Searching for ivermectin resistance in a Strongylidae population of horses stabled in Poland

A. Zak 1*, N. Siwinska 1, M. Slowikowska 1, H. Borowicz 1, K. Kubiak 1, J. Hildebrand 2, M. Popiolek 2 and A. Niedzwiedz 1

Abstract

Background: There are no available studies describing the possible resistance of strongyles to ivermectin in horses in Poland. One hundred seventy three horses from nine stud farms from South-Western Poland were studied. The effectiveness of ivermectin was studied on the 14th day after ivermectin administration using the fecal egg count reduction test, and a long-term observation of the egg reappearance period was carried out. The fecal study was carried out using a modified McMaster method, which typically detects 20 eggs per gram of stool. The results were subjected to statistical analysis that enabled quantification of the eggs in the stool samples.

Results: The study revealed high efficacy of ivermectin on the 14th day after administration without a shortening of the egg reappearance period.

Conclusion: The results indicate that strongyles resistance to ivermectin in Poland is not a serious problem.

Keywords: Strongylidae, Horses, Ivermectin resistance, Ivermectin effectiveness

Highlights

- Our results indicate that the efficacy of ivermectin measured on the 14th day after treatment is very high in horses stabled in Poland.
- There was no shortening of the egg reappearance period after ivermectin administration.
- Our results indicate that there is no ivermectin resistance in the strongylidae population in horses bred in South-Western Poland

Background

Strongyles are considered to be major gastrointestinal parasites in horses [1, 2]. Pasture grazed adult horses are usually infected with strongyles [1, 3, 4]. The clinical symptoms associated with a strongyle infection are often non-specific. They include a weight loss, poor quality horse hair, diarrhoea and recurring colic [1, 5]. The migration of the fourth-stage small strongyloae larvae (L4) and their encystation in the intestinal wall may lead to “larval cyathostomosis”. This, in turn, causes protein-losing enteropathy and diarrhoea [1, 6]. Anthelmintic drugs from three groups - the benzimidazoles (e.g. fenbendazole and oxibendazole), the tetrahydropyrimidine pyrantel and the macrocyclic lactones (ivermectin and moxidectin) are used in horses to treat strongyle infections. Long-term use of anthelmintic drugs may trigger parasite resistance in a given population. In the case of small strongyles (cyathostominis), benzimidazole resistance was reported in 14 countries while pyrantel resistance occurred in 12 countries [7]. Unlike benzimidazole and pyrantel, the efficacy of macrocyclic lactones is reported to be 99% [8]. However, there have been isolated cases of ivermectin resistance based on the fecal egg count reduction test (FECRT) and/or a shortened egg reappearance period (ERP) what is the first indicator of strongyle resistance to ivermectin [4, 8–15]. To date, the ivermectin resistance of strongyles in Poland has not been studied. The aim of the study was the assessment of ivermectin resistance of strongylidae in horses bred in Poland, considering routine horse deworming twice a year using ivermectin, without coproscopy.

* Correspondence: agnieszka.zak@up.wroc.pl
1Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 47, 50-366 Wroclaw, Poland
Full list of author information is available at the end of the article

© The Author(s). 2017 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Methods

Study design

The study was carried out in the first half of 2016 in nine stables in Lesser Poland, Greater Poland and Lower Silesian Voivodships. In total, 173 horses of both genders and various breeds, with a mean age of 11.5 years (range: 1.5–32) were studied. The first part of the study, which included an evaluation of the efficacy of ivermectin on the 14th day after its administration, using the FECRT, was carried out on all 173 horses. In the second part of the study, the egg reappearance period (ERP) was assessed in 42 horses, from two stables, selected based on an EPG value exceeding 20. All the horses were routinely dewormed twice a year in accordance with current standards using single-component pastes in spring and two-component pastes in autumn. In both groups, the horses were naturally infected with strongyles. The study included pasture grazing horses which were dewormed more than 8 weeks prior to the commencement of the study [16, 17]. The examination at T0 consisted of collecting fresh stool samples from the litter bedding, evaluating the number of eggs per gram of stool during the fecal examination and determining the contamination potential of the horses. Deworming was carried out using an equine single-component oral paste (Ecomectin, Eco Animal Health) containing 18.7 mg/g of ivermectin administered at a dosage of 200 μg/kg. The body weight of the horses was recorded using a “girth tape”. Deworming was carried out by a qualified veterinary surgeon. No dose errors were reported. Stool samples were collected 14 days after deworming from all the horses. In addition, stool samples were collected on a weekly basis from 42 horses selected for the ERP, until at least 10% of the amount of eggs recorded at T0 was reached. The fecal examination followed a modified McMaster centrifugation – enhanced method using a sugar-salt flotation solution with a specific gravity of 1.3 g/ml [18, 19]. The detection limit of the method was 20 EPG [18].

