Ivermectin treatment in lactating mares results in suboptimal ivermectin exposure in their suckling foals

Ghismon-De-Kasin Mayinda, Delphine Serreau, Amandine Gesbert, Fabrice Reigner, Jean-François Sutra, Anne Lespine, Guillaume Salle

Ivermectin treatment in lactating mares results in suboptimal ivermectin exposure in their suckling foals

Ghismon-de-Kasin Mayinda[1], Delphine Serreau[1], Amandine Gesbert[2], Fabrice Reigner[2], Jean-François Sutra[3], Anne Lespine[3], Guillaume Sallé[1]

[1], INRAE, Université de Tours, UMR1282 Infectiologie et Santé Publique, F-37380 Nouzilly

[2], INRAE, UE1297 Physiologie Animale de l'Orfrasière, F-37380 Nouzilly

[3], INRAE, Ecole Nationale Vétérinaire de Toulouse, UMR1436 INTHERES, F-31076 Toulouse

Corresponding author:

Guillaume Sallé

Guillaume.Salle@inrae.fr

INRAE Centre Val de Loire

37380 Nouzilly

France

Keywords: ivermectin, horse, strongyle, nematode, foal

Abstract

The management of equine strongyles has become problematic over the last decade because of an increased prevalence of drug-resistant isolates worldwide. Therapeutic options are therefore limited, leaving macrocyclic lactones as the most often effective drug class. However, their lipophilic properties result in a long-lasting elimination that could favour drug resistance selection. As a result, ivermectin treatment in lactating mares could promote suboptimal exposure of their foal parasites to ivermectin, thereby selecting for more resistant worms. To test for this putative transfer, we selected two groups of six foal-mare pairs, one group of mares receiving ivermectin and the other being left untreated. We compared faecal egg count trajectories in foals from the two groups and quantified plasma ivermectin concentrations in ivermectin treated mares and their foals during seven days. Our results showed limited but sustained plasmatic exposure of foals associated with non-significant faecal egg count reduction ($P = 0.69$). This suggests that ivermectin treatment in lactating mares results in suboptimal exposure to the drug in their foal.

Introduction

Equine gastro-intestinal nematodes emerge as a major threat to equine health. Cyathostominae and *Parascaris* spp. are the most problematic species as they account for most deaths in young horses while tapeworms and *Strongylus* spp. seem currently under control (Sallé et al., 2020). Cyathostominae are responsible for clinical disease occurring when encysted larval stages emerge at once (Love et al., 1999). This so-called “larval cyathostominosis” is associated with colic, protein-losing enteropathy and eventually death of the animals (Giles et al., 1985; Sallé et al., 2020). *Parascaris* spp. mostly infect foals under the age of one year and recent observations suggested that foal infection follows a bivariate pattern with worm counts peaking at five- and nine-month of age under treatment-free conditions (Fabiani et al., 2016). Complex migration of larval stages from the intestine, through the liver and lungs can result in coughing and nasal discharge (Taylor et al., 2007). Heavy infection by adult stages is associated with foal emaciation, catarrhal enteritis and it can lead to intestinal impaction or rupture (Cribb et al., 2006; Sallé et al., 2020).

Control of these parasite species by anthelmintic drugs has become problematic over the last decades because of widespread emergence of drug-resistant isolates. Fenbendazole has been found inefficient against small strongyles in most countries (Hodgkinson et al., 2008; Lyons et al., 2008; Kyvsgaard et al., 2011; Traversa et al., 2012; Kumar et al., 2016) while suboptimal ivermectin efficacy has been frequently reported for *Parascaris* sp. (Lindgren et al., 2008; Lyons et al., 2008; Näreaho et al., 2011; Laugier et al., 2012; Morris et al., 2019; Studzińska et al., 2020). Expected efficacies of ivermectin and moxidectin are usually observed against small strongyles although failures have been reported in Brazil and western Europe (Peregrine et al., 2014)..

To date, risk factors favouring the selection of drug-resistant parasite populations are not fully identified. Two recent surveys in the USA (Nielsen et al., 2018) or in France (Sallé et al., 2017)

showed limited convergence in their conclusions regarding the impact of parasite management strategies, but identified pasture rotation as a beneficial strategy to limit drug resistance development.

Ivermectin and other macrocyclic lactones are slowly eliminated, thereby exposing incoming parasite infective larvae to reduced concentrations through time (Le Jambre et al., 1999). These suboptimal drug concentrations promote the selection of resistant worms while eliminating susceptible individuals, in a so-called “tail-selection” process (Le Jambre et al., 1999). Because of their lipophilic properties (Geary, 2005), macrocyclic lactones are found in the milk of lactating individuals including horses (Gokbulut et al., 2016). As a result, suckling offsprings may be significantly exposed to the drug as quantified in cattle (Alvinerie et al., 1996). In sheep, two independent experiments demonstrated that treatment of ewes with moxidectin could impact on the parasite population in their lambs, either reducing by 70% the establishment of susceptible *Teladorsagia circumcincta* (Leathwick et al., 2015) or inducing a 51% cut in *Haemonchus contortus* excretion (Dever and Kahn, 2015). There is hence an opportunity that ivermectin treatment given to lactating mares as part of calendar treatments (Sallé et al., 2017) may affect their foal parasite communities. To the best of our knowledge, ivermectin transfer from mares to suckling foals has never been characterized.

Here, we tested the possible passage of ivermectin from the mare milk to their foals by comparing faecal egg count and plasma drug concentrations in foals whose mare were either treated or not in late summer. Our results showed non-significant faecal egg count reduction in combination with limited but sustained plasmatic exposure of foals..

Materials and methods

Experimental design

To investigate the impact of ivermectin transfer between treated mares and their foal, we used fourteen Welsh pony mares and their foals. Pregnant Welsh pony mares were allocated to two 7 ha pastures in April 2019. Pasture allocation was balanced for the stallion they were mated with, and for the foreseen date of foaling. This latter parameter was considered to obtain similar foal parasite exposure across pastures. Foaling took place from May 25 to June 23 2019. Detailed metadata are provided in supplementary Table 1.

Faecal egg count (FEC) was performed every month (from early June to late August) in the seven mare-foal pairs (supplementary Table 1) to quantify strongyle egg excretion. Foals were not sampled before they reached 14 days of age. For each pair, animals were placed in a paddock to collect fresh faecal material off the ground. In some cases, foals did not expel faecal matter directly; they were hence separated from their mother for 10 min to stimulate defecation.At the end of August (August 30, 2019), six mares with faecal egg count above 150 eggs/g (Coles et al., 2006) were administered ivermectin orally (Eraquell, Virbac, France, 200 µg/kg bodyweight) to determine whether ivermectin treatment in the mare could affect parasite egg excretion in foals. The other 8 mares and their foals remained untreated and were used as a control group. FEC was measured 18 days later (September 17, 2019) to determine treatment efficacy in both mares and foals.

FEC measure

FEC were measured from 5 g of faecal material diluted in 70 mL of a saturated NaCl solution (specific gravity = 1.18). Filtrate was loaded on a McMaster slide and eggs were counted using light microscopy at 150X magnification (detection limit of 15 eggs/g).

Ivermectin dosage and analysis

Plasmatic ivermectin concentration was measured to i) ensure that plasmatic ivermectin exposure in mares was in line with observed FECR, ii) support ivermectin transfer to the milk and iii) evaluate plasmatic exposure in foals. For this reason, we restricted the study to the first seven days. Although ivermectin residence time in adult horses expands beyond seven days (Pérez et al., 2003), restricting our study to the first seven days was sufficient to address our main objectives while reducing animal use. For ivermectin dosage, milk (4 mL silica-coated tubes, BD Vacutainer®, Plymouth, UK) and blood (4 mL heparin-coated tubes, BD Vacutainer®, Plymouth, UK) samples were taken from six mares at 1 h, 2 h, 8 h, 24 h, 48 h, 4 days and 7 days after treatment. To measure putative ivermectin transfer in their foals (n = 6), blood samples were collected from the foals at the same times from 8 h onwards. Blood samples were centrifuged at 1500 *g* and 4 °C for 10 min to harvest plasma. Both milk and plasma samples were cryopreserved at -80 °C until ivermectin concentration was subsequently measured.

