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Title: Shortened egg reappearance after ivermectin or moxidectin use in horses in the UK

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Abstract: This study reports ivermectin and moxidectin egg reappearance periods (ERP) from UK horses with persistently positive faecal egg counts (FEC), defined as positive FEC within the ERP of an anthelmintic post- treatment, or with FECs that remained positive after the normal ERP post- anthelmintic treatment. A selected population of UK pleasure horses deemed at high risk of strongyle infection were studied. The earliest ERP recorded after ivermectin or moxidectin, using first positive FEC, was 5 weeks. From 16 premises where moxidectin was used, five had ERP 12 weeks using two further metrics. For premises where moxidectin was administered to only one animal (present or tested), and evaluated as one group (n=61), ERP was 10 weeks. For premises where ivermectin was used, the ERP was 5 weeks. Premises with only one horse (present or tested), dosed with ivermectin (n=31), analysed as one group, demonstrated egg reappearance 6 weeks. This field data suggests shortened ERPs following macrocyclic lactone treatment compared to previously published values (8-10 and >13 weeks respectively) when these drugs were first marketed.

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# 1 Short communication

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# 4 Shortened egg reappearance after ivermectin or moxidectin use in horses in the UK

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# Abstract

1. This study reports ivermectin and moxidectin egg reappearance periods (ERP) from
2. UK horses with persistently positive faecal egg counts (FEC), defined as positive FEC within
3. the ERP of an anthelmintic post-treatment, or with FECs that remained positive after the
4. normal ERP post-anthelmintic treatment. A selected population of UK pleasure horses
5. deemed at high risk of strongyle infection were studied. The earliest ERP recorded after
6. ivermectin or moxidectin, using first positive FEC, was 5 weeks. From 16 premises where
7. moxidectin was used, five had ERP 12 weeks using two further metrics. For premises where
8. moxidectin was administered to only one animal (present or tested), and evaluated as one
9. group (*n*=61), ERP was 10 weeks. For premises where ivermectin was used, the ERP was
10. 5 weeks. Premises with only one horse (present or tested), dosed with ivermectin (*n*=31),
11. analysed as one group, demonstrated egg reappearance 6 weeks. This field data suggests
12. shortened ERPs following macrocyclic lactone treatment compared to previously published
13. values (8-10 and >13 weeks respectively) when these drugs were first marketed.

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34 *Keywords*: Egg reappearance; Ivermectin; Moxidectin; Strongyles; Resistance

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1. Shortened egg reappearance periods (ERP) are an early indicator of anthelmintic
2. resistance (Sangster, 1999). Original ivermectin and moxidectin ERPs have been reported as
3. 8-10 weeks and >13 weeks, respectively (Borgsteede et al., 1993; Jacobs et al., 1995). This
4. retrospective study was performed to determine ERP after ivermectin or moxidectin
5. treatment under field conditions, in UK pleasure horses with persistently positive faecal egg
6. counts (FEC) following anthelmintic treatment.

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1. Ethical approval was granted by the University of Liverpool Ethics Committee
2. (Approval Number RETH000363). Parasite control records of horses tested for ivermectin or
3. moxidectin efficacy by a commercial provider (EPLA) between 01/01/2008 – 29/08/2011
4. were reviewed (*n*=200). Parasite control programs were designed around FEC data, horse
5. age, breed, weight and pasture management. Where horses demonstrated persistently positive
6. FEC and anthelmintic ERP post-treatment data was recorded, the commercial provider placed
7. these animals under additional FEC monitoring. This involved additional FECs scheduled
8. before further anthelmintic treatment and then again within the ERP for the anthelmintic
9. (efficacy testing). This process worked backwards from the end of the anthelmintic ERP, in
10. weeks, to identify the time point of egg reappearance. All FEC results, drug dosing data and
11. dates were recorded in the horses’ records. Sample collection and anthelmintic treatment
12. were undertaken by the client, as instructed by the commercial provider. The instructions
13. given to clients for sample collection and dosing agreed with current practice. FECs were
14. processed at the University of Liverpool using a modified McMaster technique representing
15. 25 eggs per gram (epg); all eggs were reported as strongyles.

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1. FEC results and treatment data were extracted from paper records and entered into a
2. database. FECs were performed between 4-10 weeks post-ivermectin treatment and between
3. 3-13 weeks post-moxidectin; time points were set by the commercial provider. Horses with
4. initial pre-treatment FECs <150epg were excluded (Coles et al., 2006). Horses that received
5. anthelmintic 90 days before the initial FEC were also excluded.

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1. Currently there are no globally accepted published guidelines for measuring ERP
2. (Relf et al., 2014). Therefore, ERP was described using previously reported methods: (1) the
3. first positive FEC post-treatment (Lyons et al., 2008); (2) group arithmetic mean FEC >10%
4. of the group arithmetic mean FEC at Day 0 (Larsen et al., 2011); and (3) <90% FEC
5. reduction (FECR) within the ERP for the product (Larson et al., 2011).

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1. ERP metrics were evaluated at the premises level (moxidectin *n*=16; ivermectin
2. *n*=10). When only one test record per premises existed, data were reported at the animal level
3. (moxidectin *n*= 61; ivermectin *n*= 31). All single animal premises results were analysed
4. collectively.

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1. Of 200 records, 153 met the inclusion criteria, categorised as moxidectin (*n*=95) or
2. ivermectin (*n*=58). Breeds represented included Thoroughbreds, including cross breeds
3. (*n*=33), warmbloods, including cross breeds (*n*=40), draft horses (*n*=10), native ponies (*n*=22)
4. and cob types (*n*=35). In some records, breed was not recorded (*n*=13). There were 72
5. females and 81 males, ranging from 1-31 years (mean 10 ± 7.2 years). Premises were
6. predominantly self-catering livery yards.

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1. The shortest observed ERP for ivermectin or moxidectin was 5 weeks. Five of the 16
2. premises where moxidectin was used recorded ERP (measured as >10% of day 0 FEC and
3. FECR <90%) at 12-13 weeks (Table 1). For collective moxidectin individual animal data
4. (Table 2), the earliest ERP was 5 weeks, and FECR was 67% (*n*=1). At 6 weeks post-dosing,
5. the mean FECR was 87% and there was a 16% difference between pre-treatment and post-
6. treatment FEC (*n*=3). Most tests (*n*=87) were conducted between 10 and 13 weeks post-
7. moxidectin, yielding a mean FECR 80-85% and pre and post-dosing FEC differences of 11-
8. 22%. Overall for moxidectin (Table 3), 5% of the horses had an ERP of 5-7 weeks, and 95%
9. of horses had an ERP of ≤13 weeks.

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1. Premises-level results following ivermectin treatment (Table 1) indicated that 50% of
2. the premises studied had ERPs between 5 and 10 weeks. These premises had FECR results
3. ranging from 0 to 79%, and a mean FEC difference of 18-100% (*n*=13). When analysed
4. collectively, single animals had ERPs of 6-10 weeks, the FECR range was 57-89%, and pre-
5. and post-treatment FEC differences were 13-64% (*n*=31; Table 2). For all ivermectin
6. treatments collectively, two animals had egg reappearance at 5 weeks. ERP was 7-10 weeks
7. in the remainder of the horses (Table 3). Comparing the FECR method and the percentage
8. difference between pre- and post-treatment FECs, there was 99% agreement in classifying
9. egg reappearance at the same time point.
10. This study demonstrates shortened ERP following treatment with ivermectin or
11. moxidectin compared to previously published values. Our findings support those by Lyons et
12. al. (2011) and Relf et al. (2014), which reported shortened strongyle ERP following
13. ivermectin or moxidectin treatment. Our findings suggest that ERP for ivermectin was from
14. 5 weeks post-treatment, less than the originally reported 8-10 weeks (Borgsteede et al.,
15. 1993). Moxidectin ERP in our study was 11-13 weeks compared to >13 weeks and up to 24
16. weeks (Jacobs et al., 1995). Boersema et al. (1998) reported egg reappearance at 9 weeks in
17. some individual animals. Our data demonstrated egg reappearance after moxidectin treatment
18. was as low as 5 weeks in some individual animals.
19. We report egg reappearance times calculated using three different methods (Lyons et
20. al., 2008; Larsen et al., 2011; Relf et al., 2014). Currently there are no globally accepted
21. guidelines for the calculation of an ERP. Our data would suggest that both the adapted %
22. FECR method and the >10% difference between pre- and post-treatment FEC method are
23. acceptable tools for evaluating ERP. Furthermore, these two metrics used together might
24. provide a more robust metric for defining reduced efficacy.
25. The authors recognise that this study had limitations. Data were analysed
26. retrospectively, and original sampling and dosing was carried out by horse owners. Data
27. represents field cases throughout the UK, FECs were not planned experimentally and ERP
28. could have occurred earlier than we reported due to the timing of the scheduled FECs. There
29. could be alternative reasons for early egg reappearance other than reduced anthelmintic
30. efficacy, e.g. incorrect weight estimation and dosing. There was also a possibility that the
31. animals in this study had persistently positive FECs due to poor anthelmintic efficacy, rather
32. than true early egg reappearance. However, true 14 day FECR data was not available.
33. Additionally, at the premises level our sample numbers were small. Where only one record
34. per premises was available, results were analysed collectively; therefore, conclusions should
35. be drawn with caution from these data. However, it is common for horses to be kept in small
36. groups and for practical identification of anthelmintic efficacy, this dataset suggests that it is
37. possible to gain an indication of egg reappearance times on premises with very few animals.
38. Our sample population may not be representative of the whole UK horse population and
39. horses that were selected for this study had signs of early egg reappearance, thus biasing the
40. sample towards horses possibly harbouring strongyles with reduced susceptibility to
41. ivermectin or moxidectin. Notwithstanding these limitations, these data provide an insight
42. into ivermectin and moxidectin efficacy in the field. Further study is required in to define
43. current ERP for these drugs and to investigate whether using two metrics provide a more
44. robust measure of ERP. Lastly, the animals in our study comprised a range of ages. The
45. pharmacokinetics of anthelmintics can differ in younger animals compared to older animals,
46. which may have influenced our findings (Gonzalez Canga et al., 2009).
47. Our results provide evidence for reduced strongyle ERPs following ivermectin and
48. moxidectin treatment in UK pleasure horses in the field. This work agrees with other recent
49. reports of reduced efficacy of these drugs in the UK.

# Conflict of interest statement

1. Neither of the authors has any financial or personal relationships that could
2. inappropriately influence or bias the content of the paper.

# Acknowledgement

1. EPLA Ltd provided the data for this study. Preliminary data was presented at the 11th
2. Colic Research Symposium, Dublin, July 7 to10, 2014.

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# Table 1

1. Egg reappearance tests at premises level, classified by premises, drug and then by time point
2. post-dosing.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | |  | Difference |  |
| Time | between |
|  |  |  | Pre | Post | post | pre- and | Mean |
|  |  |  | FEC | FEC | dosing | post-dose | FECR |
| Premises | *n* | Drug | (mean) | (mean) | (weeks) | FECs (%) | (%) |
| 1 | 3 | Mox | 375 | 50 | 13 | 13a | 87a |
| 2 | 2 | Mox | 350 | 0 | 13 | 0 | 100 |
| 3 | 3 | Mox | 408 | 267 | 13 | 65a | 35a |
| 4 | 4 | Mox | 344 | 13 | 10 | 4 | 96 |
| 5 | 2 | Mox | 238 | 0 | 13 | 0 | 100 |
| 6 | 5 | Mox | 180 | 55 | 12 | 31a | 69a |
| 7 | 3 | Mox | 400 | 42 | 13 | 10 | 90 |
| 8 | 2 | Mox | 625 | 25 | 12 | 4 | 96 |
| 9 | 2 | Mox | 375 | 0 | 12 | 0 | 100 |
| 10 | 3 | Mox | 400 | 100 | 10 | 0 | 75 |
| 11 | 2 | Mox | 525 | 88 | 13 | 17a | 83a |
| 12 | 2 | Mox | 1563 | 0 | 10 | 0 | 100 |
| 13 | 2 | Mox | 313 | 0 | 12 | 0 | 100 |
| 14 | 2 | Mox | 850 | 263 | 13 | 31a | 69a |
| 15 | 3 | Mox | 325 | 17 | 13 | 5 | 95 |
| 16 | 7 | Mox | 364 | 14 | 13 | 4 | 96 |
| 17 | 2 | Ivm | 713 | 125 | 5 | 18a | 82a |
| 18 | 2 | Ivm | 388 | 38 | 9 | 10 | 90 |
| 19 | 2 | Ivm | 200 | 13 | 7 | 6 | 94 |
| 20 | 2 | Ivm | 413 | 38 | 8 | 9 | 91 |
| 21 | 5 | Ivm | 300 | 250 | 10 | 83a | 17a |
| 22 | 2 | Ivm | 188 | 463 | 8 | 247a | -147a |
| 23 | 2 | Ivm | 313 | 0 | 6 | 0 | 100 |
| 24 | 2 | Ivm | 263 | 0 | 7 | 0 | 100 |
| 25 | 2 | Ivm | 475 | 175 | 10 | 37a | 63a |
| 26 | 4 | Ivm | 606 | 131 | 8 | 22a | 78a |

1. FEC, faecal egg count; FECR, faecal egg count reduction; Mox, moxidectin; Ivm, ivermectin
2. a Both FECR <90% and pre-treatment and post-treatment FEC difference >10%.
3. b Egg reappearance by one metric only.

# Table 2

1. Egg reappearance data from single animal premises analysed collectively by drug and time
2. point. Grouped by drug and week for analysis. Superscripts suggest early egg reappearance.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Time post- dosing |  | Pre- dose | Post- dose | Difference between pre- and post-dose | Mean FECR |
| Drug | (weeks) | *n* | FEC (mean) | FEC (mean) | FECs (%) | (%) |
| Ivm | 4 | 2 | 550 | 0 | 0 | 100 |
| Ivm | 6 | 2 | 763 | 100 | 13a | 87a |
| Ivm | 7 | 3 | 775 | 75 | 10 | 90 |
| Ivm | 8 | 13 | 521 | 121 | 23a | 77a |
| Ivm | 9 | 3 | 533 | 342 | 64a | 36a |
| Ivm | 10 | 8 | 516 | 159 | 31a | 69a |
| Mox | 3 | 1 | 275 | 0 | 0 | 100 |
| Mox | 5 | 1 | 150 | 50 | 33a | 67a |
| Mox | 6 | 3 | 1613 | 250 | 16a | 85a |
| Mox | 7 | 1 | 325 | 50 | 15a | 85a |
| Mox | 8 | 1 | 1850 | 0 | 0 | 100 |
| Mox | 9 | 1 | 150 | 0 | 0 | 100 |
| Mox | 10 | 15 | 433 | 58 | 13a | 87a |
| Mox | 11 | 4 | 444 | 50 | 11a | 89a |
| Mox | 12 | 11 | 250 | 0 | 0 | 100 |
| Mox | 13 | 23 | 490 | 107 | 22a | 78a |

1. FEC, faecal egg count; FECR, faecal egg count reduction; Mox, moxidectin; Ivm, ivermectin
2. a Early egg reappearance

# Table 3

1. Egg reappearance data for all horses tested collectively within the data set, classified by drug
2. and then by time post dosing.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 212 |  | | | | | | |
|  |  | