) were discarded. Additionally, the total number of veterinary drugs

208   found on-site or the frequency of health register updates were not considered further as

209   determinants of anthelmintic efficacy.

210   The aim of this study was to quantify risk factors associated with reduced anthelmintic

211   efficacy, hence associating measured drug efficacy with management practices. This requires

212   the selection of variables that would maximize the total variance explained, while avoiding

213   biased estimates through collinearity (Zuur et al., 2010).

214   Pair-wise Pearson's correlations between variables were estimated to identify collinearity and

215   avoid redundancy in subsequent modelling of anthelmintic efficacy (supplementary data 2).

216   Any correlation coefficient equal to or above 0.4 was considered as indicative of collinearity.

217 As a result, veterinary advice, health management and the off-licence use of anthelmintics

218 that were accounted for by other variables, were not considered any further.

219

2.5.3. Dimensionality reduction by Multiple Correspondance Analysis (MCA)

221 While the discarding of strongly correlated variables addressed the most problematic

222 collinear relationships, significant correlations were still present between the 18 remaining

223 variables (supplementary data 2).

224 Remaining variables were related to the use of anthelmintics, the use of pasture, or the

225 general constraints applying to the premise (work load measured by the number of horses per

226 employee, and annual veterinary expenses per horse). For each of these three categories, a

227 multiple correspondence analysis was performed on the dedicated set of variables with the

228 FactoMineR v.136 package (Lê et al., 2008). Premise coordinates on the first two

229 components of the MCA were subsequently used to define three-level (premise constraints

230 variable) or four-level variables (anthelmintics and pasture usage).

231

2.5.4. Marginal modelling of drug efficacy

233 A marginal modelling approach of individual horse egg count reduction rate (FECR) was

234 applied as outlined elsewhere (Walker et al., 2014) and implemented in R (R Core Team,

235 2016) with the geepack package v1.2-1 (Højsgaard S., 2006). In that framework, individual

236 egg counts measured at a given time, before or after treatment, are assumed to be Poisson

237 distributed and thus modelled with a log-linear regression model.

238 This model includes environmental variables (premise type, region, and the three variables

239 built from the MCA) interacting with a binary variable coding for the treatment, *i.e.* taking

240 value of 1 after treatment or 0 before treatment. This variable accounts for the treatment-

241 mediated change in egg count reduction, hence estimating FECRs, while the fitted

242   interactions estimate the contribution of considered environmental conditions to FECRs

243   (Crellen et al., 2016). Exponentiated estimates therefore provide the relative risk of increased

244   (relative risk above one) / decreased (relative risk smaller than one) FECRs associated with a

245   given environmental variable. Any variable whose relative risk confidence interval does not

246   include one is declared as significantly impacting on the FECRs. A forward-backward

247   procedure was implemented with the *stepAIC( )* function of the R MASS package v7.3-47 to

248   select for the model minimizing the Akaike Information Criterion.

249   The model aimed to quantify universal risk factors, *i.e.* considered environmental effects

250   across drug class. Treatment group was thus added into the model. Drug class-specific

251   analyses based on individual treatment group data taken separately did not provide reliable

252   results and were not reported.

253 3. Results

254 3.1. Observations from questionnaire surveys

255 Detailed answers from questionnaire surveys are provided as supplementary data 1.

256

257 3.1.1. Overview of the enrolled premises

258 At least three treatment groups could be built in every location. However, herd size was

259 highly variable between sites, ranging from 21 to 250 individuals (mean herd size of 70

260 horses). This variation was mostly driven by the herd being bigger in stud farms than in

261 riding schools (average herd size of 88.3 and 52.2 respectively; p=0.008). Horses were

262 generally housed in individual boxes (n=31/39) and a few premises had an outdoor-only

263 breeding system (n=7/39). Noticeably, staff size did not strongly correlate with herd size

264 (r=0.33, p=0.04), especially in stud farms where workers were in charge of 13 horses more

265 than in riding schools ($p<10^{-4}$).

266

267 3.1.2. Horse movements on site

268 Horse movement occurred in half of the premises (n=21/39) at least once a month while

269 seven premises were rarely housing horses from other places. For these introductions, no

270 serology, no coproscopy and no anthelmintic efficacy tests were performed in any of the 39

271 premises, whereas anthelmintic drenching upon arrival was implemented in 11 riding schools

272 and seven stud farms. Only four managers reported seeking advice from their veterinarians to

273 manage these movements.

274

275 3.1.3. Pasture availability and management

276 In every site, horses had outdoor access and could generally grazed throughout the year

277 (n=27/39). Most premises had their own pastures that were grazed by horses only. Three

278   farms had access to pastures shared with other breeders, and mixed grazing of horses with

279   cattle was implemented in seven premises. Stocking density was below 3 horses/ha in 75% of

280   sites. However, it was as high as 14 horses/ha and was generally higher in riding schools than

281   in stud farms (average densities of 1.3 and 4.3 horses/ha respectively, p=2x10^{-4}). Rotation

282   between pastures was implemented in 29 locations at least once a year and driven by grass

283   growth. Faeces removal was implemented in one premise. Manure spreading was performed

284   in one third (n=10/39) of the surveyed sites.

285

286   ### 3.1.4. Health management and interactions with veterinarians

287   About two-thirds of the premises relied on specialized equine practitioners (n=24/39), who

288   were often called after managers had already attempted to manage health problem themselves

289   (n=28/39). Half of the premises (n=20/39) were consulting several practices to cross-validate

290   advice, or benefit from several skills, or both.

291   Yearly veterinary expenses per horse varied from less than 100 € (n=15), between 100 and

292   200 € (n=14), or more than 200 € (n=10).

293   Importantly, managers reporting to be more independent in health management were over-

294   represented in sites not implementing any measure upon horse introduction (13/39) and in the

295   sites spending less than 100€/horse/year (r=-0.39 and -0.41 respectively; supplementary table

296   1). Health management was hence confounded by the two other variables and not considered

297   further.

298   Mandatory on-site health register was variably used, *i.e.* 20 managers fulfilled it regularly

299   (systematically or on a regular basis), while 19 rarely did it (never or doing it from time to

300   time). The number of veterinary drugs found within on-site pharmacy greatly varied from

301   null to 15, with two-thirds of premises having 5 drugs or less with a slight trend of more

302   medications found in horse riding schools (Kruskal-Wallis test, p=0.07).

303

3.1.5. Drenching strategy for intestinal nematodes

304

305 Anthelmintic dosing was usually based on a visual weight estimate (n=27/39) that could be

306 combined with girth tape (n=9/39), and rarely with a scale (n=2/39). Grouped-based

307 drenching was carried out in 11 sites. Time of drenching was registered most of the time

308 (n=31). Drenching frequency occurred two (n=13), three (n=11) or four times (n=14) a year,

309 and drenching programs alternated between drug classes.

310 Noticeably, a limited fraction of premises (n=6) reported off-license use of anthelmintics

311 despite most of the managers seeking advice from their veterinarians (5 out 6). These

312 involved ivermectin (n=3), doramectin (n=2) or praziquantel (n=2) licensed for ruminants.

313 The off-licence use of anthelmintics was only reported in premises implementing two (n=5)

314 or three (n=1) annual treatments, resulting in a negative correlation between these two

315 variables (r=-0.41, supplementary data 2). The off-licence variable was hence not considered

316 further in the modelling analysis.

317 Anthelmintics were bought from veterinarians in 62% of cases, while three and 16 managers

318 reported buying from the internet or their pharmacist, respectively. In the latter case, only

319 three managers were aware of the legal requirement of showing a veterinarian's prescription

320 to the pharmacist.

321 The delivery of anthelmintics by veterinarians occurred more frequently when they

322 contributed to the drenching scheme design, *i.e.* 79 % of cases against 40% when they did not

323 (r=0.4, supplementary table 2). In addition, stud farm managers relied more on their

324 veterinarian's advice for drenching in comparison to their riding school counterparts (84% of

325 stud farms vs. 67 % of riding schools; r=0.45, supplementary table 2). Premises that did not

326 rely on veterinary advice also did not implement any measure upon horse arrival (r=0.47,

327 p=0.003; supplementary table 2). Veterinary advice was hence accounted for three other

328 variables, and was not considered further in the analysis.

329 FEC-based drenching regimen was implemented in 14 premises.

330 **3.2. Results of anthelmintic efficacy tests**

331 This design provided enough resolution to detect prevalence as low as 1%, with precision of

332 0.05 and assuming FEC sensitivity of 70% and specificity of 90%.

333 A total of 688 horses from 39 premises were sampled at least once during this experiment.

334 Out of these, 601 horses excreting more than 150 epg before treatment were enrolled for the

335 anthelmintic resistance test (Table 1). Control groups were available in 24 out of the 39

336 retained farms (Table 1). Average FEC before treatment was 912 ± 762 epg (supplementary

337 data 3).

338 Estimated Faecal Egg Count Reduction (FECR) and associated variation have been reported

339 in Table 1 while premise-level estimated FECR have been attached as supplementary data 4.

340 FECRs measured in the 24 sites where control group was available showed highly consistent

341 results between the two implemented calculation methods for ivermectin and fenbendazole

342 (Pearson's correlation coefficient of 100 and 82%, respectively). This correlation however

343 dropped to 65% for pyrantel.

344 Estimated FECRs demonstrated the almost generalized inefficacy of fenbendazole with an

345 average FECR of 46.2% (sd=33.5%) or 42.8% (sd = 33.4%) for method 1 and 2, respectively

346 and confidence intervals not including 100% efficacy in Burgundy and Aquitaine (Table 1,

347 supplementary data 4). Nevertheless, two riding schools and one stud farm located in

348 Normandy exhibited FECRs of at least 90% (supplementary data 4).

349 Observed trends for ivermectin were the exact opposite of these, as the mean estimated

350 FECRs were 98.1% (sd: 8.6%) and 96.3% (sd: 14.5%) according to methods 1 and 2,

351 respectively (Table 1, supplementary data 4). Seven horses from three riding schools and

352   three stud farms exhibited FECRs lower than 90% after ivermectin treatment, resulting in

353   bigger confidence intervals in Aquitaine and Normandy (Table 1). Egg reappearance was

354   investigated 30 days after ivermectin drenching, *i.e.* based on FEC from 157 available horses.

355   At this time, only nine horses excreted strongyle eggs (mean FEC of 14.6 epg). These were

356   found in eight farms (three from Burgundy, four from Aquitaine and one from Loire Valley)

357   and one riding school from Aquitaine. Only three of these horses had egg excretion levels

358   above 50% of their before treatment FEC.

359   Pyrantel exhibited an intermediate profile in comparison to the two other drugs as average

360   FECRs were close to the 90% threshold, *i.e.* 92.5% (sd: 15.4%) and 90.3% (sd: 19.6%) for

361   methods 1 and 2, respectively (Table 1).

362

363   **3.3. Risk factors associated with anthelmintic efficacy**

364   The variation in FEC before and after treatment was explicitly modelled by considering every

365   individual FEC and by correcting for the farm environmental variables. This is to better

366   capture the inter-individual variations associated with FEC while estimating the relative risk

367   associated with environmental variables. Relative risks associated with reduced FECR were

368   estimated across drug categories, any relative risk above 1 indicating an increased egg count

369   after treatment and thus reduced efficacy. Drug-specific risk factors were subsequently

370   estimated considering observations from each treatment group independently.

371

372   **3.3.1. Multivariate analysis and summary variables**

373   An MCA was applied to the set of variables related to the use of pasture and anthelmintics

374   (figure 1) or the constraints applying to the premise (figure 2) to avoid fitting collinear

375   variables in the drug efficacy model.

376   The MCA applied to the variables related to pasture usage accounted for 42% of the between-

377   variable variance (figure 1A) and distinguished between four contrasted typologies. The first

378   typology regrouped premises where grazing options were limited, *i.e.* mostly indoor housing

379   and seasonal grazing with rare rotation between pastures. While typologies 1 and 3 were

380   mostly driven by the housing system, the split between the other two systems was accounted

381   for by the pasture surface availability (figure 1A). The 2nd pasture usage typology clustered

382   together premises with the lowest stocking density, that were also able to perform manure

383   spreading and frequent rotation between pastures. These four typologies were used to build a

384   pasture usage variable for subsequent modelling.

385   The same approach resolved 31% of the variation in anthelmintics usage between premises

386   (figure 1B). In this case, typologies 2 and 4 distinguished between premises with the most

387   extreme behaviors regarding drug use. Premises falling under typology 2 applying strategies

388   usually regarded as the most sustainable for drug resistance and typology 1 represented an

389   intermediate situation between typologies 2 and 4. Typology 3 grouped together premises

390   relying on a single annual macrocyclic lactone treatment. These four typologies were used to

391   build an anthelmintic usage variable for subsequent modelling.

392   The two remaining variables to be considered addressed the annual veterinary expenses and

393   the workload in premises (figure 2). These two variable were summarized as a so-called

394   "constraint" variable that distinguished between three situations. Typology 2 described the

395   most heavily constrained premises with highest workload and veterinary expenses (figure 2),

396   whereas typology1 and 3 accounted for the smallest workload and most reduced veterinary

397   expenses respectively (figure 2). Typology 1, defined by more limited workload and

398   intermediate veterinary expenses, mostly encompassed riding schools. However, he

399   correlation between the premise type and the three constraint levels was not significant

400   ($r=0.26$, $p=0.1$, supplementary table 5).

401   No residual significant correlations existed between variables considered for modeling

402   (supplementary table 5).

403

404   ### 3.3.2. Risk-factors across anthelmintic drug class

405   Modeling of drug efficacy relied on a set of six variables, *i.e.* day of treatment, treatment

406   group, region and the three summary variables derived from MCA.

407   A first analysis investigated universal factors associated with drug efficacy, measured by

408   FECR, that would be true across anthelmintic drugs and would not depend on the drug mode

409   of action. Relative risks associated with the retained variables have been provided in table 2.

410   Noticeably, the interaction term between the day of treatment and premise type or the region

411   of interest or premise constraints were not retained by the model selection procedure,

412   suggesting these variables were not providing information to the modelling of drug efficacy

413   that had not been already accounted for by other variables.

414   In line with estimated FECRs, pyrantel and ivermectin were less at risk of reduced FECRs

415   than fenbendazole considered as the reference level (Table 2).

416   Premises falling under the second typology of pasture usage (figure 1A) demonstrated a

417   significant reduction of drug resistance risk (OR=0.53, p=0.001) in comparisons to other

418   modalities (Table 2). This typology equally matched riding schools (n=6) or stud farms (n=5)

419   but was over-represented in Normandy (6/11). This certainly explains why the site location

420   was not retained in the model selection procedure.

421   The second typology of anthelmintic usage (figure 1B), that regroup strategies usually

422   thought of as more sustainable toward drug resistance, was significantly associated with a

423   reduced risk of drug resistance (OR=0.57, p=0.02). No significant differences could be made

424   between other typologies (Table 1).

425

4. Discussion

Current knowledge about anthelmintic resistance in equine strongyles is usually scattered across drug efficacy reports and questionnaire surveys about parasite management (Nielsen, 2012). This leaves a major knowledge gap in the critical assessment of factors underpinning anthelmintic resistance in equids. Our study aims to fill in this gap with the report of an association between anthelmintic FECRs and management practices in horses.

The drug efficacy landscape in the present study remained similar to what was reported in a previous study in France (Traversa et al., 2012) and what was described in other countries (Matthews, 2014). In summary, fenbendazole cannot be used for the management of small strongyles any more, in contrast to ivermectin whose efficacy remained above 95%. Pyrantel had an intermediate efficacy FECR pattern with a 90% reduction of egg-excreting animals.

However, two original findings tend to depart from this general pattern. First, the risk of fenbendazole resistance was significantly lowered in Normandy with a few premises (3/39) still harbouring fenbendazole-susceptible strongyle populations. This was in line with a previous study (1/18) conducted in France (Traversa et al., 2012). However, it was not possible to identify an obvious consistent factor that would explain this sustained fenbendazole efficacy. In-depth investigation of practices and analysis of parasitic community structure with a nemabiome approach (Avramenko et al., 2015) may help better understanding this feature and then confirming fenbendazole-susceptibility by interrogating beta-tubulin sequences and allelic frequencies (Lake et al., 2009). Second, our results showed that ivermectin efficacy may not be sustained at its current level in the near future as egg excretion already took place 30 days after treatment in a limited number of horses across all regions. In addition, larger FECR confidence intervals were encountered in Normandy and Aquitaine, suggestive of a higher variability in ivermectin efficacy. Original ERP was 9 weeks for ivermectin (Boersema et al., 1996) but indications of shortened ERP have been

451   found in Germany (von Samson-Himmelstjerna et al., 2007), the UK (Daniels and Proudman,

452   2016), Belgium, the Netherlands and Italy (Geurden et al., 2014) and had never been reported

453   in France. In this study, the quantitative determination of ERP was not possible due to

454   practical reasons. Instead we focused on the 30th day post ivermectin treatment to minimize

455   interferences of our design with activities on premises and to ensure that most of the treated

456   horses would still be available for sampling. Despite this, a few horses had already been sold

457   or sent to other premises for training.

458   Beyond the crude estimation of drug efficacy, this study aimed to identify major determinants

459   underpinning egg reduction rate, and to estimate their respective relative contributions to

460   provide evidence-based recommendations in the field.

461   Pasture-related variables were significant contributors to the variation in drug efficacy

462   measured by egg reduction rate. Noticeably, sites with typology 2, that had the lowest

463   stocking density, were able to implement frequent pasture rotation and to perform manure

464   spreading, had a significantly decreased risk of drug resistance. Stocking density has been

465   advocated as a factor driving drug resistance even if it could not be associated with more

466   elevated infection rate in a German epidemiological study (Fritzen et al., 2010). On the

467   contrary, frequent pasture rotation is one of the evasion strategy recommended to minimize

468   pasture contamination (Michel, 1985). Therefore, it is probable that the combination of both a

469   reduced horse density with frequent rotation between pastures minimized the use of drugs in

470   these sites. The effect of manure spreading in the context of drug resistance remains

471   uncertain. It could simply correlate with the available grazing surface and mirror the stocking

472   density but this correlation was not significant in this dataset (r=-0.28, p=0.09).

473   Interestingly, drug resistance was significantly reduced in sites implementing a combination

474   of FEC-based drenching programs, determination of drug dosage on an individual basis and a

475   high level of biosecurity, *i.e.* little horse movement combined with a qurarantine and

476   drenching upon horse introduction. Evidence-based drenching and individual drug dosage

477   have long been advocated for in ruminant and equine systems as a sustainable parasite

478   management practices, as the former is thought to reduce selection pressure (Kenyon et al.,

479   2009) and the latter is thought to prevent under dosing whose impact on drug resistance

480   development relies on many parameters (Smith et al., 1999; Silvestre et al., 2001). Our

481   findings thus provided evidence to promote their enforcement in the field.

482   Notably, the more sustainable anthelmintic usage typology (typology 2) also accounted for

483   the delivery of anthelmintic treatment by veterinarians. Under French regulations, veterinary

484   medications can be delivered by veterinarians or pharmacists upon display of a veterinary

485   prescription (Anonymous, 2007). This regulation was generally applied across the considered

486   study sites and well correlated with the involvement of veterinarians into the drenching

487   scheme design. Recent study in the UK suggests that practitioners may provide useful advice

488   on drug use (Easton et al., 2016) and thus reinforce their role in sustainable parasite control.

489   Such partnerships between veterinarians and horse owners should thus be encouraged in

490   France as 40% of the considered sites designed their own drenching scheme.

491   These first insights into determinants of drug efficacy only focused on environmental factors,

492   putting aside intrinsic worm characteristics, like species composition, that should be

493   investigated further. Recent advances in parasite metagenomics would help addressing this

494   question.

495

496 **Conclusions**

497   This study reports the first risk estimation analysis between management practices and drug

498   efficacy in equine strongyles. While drug resistance prevalence remains in agreement with

499   previous surveys from France and other countries, *i.e.* a generalized failure of fenbendazole, a

500   decreasing efficacy of pyrantel and reasonably high efficacy of ivermectin despite evidence

501   of reduced egg reappearance period. Most importantly, we have quantified the relative risks

502   and benefits associated with equine farms management practices. These estimations provided

503   support to advocate for FEC-based treatment regimens as well as individual determination of

504   anthelmintic dosage. In addition, tight biosecurity enforced by reduced horse movements and

505   a combinaison of anthelmintic treatment with quarantine upon horse introduction should be

506   recommended. Anthelmintics delivery by veterinarians was also among beneficial factors

507   relative to drug resistance. Also, sites with frequent pasture rotations and stocking rate below

508   five horses/ha displayed a reduced risk of drug resistance.

509

510 **Figure 1. Multiple correspondence analysis of the variables related to pasture (A) and**

511 **anthelmintics (B) uses**

512 The first two components of the analysis are plotted and distinguish between four different

513 typologies annotated in black (e.g. pasture.use1). Environmental variables are represented by

514 red triangles while dots represent corresponding premises, colored according to the typology

515 they belong to. Ellipses represent the 95% credible interval associated with each typology.

516

517 **Figure 2. Multiple correspondence analysis of the variables related to structural**

518 **constraints applying to premises**

519 The first two components of the analysis are plotted and distinguish between three different

520 typologies annotated in black (e.g. Constraint.1). Levels corresponding to the considered

521 workload and veterinary expenses variables are represented by red triangles while dots

522 represent corresponding premises, colored according to the typology they belong to. Ellipses

523 represent the 95% credible interval associated with each typology.

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529 **Table 1. Average Egg Reduction Rate estimated across premise type and regions**

Region		Ivermectin			Pyrantel			Fenbendazole		
		RS*	SF*	Across region	RS	SF	Across region	RS	SF	Across region
Aquitaine	N	26	27	53	29	26	55	29	28	57
3 RS (14)	mean	100	99,9	99,9	75,3	86,7	81	18,8	63	40,9
3 SF (15)	sd	0	0,1	0,1	39,5	16,4	27,8	32,5	41,1	41,1
	CI	[100-100]	[91.8-100]	[97.6-100]	[39.4-100]	[41.3-100]	[0-100]	[0-87]	[0-98.9]	[0-98.3]
Burgundy	N	15	17	32	13	19	32	16	19	35
3 RS (11)	mean	99,8	89,5	93,9	99,2	96,3	97,6	31,2	55,9	45,3
4 SF (19)	sd	0,3	21,1	15,9	1,4	4,3	3,5	27,9	26	27,8
	CI	[99.4-100]	[0-100]	[0-100]	[100-100]	[83.6-100]	[51.4-100]	[0-77.5]	[0-96.9]	[0-97.1]
Loire Valley	N	18	16	34	19	15	34	19	13	32
3 RS (14)	mean	100	100	100	97,2	93,3	95,2	32,5	68,2	50,4
3 SF (14)	sd	0	0	0	2,6	9,3	6,5	29,6	9,6	27,8
	CI	[100-100]	[100-100]	[100-100]	[85.3-100]	[50-100]	[19.6-100]	[0-78.6]	[0-100]	[0-100]
Normandy	N	18	22	40	19	23	42	21	22	43
2 RS (10)	mean	99	99,9	99,5	90,8	99,3	95,9	45,9	50,8	48,9
3 SF (15)	sd	1,4	0,2	0,9	12,3	1	7,7	64,9	46,9	46,5
	CI	[82.2-100]	[95-100]	[0-100]	[80.3-100]	[92.8-100]	[60.3-100]	[0-100]	[0-100]	[0-100]
Across	N	77	82	159	80	83	163	85	82	167
premise	mean	99,8	96,7	98,1	90,6	94,1	92,5	30,8	59,2	46,2
type	sd	0,6	11,7	8,6	20,8	9,3	15,4	32,5	29,6	33,5
	CI	[98.8-100]	[95.2-100]	[95-100]	[39.4-100]	[58.9-100]	[41.3-100]	[0-92.3]	[0-100]	[0-100]

530
531

532 **Table 1 continued**

Method 2: FECRT = 100 x (1 - FEC14/FEC0)

Region		Ivermectin RS*	SF*	Across region	Pyrantel RS	SF	Across region	Fenbendazole RS	SF	Across region
Aquitaine	mean	100	98,7	99,4	93,8	73,4	84,4	13,9	51,6	31,3
7 RS	sd	0	2,9	2	6,9	36,2	26,1	14,7	41,8	34,9
6 SF	CI	[100-100]	[70.8-100]	[79.2-100]	[0-100]	[0-100]	[0-100]	[0-79.6]	[0-98.7]	[0-98.5]
Burgundy	mean	99,6	88,1	93,8	98,9	95,6	97,3	22,3	42,5	32,4
4 RS	sd	0,5	23,8	16,8	1,5	4,8	3,7	30,2	37	33,1
4 SF	CI	[96.3-100]	[0-100]	[0-100]	[91.4-100]	[78.6-100]	[78.6-100]	[0-88.2]	[0-92.4]	[0-92.4]
Loire Valley	mean	100	79,5	89,8	96,5	74,1	85,3	36,1	60,9	48,5
4 RS	sd	0	41	29	2,4	33,8	25,2	15,2	15,5	19,4
4 SF	CI	[100-100]	[0-100]	[0-100]	[87.7-100]	[26.7-100]	[26.7-100]	[0-78.6]	[0-100]	[0-100]
Normandy	mean	99,4	99,9	99,6	97,5	95,5	96,5	83,4	39,7	61,6
5 RS	sd	1,3	0,2	0,9	3,2	8,9	6,4	18,2	36,2	35,5
5 SF	CI	[60-100]	[99.2-100]	[99.2-100]	[85.1-100]	[38.7-100]	[85.1-100]	[0-100]	[0-100]	[0-100]
Across	mean	99,8	92,7	96,3	96,3	84	90,3	37,4	48,5	42,8
premise	sd	0,7	21,1	15	4,8	26,5	19,6	33,6	33,3	33,5
type	CI	[96.3-100]	[0-100]	[79.2-100]	[52.6-100]	[38.7-100]	[39.7-100]	[0-100]	[0-100]	[0-100]

533

534 Drug-specific average Egg Reduction Rates (mean) and standard deviations (sd) measured 14 days after treatment have been collated for each

535 drug and region of interest for the two egg reduction rate calculation methods used. CI stands for Cross-sectional confidence intervals. N

536 indicates the number of horses available, while RS and SF stand for riding-school and stud farm respectively. Figures in brackets under the

537 Region column stands for the number of horses allocated to the control group in Riding Schools or Stud Farms accordingly.

538   **Table 2. Estimated odd ratios associated with retained environmental variables**

539   For each of the retained management practice, the relative risk of reduced (below 1) or

540   increased (higher than 1) risk of anthelmintic resistance is provided. Associated 95%

541   confidence intervals lower and upper limits are given as well as the associated *p* value.

542

Variable	Odd ratio	Lower Odd Ratio	Upper Odd Ratio	*p*
Ivermectin vs. Fenbendazole	0,02	0,01	0,08	$<10^{-2}$
Pyrantel vs. Fenbendazole	0,14	0,08	0,22	$<10^{-2}$
Pasture.use2 vs Pasture.use1	0,53	0,36	0,79	$<10^{-2}$
Pasture.use3 vs Pasture.use1	0,78	0,52	1,18	0,24
Pasture.use4 vs Pasture.use1	0,78	0,55	1,11	0,17
AH.use2 vs AH.use1	0,57	0,36	0,92	0,02
AH.use3 vs AH.use1	0,89	0,62	1,27	0,52
AH.use4 vs AH.use1	1,10	0,78	1,57	0,58

543

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545

546   **Supplementary Data 1. Retained variables and data distribution across premises**

547   **Supplementary Data 2. Pair-wise Pearson's correlations between variables**

548   **Supplementary Data 3. Average faecal egg count by premise type and region before**

549   **anthelmintic treatment**

550   **Supplementary Data 4. Farm-level egg reduction rates with associated confidence**

551   **intervals**

552   **Supplementary Data 5. Correlation between variables retained for the modelling of**

553   **Faecal Egg Count Reduction**

554 **Acknowledgements**

564

565

566   **References**

567   Anonymous, 2007. Code de la Santé Publique - Article R5141-112, in: Française, R. (Ed.).
568   Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S.,
569   2015. Exploring the Gastrointestinal "Nemabiome": Deep Amplicon Sequencing to Quantify
570   the Species Composition of Parasitic Nematode Communities. PLoS One 10, e0143559.
571   Boersema, J.H., Eysker, M., Maas, J., vanderAar, W.M., 1996. Comparison of the
572   reappearance of strongyle eggs in foals, yearlings, and adult horses after treatment with
573   ivermectin or pyrantel. Vet Quart 18, 7-9.
574   Boersema, J.H., Eysker, M., van der Aar, W.M., 1998. The reappearance of strongyle eggs in
575   the faeces of horses after treatment with moxidectin. Vet Q 20, 15-17.
576   Canever, R.J., Braga, P.R., Boeckh, A., Grycajuck, M., Bier, D., Molento, M.B., 2013. Lack of
577   Cyathostomin sp. reduction after anthelmintic treatment in horses in Brazil. Vet Parasitol
578   194, 35-39.
579   Chernick, M.R., 1999. Bootstrap Methods: A practitioner's guide.
580   Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992.
581   World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for
582   the detection of anthelmintic resistance in nematodes of veterinary importance. Vet
583   Parasitol 44, 35-44.
584   Corbett, C.J., Love, S., Moore, A., Burden, F.A., Matthews, J.B., Denwood, M.J., 2014. The
585   effectiveness of faecal removal methods of pasture management to control the
586   cyathostomin burden of donkeys. Parasit Vectors 7, 48.
587   Corning, S., 2009. Equine cyathostomins: a review of biology, clinical significance and
588   therapy. Parasit Vectors 2 Suppl 2, S1.
589   Crellen, T., Walker, M., Lamberton, P.H., Kabatereine, N.B., Tukahebwa, E.M., Cotton, J.A.,
590   Webster, J.P., 2016. Reduced Efficacy of Praziquantel Against Schistosoma mansoni Is
591   Associated With Multiple Rounds of Mass Drug Administration. Clin Infect Dis 63, 1151-
592   1159.
593   Daniels, S.P., Proudman, C.J., 2016. Shortened egg reappearance after ivermectin or
594   moxidectin use in horses in the UK. Vet J 218, 36-39.
595   Dash, K.M., Hall, E., Barger, I.A., 1988. The role of arithmetic and geometric mean worm egg
596   counts in faecal egg count reduction tests and in monitoring strategic drenching programs in
597   sheep. Aust Vet J 65, 66-68.
598   Day, T., Read, A.F., 2016. Does High-Dose Antimicrobial Chemotherapy Prevent the
599   Evolution of Resistance? PLoS Comput Biol 12, e1004689.
600   Easton, S., Pinchbeck, G.L., Tzelos, T., Bartley, D.J., Hotchkiss, E., Hodgkinson, J.E., Matthews,
601   J.B., 2016. Investigating interactions between UK horse owners and prescribers of
602   anthelmintics. Prev Vet Med 135, 17-27.
603   Fritzen, B., Rohn, K., Schnieder, T., von Samson-Himmelstjerna, G., 2010. Endoparasite
604   control management on horse farms--lessons from worm prevalence and questionnaire
605   data. Equine Vet J 42, 79-83.
606   Geurden, T., van Doorn, D., Claerebout, E., Kooyman, F., De Keersmaecker, S., Vercruysse, J.,
607   Besognet, B., Vanimisetti, B., di Regalbono, A.F., Beraldo, P., Di Cesare, A., Traversa, D.,
608   2014. Decreased strongyle egg re-appearance period after treatment with ivermectin and
609   moxidectin in horses in Belgium, Italy and The Netherlands. Vet Parasitol 204, 291-296.
610   Harrell, F.E., Dupont, C., 2017. Hmisc: Harrell Miscellaneous.

611   Hinney, B., Wirtherle, N.C., Kyule, M., Miethe, N., Zessin, K.H., Clausen, P.H., 2011a.
612   Prevalence of helminths in horses in the state of Brandenburg, Germany. Parasitol Res 108,
613   1083-1091.
614   Hinney, B., Wirtherle, N.C., Kyule, M., Miethe, N., Zessin, K.H., Clausen, P.H., 2011b. A
615   questionnaire survey on helminth control on horse farms in Brandenburg, Germany and the
616   assessment of risks caused by different kinds of management. Parasitol Res 109, 1625-1635.
617   Højsgaard S., H., U., Yan, J., 2006. The R Package geepack for Generalized Estimating
618   Equations. Journal of Statistical Software 15, 1-11.
619   Humphry, R.W., Cameron, A., Gunn, G.J., 2004. A practical approach to calculate sample size
620   for herd prevalence surveys. Prev Vet Med 65, 173-188.
621   Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: Global worming and
622   anthelmintic resistance. Veterinary Parasitology 186, 70-78.
623   Kennedy, D.A., Read, A.F., 2017. Why does drug resistance readily evolve but vaccine
624   resistance does not? Proc Biol Sci 284.
625   Kenyon, F., Greer, A.W., Coles, G.C., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B.,
626   Varady, M., Van Wyk, J.A., Thomas, E., Vercruysse, J., Jackson, F., 2009. The role of targeted
627   selective treatments in the development of refugia-based approaches to the control of
628   gastrointestinal nematodes of small ruminants. Vet Parasitol 164, 3-11.
629   Kotze, A.C., Hunt, P.W., Skuce, P., von Samson-Himmelstjerna, G., Martin, R.J., Sager, H.,
630   Krucken, J., Hodgkinson, J., Lespine, A., Jex, A.R., Gilleard, J.S., Beech, R.N., Wolstenholme,
631   A.J., Demeler, J., Robertson, A.P., Charvet, C.L., Neveu, C., Kaminsky, R., Rufener, L.,
632   Alberich, M., Menez, C., Prichard, R.K., 2014. Recent advances in candidate-gene and whole-
633   genome approaches to the discovery of anthelmintic resistance markers and the description
634   of drug/receptor interactions. Int J Parasitol Drugs Drug Resist 4, 164-184.
635   Lake, S.L., Matthews, J.B., Kaplan, R.M., Hodgkinson, J.E., 2009. Determination of genomic
636   DNA sequences for beta-tubulin isotype 1 from multiple species of cyathostomin and
637   detection of resistance alleles in third-stage larvae from horses with naturally acquired
638   infections. Parasites & Vectors 2.
639   Lê, S., Josse, J., Husson, F., 2008. FactoMineR: A Package for Multivariate Analysis. Journal of
640   Statistical Software 25, 1-18.
641   Lendal, S., Larsen, M.M., Bjorn, H., Craven, J., Chriel, M., Olsen, S.N., 1998. A questionnaire
642   survey on nematode control practices on horse farms in Denmark and the existence of risk
643   factors for the development of anthelmintic resistance. Vet Parasitol 78, 49-63.
644   Lichtenfels, J.R., Kharchenko, V.A., Dvojnos, G.M., 2008. Illustrated identification keys to
645   strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Vet
646   Parasitol 156, 4-161.
647   Lind, E.O., Rautalinko, E., Uggla, A., Waller, P.J., Morrison, D.A., Hoglund, J., 2007. Parasite
648   control practices on Swedish horse farms. Acta Vet Scand 49, 25.
649   Love, S., Murphy, D., Mellor, D., 1999. Pathogenicity of cyathostome infection. Vet Parasitol
650   85, 113-121; discussion 121-112, 215-125.
651   Lyons, E.T., Tolliver, S.C., Ionita, M., Collins, S.S., 2008. Evaluation of parasiticidal activity of
652   fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate in horse foals with emphasis
653   on ascarids (Parascaris equorum) in field studies on five farms in Central Kentucky in 2007.
654   Parasitol Res 103, 287-291.
655   Maddox, T.W., Pinchbeck, G.L., Clegg, P.D., Wedley, A.L., Dawson, S., Williams, N.J., 2012.
656   Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 2: Risk factors for
657   faecal carriage of antimicrobial-resistant Escherichia coli in horses. Equine Vet J 44, 297-303.

658   Martinez-Valladares, M., Famularo, M.R., Fernandez-Pato, N., Cordero-Perez, C., Castanon-
659   Ordonez, L., Rojo-Vazquez, F.A., 2012. Characterization of a multidrug resistant Teladorsagia
660   circumcincta isolate from Spain. Parasitol Res 110, 2083-2087.
661   Matthews, J.B., 2014. Anthelmintic resistance in equine nematodes. Int J Parasitol Drugs
662   Drug Resist 4, 310-315.
663   Michel, J.F., 1985. Epidemiology and Control of Gastrointestinal Helminths in Domestic
664   Animals, in: Bossche, H.V., Thienpont, D., Janssens, P.G. (Eds.), Chemotherapy of
665   Gastrointestinal Helminths. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 67-123.
666   Molento, M.B., Antunes, J., Bentes, R.N., Coles, G.C., 2008. Anthelmintic resistant
667   nematodes in Brazilian horses. Vet Rec 162, 384-385.
668   Murphy, D., Love, S., 1997. The pathogenic effects of experimental cyathostome infections
669   in ponies. Vet Parasitol 70, 99-110.
670   Nielsen, M.K., 2012. Sustainable equine parasite control: perspectives and research needs.
671   Vet Parasitol 185, 32-44.
672   Nielsen, M.K., Jacobsen, S., Olsen, S.N., Bousquet, E., Pihl, T., 2016. Nonstrangulating
673   intestinal infarction associated with Strongylus vulgaris in referred Danish equine cases.
674   Equine Vet J 48, 376-379.
675   Nielsen, M.K., Vidyashankar, A.N., Olsen, S.N., Monrad, J., Thamsborg, S.M., 2012.
676   Strongylus vulgaris associated with usage of selective therapy on Danish horse farms-is it
677   reemerging? Vet Parasitol 189, 260-266.
678   O'Meara, B., Mulcahy, G., 2002. A survey of helminth control practices in equine
679   establishments in Ireland. Vet Parasitol 109, 101-110.
680   Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic resistance in
681   important parasites of horses: does it really matter? Vet Parasitol 201, 1-8.
682   R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation
683   for Statistical Computing, Vienna.
684   Relf, V.E., Lester, H.E., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2014. Anthelmintic
685   efficacy on UK Thoroughbred stud farms. Int J Parasitol 44, 507-514.
686   Relf, V.E., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2012. A questionnaire study on
687   parasite control practices on UK breeding Thoroughbred studs. Equine Vet J 44, 466-471.
688   Sallé, G., Cabaret, J., 2015a. Meta-analysis of cyathostomins community structure and
689   diversity, International Conference of the World Association for the Advancement of
690   Veterinary Parasitology, Liverpool, The United-Kingdom, p. 220.
691   Sallé, G., Cabaret, J., 2015b. A survey on parasite management by equine veterinarians
692   highlights the need for a regulation change. Vet Rec Open 2, e000104.
693   Silvestre, A., Cabaret, J., Humbert, J.F., 2001. Effect of benzimidazole under-dosing on the
694   resistant allele frequency in Teladorsagia circumcincta (Nematoda). Parasitology 123, 103-
695   111.
696   Slocombe, J.O., de Gannes, R.V., 2006. Cyathostomes in horses in Canada resistant to
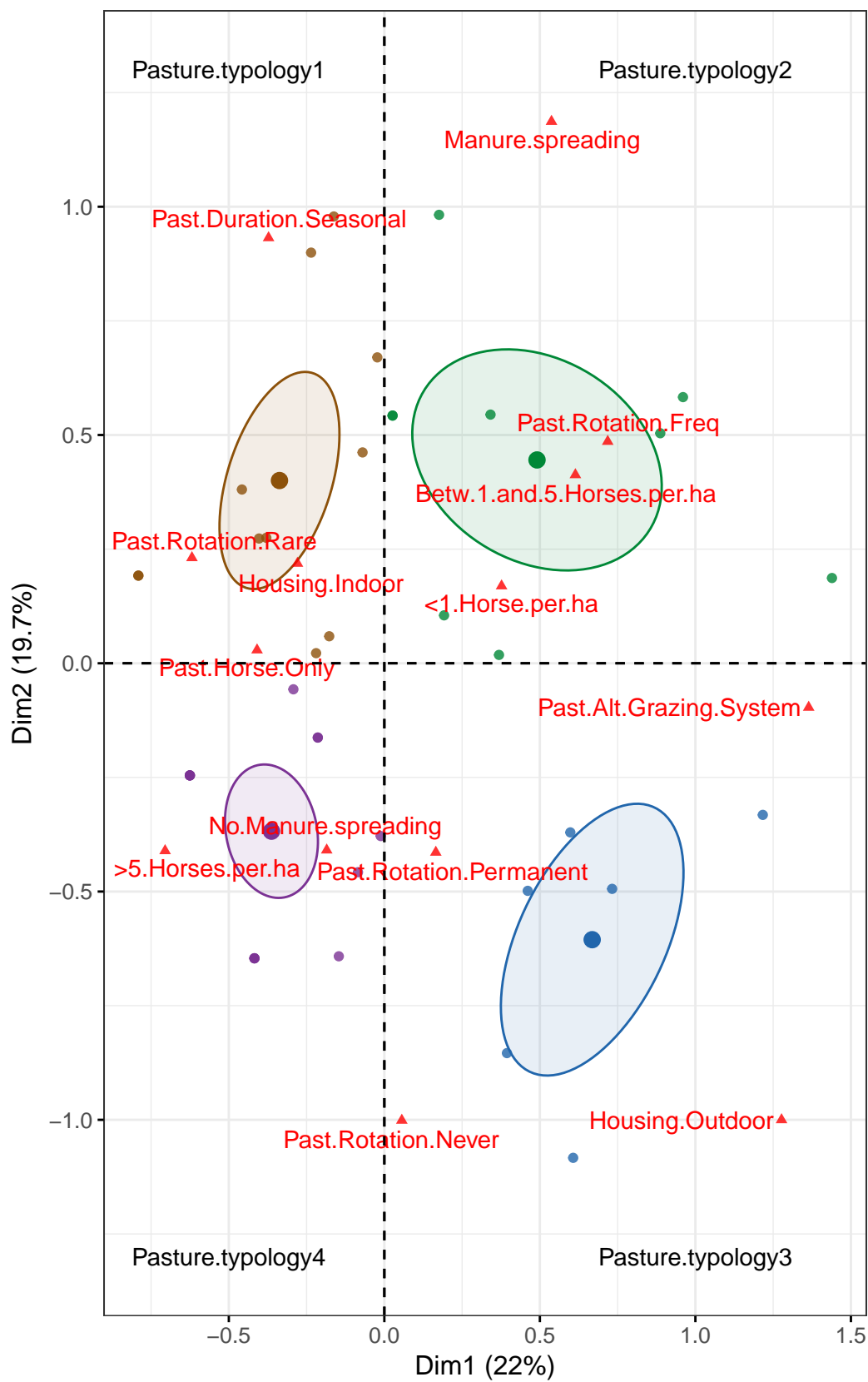697   pyrantel salts and effectively removed by moxidectin. Vet Parasitol 140, 181-184.
698   Smith, G., Grenfell, B.T., Isham, V., Cornell, S., 1999. Anthelmintic resistance revisited:
699   under-dosing, chemoprophylactic strategies, and mating probabilities. Int J Parasitol 29, 77-
700   91; discussion 93-74.
701   Traversa, D., Castagna, G., von Samson-Himmelstjerna, G., Meloni, S., Bartolini, R., Geurden,
702   T., Pearce, M.C., Woringer, E., Besognet, B., Milillo, P., D'Espois, M., 2012. Efficacy of major
703   anthelmintics against horse cyathostomins in France. Vet Parasitol 188, 294-300.

704 Traversa, D., Klei, T.R., Iorio, R., Paoletti, B., Lia, R.P., Otranto, D., Sparagano, O.A.,
705 Giangaspero, A., 2007. Occurrence of anthelmintic resistant equine cyathostome
706 populations in central and southern Italy. Prev Vet Med 82, 314-320.
707 Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Milillo, P., Schurmann, S., Barnes,
708 H., Otranto, D., Perrucci, S., di Regalbono, A.F., Beraldo, P., Boeckh, A., Cobb, R., 2009.
709 Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United
710 Kingdom and Germany. Parasit Vectors 2 Suppl 2, S2.
711 Tzelos, T., Barbeito, J.S., Nielsen, M.K., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2017.
712 Strongyle egg reappearance period after moxidectin treatment and its relationship with
713 management factors in UK equine populations. Vet Parasitol 237, 70-76.
714 von Samson-Himmelstjerna, G., Fritzen, B., Demeler, J., Schurmann, S., Rohn, K., Schnieder,
715 T., Epe, C., 2007. Cases of reduced cyathostomin egg-reappearance period and failure of
716 Parascaris equorum egg count reduction following ivermectin treatment as well as survey
717 on pyrantel efficacy on German horse farms. Vet Parasitol 144, 74-80.
718 Walker, M., Churcher, T.S., Basanez, M.G., 2014. Models for measuring anthelmintic drug
719 efficacy for parasitologists. Trends Parasitol 30, 528-537.
720 Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common
721 statistical problems. Methods Ecol Evol 1, 3-14.
722

A

Pasture.typology1

Pasture.typology2

Manure.spreading

Past.Duration.Seasonal

Past.Rotation.Freq

Betw.1.and.5.Horses.per.ha

Past.Rotation.Rare

Housing.Indoor

<1.Horse.per.ha

Past.Horse.Only

Past.Alt.Grazing.System

No.Manure.spreading

>5.Horses.per.ha

Past.Rotation.Permanent

Housing.Outdoor

Past.Rotation.Never

Pasture.typology4

Pasture.typology3

Dim1 (22%)

Dim2 (19.7%)

B

AH.typology1

AH.typology2

Mvts.Rare

3.per.year

Drenching.Not.Registered

Qua.Only

Drench.Only

Qua.Drench

Provider.Vet

IndividualDose

FEC–based

AH.Alternation

Mvts.Monthly

4.per.year

NoFEC

Drenching.Registered

GroupDose

AH.SingleDrug

1.per.year

Provider.Other

Mvts.Sometimes

Nothing.Upon.Arrival

AH.typology4

AH.typology3

Dim1 (16.5%)

Dim2 (14.6%)