Ivermectin concentrations were determined by High-Performance Liquid Chromatography with fluorescence detection according to previously validated methods in plasma (Alvinerie et al., 1999) and in milk (Alvinerie et al., 1993). Data were analyzed by a non-compartmental approach using the KineticaTm software (version 4.2, innaPhase, Philadelphia, USA). Apparent and maximal concentration (C_{max}) and time of C_{max} (T_{max}) were then determined. The partial area under the plasma concentration time curve (*AUC*) and the mean residence time (*MRT*) were

calculated from 0 to 7 days by the linear trapezoidal rule. Data are expressed as mean ± standard deviation (SD) of 6 animals for mares and foals respectively.

Statistical analysis

Statistical analysis on FEC data was implemented in R v4.0.2 (R Core Team, 2016).

Treatment efficacy was assessed by FEC reduction in the treated group corrected for FEC trajectory in the control group (Presidente, 1985) : $100 \times (1 - \frac{FEC14\ treated}{FEC0\ treated}\ x\ \frac{FEC0\ control}{FEC14\ control})$, where FEC_{14} and FEC_0 correspond to group average FEC measured before and after treament respectively. This formula was applied to measure ivermectin efficacy in mares and in foals whose mares had been treated. Efficacy in foals was estimated from the only foals with patent strongyle infection (n = 5 in the mare treated group and 6 in the control group). Efficacy below 0% were censored to 0. Confidence intervals (95% CI) were estimated using the R-version of the eggCounts package v2.3 (Wang et al., 2018).

In addition, to determine whether FEC reduction was significantly different between experimental groups, we modeled FEC data with a marginal modeling approach using the geeM package v.0.10.1 (McDaniel et al., 2013). The implemented model was: $FEC_{ij} \sim \beta_0 + \beta_1 \text{ x } day_{ij} + \beta_2 \text{ x } group_{ij} + \beta_3 \text{ x } day_{ij} \text{ x } group_{ij}$, where FEC_{ij} stands for the j^{th} FEC record in individual *i*, with an auto-regressive correlation structure between FEC_{ij} and FEC_{ij+1}, β_0 is the intercept, β_1 to β_3 are the regression coefficients describing the covariate effects (day, treatment group and their interaction). Because of the over-dispersed nature of FEC, a negative-binomial function was used as a link function between the linear combinations of covariates and observed FECSignificance of the interaction term (*P-value* < 0.05) would indicate egg count reduction between groups.

Data and R script used for this study are under the following repository: https://github.com/guiSalle/Ivermectin-in-lactating-ponies.

Results

FEC trajectory over the grazing season in mares and foals

Summary statistics for FEC measured in mares and foals are shown in Table 1 and detailed trajectories are available in supplementary Table 1 and supplementary Fig.Fig. 1. Mares showed increased excretion of strongyle eggs from June to mid-August, reaching 464 eggs/g (range between 0 and 2520 eggs/g) on average at that time. In foals, strongyle egg excretion peaked at the end of August (227 eggs/g on average, ranging from 0 to 570 eggs/g). Of note, one foal remained FEC negative throughout the study period.

Ivermectin treatment in mares did not significantly affect foal strongyle egg excretion

FEC reduction was measured between foals whose mare received ivermectin treatment or not over an 18-day period. Fig.Following suppressive ivermectin treatment in six mares (Table 1), reduced egg excretion was found in four foals (Fig.Table 1), although FEC increased in one foal from 30 to 45 egg/g (one foal remained FEC negative). Average FEC reduction in the foals whose mares were treated was 67% (95% CI 6.2 - 85%). However, foals from the control group also demonstrated 55% reduction of their FEC (95% CI 10.4 - 90.8%). FEC reduction accounting for the control group FEC trajectory was hence 0%. In agreement with this result, the marginal modeling approach found no evidence of statistical difference in FEC reduction between both groups (reduction ratio of 1.33 between foals whose mares received ivermectin and the control group; 95% CI 0.31 - 5.64, $P = 0.69$).

Ivermectin accumulates in mare milk but not in foal plasma

To control that mare exposure to ivermectin was achieved and to explore exposure in their respective foal, plasmatic ivermectin concentration was measured in mare-foal pairs as represented on Fig.Fig. 1 and supplementary Fig. 2. Maximal ivermectin concentrations in mares ranged between 18.69 and 43.33 ng/mL (Fig. 1, Table 2) and occurred 8 h after treatment (Fig.Fig. 1).