Statistical analysis

At T0, the contamination potential of the horses older than 3 years was determined using the 2013 AAEP Parasite Control Guidelines. In each yard, the horses were classified into groups based on the varying degree of strongyle egg shedding: low contaminators – EPG <200, moderate contaminators – EPG range: 201–500 and high contaminators - EPG >500 [4]. The chi square independence test was used to compare contamination potentials in different age and sex groups. Statistical significance was set at P < 0.05.

The primary outcome measure in this study was the analysis of the length of the ERP. The ERP was defined as the time between the treatment and the first breach-of a 90% efficacy threshold [20].

The secondary outcome measure was the percentage reduction in the arithmetic mean of the strongyle FEC on day 14 (after) relative to day 0 (before): \((FEC_{D0} - FEC_{D14})/ FEC_{D0}\) with the 95% confidence interval (CI). Anthelmintic efficacy was defined as a reduction in FEC ≥95% with the lower 95% CI ≥90% was considered as efficacious [21, 22]. Anthelmintic resistance was present if (1) the percentage reduction in FEC was ≤95% and (2) the lower 95% CI was <90%. Suspected anthelmintic resistance was present if (1) the percentage reduction in FEC was ≤95% or (2) the lower 95% CI was ≤90%.

In addition, the results of the efficacy of ivermectin on the 14th day after its administration underwent statistical analysis using a specifically designed “eggCounts” package in R (using Bayesian hierarchical models for faecal egg count data) and a web interface, (http://www.math.uzh.ch/as/index.php?id=eggCounts) enabling the elimination of the Poisson errors in the counting technique. [23]. A correction factor = 20 and zero-inflation were used in the calculations.

Results

All the animals were dosed correctly, and no abnormal health events linked to the treatment were recorded in this study. The mean EPG in the horses in this study was 131.56 (CI = 95.58–167.55) and ranged from 0 to 2300. The contamination potential of the horses older than 3 years was classified as 78.48% - low contaminators, 15.69% moderate contaminators and 5.83% high contaminators. There was no statistically significant correlation between the sex, the age and the contamination potential in the studied horses (p = 0.05). No Parascaris equorum eggs were detected in the feces.

The analysis of the efficacy of ivermectin on the 14th day after its administration indicated that it was effective in 99.9% (CI = 95%). Detailed results are presented in Table 1. The results are presented in the form of graphs in Fig. 1.

The ERP ranged from seven to 16 weeks from the time of ivermectin administration. The ERP was not shortened, and the complete results are presented in Table 2.

Discussion

The modified centrifugation – enhanced McMaster method according to Roepstoft and Nansen (1998) is considered the most accurate McMaster fecal egg counting technique in horses [19]. However, it is not possible to avoid errors associated with the choice of the fecal examination method.