Milk ivermectin concentration followed a similar pharmacokinetic pattern than that found in plasma (Pearson's correlation $r = 0.61$, $P < 0.001$) and also peaked at 8 h post-treatment in most animals (Fig. 1). In two mares, this was slightly delayed at 24 h or 48 h post-treatment (Fig.Fig. 1). It was slightly lower than plasma concentrations, with AUC_{milk} to AUC_{plasma} ratios ranging between 0.27 and 1.1 (mean 0.57 ± 0.27).

On the contrary, only limited concentrations of ivermectin were measured in foal plasma (Fig. 1) with maximal concentrations ranging between 0.06 and 0.66 ng/mL (Table 2). These values showed weak correlations with their mother plasmatic (Spearman's $\rho = 0.03$, $P = 0.85$) or milk concentrations (Spearman's $\rho = 0.06$, $P = 0.85$). The total exposure of foal (AUC) represented around 3% of the mare total drug exposure, and 7 % of that found in milk (Table 2).

Discussion

Calendar drenching schemes in horses often include an ivermectin treatment given in late summer. Because of its lipophilic properties, ivermectin can be excreted into mare milk (Gokbulut et al., 2016). This leaves the opportunity for a suboptimal ivermectin exposure in foals. However, the transfer from mare milk to foal plasma was significant but low and under the minimal active concentration. In line with this, no FEC reduction occurred in foals whose mares were administered ivermectin relative to that with an untreated mother.

The pharmacokinetic data of ivermectin in mare plasma were in good agreement with previous studies conducted in horses that received ivermectin orally (Gokbulut et al., 2016; Pérez et al., 2003). In addition, significant ivermectin excretion in pony mare milk was found with AUC_{milk}/AUC_{plasma} ratio of 0.57. This is consistent with ivermectin lipophilic properties (Prichard et al., 2012). This remains however in the common range of values (AUC_{milk}/AUC_{plasma} between 0.7 and 1.0) reported for cows (Toutain et al., 1988) but lower than in ewes where AUC_{milk}/AUC_{plasma} can be as high as 2.5 (Imperiale et al., 2004). The lower ratio observed for our pony population when compared to other species is consistent with the lower lipid content found in equid milk, *e.g.* 7.4 g/L in ponies (Pagan and Hintz, 1986) and between 5 to 20 g/L in horses (Malacarne et al., 2002) that contrasts with cow or ewe milk that exhibit fat content in the range of 37 g/L (Malacarne et al., 2002) and 71 g/L respectively (Wohlt et al., 1984). However, the AUC_{milk}/AUC_{plasma} values calculated in our study were much higher than the 0.19 value previously reported in horses (Gokbulut et al., 2016). Although unclear, the basis for such difference may come from different factors including variation in milk lipid content between animals. Variation in the expression level of the efflux transporter ABCG2 that is located in mammary glands during lactation and plays an important role in the milk partitioning of drugs (Jonker et al., 2005) including endectocides (Jani et al., 2011) may also contribute to this discrepancy.

Ivermectin was detected in foal plasma throughout the seven-day trial. This level matched the elevated ivermectin content found in milk of ivermectin-treated mares. To our knowledge, this is the first attempt to quantify ivermectin transfer from mare to their foal. However, ivermectin concentrations estimated in foal plasma were low, with C_{max} found below the minimal active concentration of 1 ng/mL (Geary 1983). This is compatible with the lack of FEC reduction in the group of foals whose mothers had received ivermectin. It is yet to be determined whether this residual concentration results in a suboptimal exposure of parasite populations or is of no biological relevance. Underdosing has been indeed advocated as a major factor favoring the emergence of drug resistant isolates (Le Jambre et al., 1999). More importantly, it should also favor the development of resistance in *Parascaris* spp. populations whose seasonal prevalence peak (Fabiani et al., 2016; Sallé et al., 2020) would match autumn treatment in the mare. Collection of larval populations to monitor their sensitivity to ivermectin using *in vitro* test could validate their putative increased resistance level.

The low ivermectin plasmatic concentration measured in the foal was however contrasting with the 0.57 AUC_{milk}/AUC_{plasma} transfer measured in the mare. This may be underpinned by the high fractional turnover rates of body water measured in foals as a result of a low energy to water content ratio (Oftedal et al., 1983). This could contribute to dilute ivermectin in the foal plasmatic compartment. In contrast, mean residence time in foal plasma expanded over two weeks. The close vicinity of measured concentration with the detection limit of our method certainly contributes to inflate mean residence time. It may also reflect a slow elimination process resulting from an immature drug detoxification system in the young animal as demonstrated in humans (Brouwer et al., 2015). This increased half-life of the drug would promote parasite exposure to suboptimal drug concentrations and potentially aggravate drug resistance development.

Conclusion

Our data suggests that ivermectin treatment in lactating mares leads to limited transfer into their suckling foals. This had no effect on strongyle egg excretion in their progenies. The extent of parasite exposure to ivermectin remains to be determined.

Acknowledgements

GdK Mayinda benefited from a Région Normandie (PAMIEBO project) stipend and G. Sallé is a grateful recipient of an Institut Français du Cheval et de l'Équitation grant (CYATHOMIX project).

Animal welfare statement

Experimental procedures were carried out following the guidelines of the European Council Directive (2010/63/UE) and with French laws and regulations (Articles R214-87 to R214-137 of the Rural Code and decree n°2013-118 dated February 1, 2013 published on February 7, 2013). Our project was approved by French Ministry of Higher Education and Research and the Val de Loire ethics committee under license #20513-2019043009343644 v5.

References

Alvinerie, M., Sutra, J.F., Galtier, P., 1993. Ivermectin in goat plasma and milk after subcutaneous injection. Vet. Res. 24, 417–421.

Alvinerie, M., Sutra, J.F., Galtier, P., Lifschitz, A., Virkel, G., Sallovitz, J., Lanusse, C., 1999. Persistence of ivermectin in plasma and faeces following administration of a sustained-release bolus to cattle. Res. Vet. Sci. 66, 57–61.

Alvinerie, M., Sutra, J.F., Lanusse, C., Galtier, P., 1996. Plasma profile study of moxidectin in a cow and its suckling calf. Vet. Res. 27, 545–549.

Brouwer, K.L.R., Aleksunes, L.M., Brandys, B., Giacoia, G.P., Knipp, G., Lukacova, V., Meibohm, B., Nigam, S.K., Rieder, M., de Wildt, S.N., Pediatric Transporter Working Group, 2015. Human Ontogeny of Drug Transporters: Review and Recommendations of the Pediatric Transporter Working Group. Clin. Pharmacol. Ther. 98, 266–287.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 136, 167–85.

Cribb, N., Coté, N., Bouré, L., Peregrine, A., 2006. Acute small intestinal obstruction associated with *Parascaris equorum* infection in young horses: 25 cases (1985–2004). N. Z. Vet. J. 54, 338–343.

Dever, M.L., Kahn, L.P., 2015. Decline in faecal worm egg counts in lambs suckling ewes treated with lipophilic anthelmintics: Implications for hastening development of anthelmintic resistance. Vet. Parasitol. 209, 229–234.

Fabiani, J.V., Lyons, E.T., Nielsen, M.K., 2016. Dynamics of Parascaris and Strongylus spp. parasites in untreated juvenile horses. Vet Parasitol 230, 62–66. https://doi.org/10.1016/j.vetpar.2016.11.002T., 2005. Ivermectin 20 years on: maturation of a wonder drug. Trends Parasitol. 21, 530–532.

Giles, C.J., Urquhart, K.A., Longstaffe, J.A., 1985. Larval cyathostomiasis (immature trichonema-induced enteropathy): a report of 15 clinical cases. Equine Vet J 17, 196–201.

Gokbulut, C., Ozuicli, M., Aksit, D., Aksoz, E., Korkut, O., Yalcinkaya, M., Cirak, V.Y., 2016. Comparative plasma and milk dispositions, faecal excretion and efficacy of per os ivermectin and pour-on eprinomectin in horses. J. Vet. Pharmacol. Ther. 39, 584–591.

Hodgkinson, J.E., Clark, H.J., Kaplan, R.M., Lake, S.L., Matthews, J.B., 2008. The role of polymorphisms at beta tubulin isotype 1 codons 167 and 200 in benzimidazole resistance in cyathostomins. Int J Parasitol 38, 1149–60.

Imperiale, F.A., Busetti, M.R., Suárez, V.H., Lanusse, C.E., 2004. Milk excretion of ivermectin and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and ripening period. J. Agric. Food Chem. 52, 6205–6211.

Jani, M., Makai, I., Kis, E., Szabó, P., Nagy, T., Krajcsi, P., Lespine, A., 2011. Ivermectin interacts with human ABCG2. J. Pharm. Sci. 100, 94–97.

Jonker, J.W., Merino, G., Musters, S., van Herwaarden, A.E., Bolscher, E., Wagenaar, E., Mesman, E., Dale, T.C., Schinkel, A.H., 2005. The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. Nat. Med. 11, 127–129.

Kumar, Sunil, Garg, R., Kumar, Saroj, Banerjee, P.S., Ram, H., Prasad, A., 2016. Benzimidazole resistance in equine cyathostomins in India. Vet. Parasitol. 218, 93–97.

Kyvsgaard, N.C., Lindbom, J., Andreasen, L.L., Luna-Olivares, L.A., Nielsen, M.K., Monrad, J., 2011. Prevalence of strongyles and efficacy of fenbendazole and ivermectin in working horses in El Sauce, Nicaragua. Vet Parasitol 181, 248–254.

306 Laugier, C., Sevin, C., Menard, S., Maillard, K., 2012. Prevalence of Parascaris equorum
307 infection in foals on French stud farms and first report of ivermectin-resistant P. equorum
308 populations in France. Vet Parasitol 188, 185–189.
309 Le Jambre, L.F., J. Dobson, R., Lenane, I.J., Barnes, E.H., 1999. Selection for anthelmintic
310 resistance by macrocyclic lactones in Haemonchus contortus. Int. J. Parasitol. 29, 1101–
311 1111.
312 Leathwick, D.M., Miller, C.M., Fraser, K., 2015. Selection for anthelmintic resistant Teladorsagia
313 circumcincta in pre-weaned lambs by treating their dams with long-acting
314 moxidectin injection. Int. J. Parasitol. Drugs Drug Resist. 5, 209–214.
315 Lindgren, K., Ljungvall, Ö., Nilsson, O., Ljungström, B.-L., Lindahl, C., Höglund, J., 2008.
316 Parascaris equorum in foals and in their environment on a Swedish stud farm, with notes
317 on treatment failure of ivermectin. Vet. Parasitol. 151, 337–343.
318 Love, S., Murphy, D., Mellor, D., 1999. Pathogenicity of cyathostome infection. Vet Parasitol 85,
319 113–122.
320 Lyons, E.T., Tolliver, S.C., Ionita, M., Collins, S.S., 2008. Evaluation of parasiticidal activity of
321 fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate in horse foals with
322 emphasis on ascarids (Parascaris equorum) in field studies on five farms in Central
323 Kentucky in 2007. Parasitol Res 103, 287–291.
324 Malacarne, M., Martuzzi, F., Summer, A., Mariani, P., 2002. Protein and fat composition of
325 mare's milk: some nutritional remarks with reference to human and cow's milk. Int. Dairy
326 J. 12, 869–877.
327 McDaniel, L.S., Henderson, N.C., Rathouz, P.J., 2013. Fast pure R implementation of GEE:
328 application of the Matrix package. R J. 5, 181–187.
329 Morris, L.H., Colgan, S., Leathwick, D.M., Nielsen, M.K., 2019. Anthelmintic efficacy of single
330 active and combination products against commonly occurring parasites in foals. Vet.
331 Parasitol. 268, 46–52.
332 Näreaho, A., Vainio, K., Oksanen, A., 2011. Impaired efficacy of ivermectin against Parascaris
333 equorum, and both ivermectin and pyrantel against strongyle infections in trotter foals in
334 Finland. Vet. Parasitol. 182, 372–377.
335 Nielsen, M.K., Branan, M.A., Wiedenheft, A.M., Digianantonio, R., Scare, J.A., Bellaw, J.L.,
336 Garber, L.P., Kopral, C.A., Phillippi-Taylor, A.M., Traub-Dargatz, J.L., 2018. Anthelmintic
337 efficacy against equine strongyles in the United States. Vet Parasitol 259, 53–60.
338 Oftedal, O.T., Hintz, H.F., Schryver, H.F., 1983. Lactation in the Horse: Milk Composition and
339 Intake by Foals. J. Nutr. 113, 2096–2106.
340 Pagan, J.D., Hintz, H.F., 1986. Composition of milk from pony mares fed various levels of
341 digestible energy. Cornell Vet. 76, 139–148.
342 Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic resistance in
343 important parasites of horses: does it really matter? Vet Parasitol 201, 1–8.
344 Pérez, R., Godoy, C., Palma, C., Cabezas, I., Munoz, L., Rubilar, L., Arboix, M., Alvinerie, M.,
345 2003. Plasma Profiles of Ivermectin in Horses following Oral or Intramuscular
346 Administration. J. Vet. Med. Ser. A 50, 297–302.
347 Presidente, P.J., 1985. Methods for detection of resistance to anthelmintics, in: Resistance in
348 Nematodes to Anthelmintic Drugs. CSIRO Division of Animal Health, Glebe, NSW,
349 Australia, pp. 13–28.
350 Prichard, R., Ménez, C., Lespine, A., 2012. Moxidectin and the avermectins: Consanguinity but
351 not identity. Int. J. Parasitol. Drugs Drug Resist. 2, 134–153.
352 R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation
353 for Statistical Computing, Vienna.
354 Sallé, G., Cortet, J., Bois, I., Dubes, C., Guyot-Sionest, Q., Larrieu, C., Landrin, V., Majorel, G.,
355 Wittreck, S., Woringer, E., Courouce, A., Guillot, J., Jacquiet, P., Guegnard, F.,

Blanchard, A., Leblond, A., 2017. Risk factor analysis of equine strongyle resistance to anthelmintics. Int J Parasitol Drugs Drug Resist 7, 407–415.

Sallé, G., Guillot, J., Tapprest, J., Foucher, N., Sevin, C., Laugier, C., 2020. Compilation of 29 years of postmortem examinations identifies major shifts in equine parasite prevalence from 2000 onwards. Int. J. Parasitol. 2, 125-132

Studzińska, M.B., Sallé, G., Roczeń-Karczmarz, M., Szczepaniak, K., Demkowska-Kutrzepa, M., Tomczuk, K., 2020. A survey of ivermectin resistance in Parascaris species infected foals in south-eastern Poland. Acta Vet. Scand. 62, 28:1-5.

Taylor, M.A., Coop, R.L., Wall, R.L., 2007. Veterinary Parasitology, 3rd Edition. Blackwell publishing, UK.

Toutain, P.L., Campan., M., Galtier, P., Alvinerie, M., 1988. Kinetic and insecticidal properties of ivermectin residues in the milk of dairy cows. J. Vet. Pharmacol. Ther. 11, 288–291.

Traversa, D., Castagna, G., von Samson-Himmelstjerna, G., Meloni, S., Bartolini, R., Geurden, T., Pearce, M.C., Woringer, E., Besognet, B., Milillo, P., D'Espois, M., 2012. Efficacy of major anthelmintics against horse cyathostomins in France. Vet Parasitol 188, 294–300.

Wang, C., Torgerson, P.R., Kaplan, R.M., George, M.M., Furrer, R., 2018. Modelling anthelmintic resistance by extending eggCounts package to allow individual efficacy. Int. J. Parasitol. Drugs Drug Resist. 8, 386–393.

Wohlt, J.E., Foy, W.L., Kniffen, D.M., Trout, J.R., 1984. Milk yield by Dorset ewes as affected by sibling status, sex and age of lamb, and measurement. J. Dairy Sci. 67, 802–807.

Figure captions

Figure 1. Kinetics of ivermectin concentration in six treated mares and their foals

Ivermectin was administered to six mares and its plasmatic level measured over seven days. The plot represents ivermectin concentration through time (starting before treatment and ending 7 days post treatment), in mare plasma (green) and milk (light green) or in their respective foal plasma (purple). Each point represents the mean of six replicates with standard deviation shown for the upper range.

Table 1. Summary statistics of strongyle faecal egg count in foals and mare

Summary statistics (mean [minimum – maximum]) of faecal egg count measured in mares and their foals from the treated (n = 6) or control group (n = 8). They are given before or after ivermectin treatment in mares. Within-group faecal egg count reduction (FECR) is provided. FECR values were all negative in foals and censored to 0%; in mares FECR values were all 100%. As a result, no confidence interval is given.

Table 2. Pharmacokinetic parameters of ivermectin in plasma and milk estimated for mares and foals.

Parameters were estimated from six samples collected over a seven-day trial.

PK: pharmacokinetic; AUC: area under the plasma concentration time curve; MRT: mean residence time