Table 1: Summary statistics for each variable - generated using an “eggCounts” package in R (http://www.math.uzh.ch/as/index.php?id=eggCounts).

|         | 2.5% | 50%  | 97.5% |
|---------|------|------|-------|
| fecr    | 0.997| 0.999| 1     |
| meanEPC | 123.606 | 131.308 | 140.243 |
| meanEPC | 0.003 | 0.078 | 0.433 |
Hence, a specifically designed “eggCounts” package in R and a user friendly web interface were used to eliminate errors associated with the inaccuracy of the test method as well as the uneven and random distribution of the eggs in the stool sample [23]. That allowed an accurate interpretation of the results, which were more reliable. Our results indicate a high efficacy of ivermectin on the 14th day after its administration. Other reports confirm our findings and state an ivermectin efficacy of almost 100% on the 14th day after administration, or describe single cases of partial or total resistance [8–15]. The first indicator of strongyle resistance to ivermectin is a shortened ERP [4, 24]. It is difficult to compare the results of studies assessing the ERP, due to a lack of a uniform definition of the ERP. The EPG after treatment, which is considered a criterion determining the ERP, varies. Some investigators calculate the ERP as the first week in which eggs appear in the stool [25–28]. Other authors consider the EPG as 100 [16], 200 [29], <80% of the FECR [30] or <90% of the FECR [31–33]. In our study, ERP was considered to be the time a < 90% FECR was obtained. Unshortened ERP and a high efficacy of ivermectin on the 14th day after its administration rule out strongyle ivermectin resistance in horses bred in Poland. In order to slow down the development of resistance, it seems necessary to introduce a selective therapy in horses that have an egg per gram count exceeding 500, proposed by the 2013 AAEP Parasite Control Guidelines, or the deworming of horses that either have a FEC exceeding 200 EPG or that shed Parascaris equorum eggs, which is used in Denmark [20]. This study was prompted by reports of incorrect use of horse deworming programmes in Poland. Although this data is not available in literature, reports of incorrect deworming were received from several horse breeders and owners. The use of off label ivermectin in horses orally, intended for intravenous or intramuscular use in pigs and ruminants, may cause ivermectin-resistance. There are no reports of the pharmacokinetic and pharmacodynamic properties of intravenous or intramuscular ivermectin administered orally. Ivermectin was most probably used off label for economical reasons. Currently, great emphasis is placed on deworming procedures in Poland. Veterinary professionals and horse owners are encouraged to use selective deworming schemes in order to limit future ivermectin resistance.

**Conclusions**

We found no strongyle ivermectin-resistance in horses bred in Poland based on the high efficacy of ivermectin on the 14th day after its administration and no shortening of the ERP. Despite these results, it is necessary to undertake measures that will limit future strongyle resistance, such as the use of correct deworming methods and selective deworming.

**Table 2** Results of ERP study – normal range for ivermectin is 42–56 days

| Farm | number of horses | mean EPG | EPG range   | ERP (days) – number of horses |
|------|------------------|----------|-------------|-----------------------------|
|      |                  |          |             | D49 | D56 | D63 | D70 | D77 | D84 | D91 | D98 | D105 | >D105 |
| 1.   | 14               | 350      | 20–1760     | 0   | 1   | 1   | 1   | 3   | 2   | 0   | 1   | 3   | 3     |
| 2.   | 28               | 92.14    | 20–900      | 1   | 5   | 8   | 3   | 6   | 1   | 1   | 1   | 0   | 2     |
Abbreviations

EPG: Egg Per Gram; ERP: Egg Reappearance Period; FEC: Fecal Egg Counts; FECR: Fecal Egg Count Reduction; FECRT: Fecal Egg Count Reduction Test

Acknowledgements

This research was supported by Calier Polska, Eco Animal Health. The results of this study has been presented in preliminary form at the oral report session at 15th Congress of Polish Society of Veterinary Sciences, Lublin, Poland, 22-24 September 2016.

Funding

This research did not receive any specific grant from funding agencies in the public or not-for-profit sectors. This research was supported by Calier Polska, Eco Animal Health by donating deworming past for research.

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Authors’ contributions

AZ participated in the design of the study, in the fecal egg count test and was a major contributor in writing the manuscript. NS, MS, HB participated in the design of the study, in the collection of the samples, in the fecal egg count test and in analysis and interpretation of data. JH and MP participated in the design of the study and performed the statistical analysis: AN and KK conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors were involved in continuous discussion regarding manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

In accordance with the Experiments on Animals Act from January 15th 2015 (Journal of Laws of the Republic of Poland, 2015, item. 266), concerning the welfare of the animals used for research or teaching purposes, the provisions shall not apply to: 1. veterinary services as defined by the Act from December 21st 2001 as amended in item 3), as well as agricultural activity, raising and breeding livestock according to the Animal Welfare Act, not designed to carry out medical procedures; 2. clinical veterinary studies carried out according to Article 37a-37a of the Act from September 6th 2001 – Pharmacetical Law (Journal of Laws from 2008, No. 45, item 271 as amended in item 4),; 3. activity aimed at identifying animals; 4. capturing wild animals for biometric and systematic assessment; 5. veterinary procedures which to not cause pain, suffering, distress or permanent health impairment equal to or more invasive than the insertion of a needle. Hence, the study entitled “Searching for ivermectin resistance in a Strongylidae population of horses stabled in Poland” does not require the approval of the Ethics Committee. All procedures were performed during the study with the owner consent.

Consent for publication

Not applicable.

Competing interests

The authors state that there are no conflicts of interest. None of the aforementioned companies had any involvement, direct or indirect, in the development of the manuscript and the works reflects the opinions solely of the authors.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, pl. Gruwalski 47, 50-366 Wrocław, Poland. 2Department of Parasitology, Institute of Genetics and Microbiology, University of Wrocław, ul. S. Przybyszewskiego 63/77, 51-148 Wrocław, Poland.

Received: 10 February 2017 Accepted: 26 June 2017
Published online: 03 July 2017

References

1. Love S, Murphy D, Mellor D. Pathogenicity of cyathostome infection. Vet Parasitol. 1999;85:11–21.
2. Kaplan RM, Vidyashankar AN. An inconvenient truth global worming and anthelmintic resistance. Vet Parasitol. 2012;186:70–8.
3. Kaplan RM. Anthelmintic resistance in nematodes of horses. Vet Res. 2002;33:491–507.
4. Nielsen MK, Mittel L, Grice A, Erdine M, Graves E, Vaala W, Tully RC, French DD, Bowman R, Kaplan RM. AAEP Parasite Control Guidelines. 2013. https://aap.org/sites/default/files/Guidelines/AAPParasiteControlGuidelines_0.pdf.
5. Mair TS, Sutton DG, Love S. Caecocacal and caecocolic intussusceptions associated with larval cyathostominosis in four young horses. Equine Vet J. 2003;32:77–80.
6. Corming S. Equine cyathostomins: a review of biology, clinical significance and therapy. Parasit Vectors. 2009;2:1–6.
7. Peregrine AS, Molento MB, Kaplan RM, Nielsen MK. Anthelmintic resistance in important parasites of horses does it really matter? Vet Parasitol; 2014;1–8.
8. Boersema JH, Eysker M, Mass J, van der Aar WM. Comparison of the reappearance of strongyle eggs in foals, yearlings, and adult horses after treatment with ivermectin or pyrantel. Vet Quart. 1996;18:7–9.
9. Canever RJ, Braga PRC, Boechli A, Grycjaucy M, Sier D, Molento MB. Lack of Cyathostomin sp. reduction after anthelmintic treatment in horses in Brazil. Vet Parasitol. 2013;194:35–9.
10. Geurden T, van Doorn D, Claerebout E, Kooyman F, De Keersmaecker S, Vercruyse J, Besognet B, Vaninneti B, Di Regabono AF, Beraldo P, Di Cesare A, traversa D. Decreased Strongyle egg reappearance period after treatment with ivermectin and moxidectin in horses in Belgium, Italy and Netherlands. Vet Parasitol. 2004;129:291–6.
11. Kaplan RM, Kies TR, Lyons ET, Lester G, Courtney CH, French D, Tolliver SC, Vidyashankar AN, Zhao Y. Prevalence of anthelmintic resistant cyathostomines on horse farms. J Am Vet Med Assoc. 2004;225:103–10.
12. Lester HE, Spanton J, Stratford CH, Bartley DJ, Morgan ER, Hodgkinson JE, Courmbe K, Mair T, Swan B, Lemo L, Cookson R, Matthews JB. Anthelmintic efficacy against cyathostominos in horses in southern England. Vet Parasitol. 2013;197:189–96.
13. Stratford CH, Lester HE, Pickles KJ, Mcgourn BC, Matthews JB. An investigation of anthelmintic efficacy against strongyloides on equine yards in Scotland. Equine Vet J. 2014;46:17–24.
14. traversa D., Von Samson-Himmelstjerna G., Demeler J., Milillo P., Schürmann S., Barnes H., Ontrato D., Penucci S., Regalbano AF., Beraldo P., Boedelch A., Colb R. Anthelmintic resistance in cyathostomine populations from horse yards in Italy, United Kingdom and Germany. Parasites & Vectors. 2009;suppl2:1–7.
15. traversa D., Castagna G., von Samson-Himmelstjerna G., Meloni S., Bartolini R., Geurden T., Pearce M., Wringer E., Besognet B., Milillo P., D’Espois D. Efficacy of major anthelmintics against horse cyathostomins in France. Vet Parasitol. 2014;188:294–300.
16. van Domm DCR, Eysker M, Kooyman FNI, Wagenaar JA, Ploeger HW. Searching for ivermectin resistance in Dutch horses. Vet Parasitol 2012;185:335–8.
17. Demeulenae re D., Vercruyse J., Dorny P., Clairebeaut E. Comparative studies of ivermectin and moxidectin in the control of naturally acquired cyathostomosis on horse farms. Vet. Parasitol. 1992;44:35
18. Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, Waller PJ. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol. 1992;44:35–44.
19. von Samson – Himmelstjerna G. Anthelmintic resistance in equine parasites – detection, potential clinical relevance and implications for control. Vet. Parasitol. 2012;1852–8.
23. Torgerson PR, Paul M, Furrer R. Evaluating faecal egg count reduction using a specifically designed package ‘eggCounts’ in R and a user friendly web interface. Int J Parasitol. 2014;44(5):299–303.

24. Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson – Himmelstjerna G, Silvestre A, Taylor MA, Verschuysse J. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 2006;136:167–85.

25. Dudeney A, Campbell C, Coles G. Macroyclic lactone resistance in cyathostomins. Vet Rec. 2008;163:163–4.

26. Little D, Flowers JR, Hammerberg BH, Gardner SY. Management of drug-resistant cyathostominosis on a breeding farm in central North Carolina. Equine Vet J. 2013;35:246–51.

27. Lyons ET, Tolliver SC, Ionita M, Lewellen A, Collins SS. Field studies indicating reduced activity of ivermectin on small strongyles in horses on a farm in Central Kentucky. Parasitol Res. 2008;103:209–15.

28. Molento MB, Antunes J, Bentes RN, Coles GC. Anthelmintic resistant nematodes in Brazilian horses. Vet Rec. 2008;162:384–5.

29. Mercier P, Chick B, Alves-Branco F, White CR. Comparative efficacy, persistent effect, and treatment intervals of anthelmintic pastes in naturally infected horses. Vet Parasitol. 2001;99:29–39.

30. Tarigo-Martinez JL, Wyatt AR, Kaplan RM. Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses. J Am Vet Med Assoc. 2001;218:1957–60.

31. Boersema JH, Borgsteede FHM, Eysker M, Saedt I. The reappearance of strongyle eggs in feces of horses treated with pyrantel embonate. Vet Quart. 1995;17:18–20.

32. Borgsteede FHM, Boerma JH, Gaasenbeek CPH, Vanderburg WPJ. The reappearance of eggs in feces of horses after treatment with ivermectin. Vet Quart. 1993;15:24–6.

33. von Samson-Himmelstjerna G, Fritzen B, Demeler J, Schuenemann S, Rohn K, Schnieder T, Epe C. Cases of reduced cyathostomin egg reappearance period and failure of Parascaris equorum egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. Vet Parasitol. 2007;144:74–80.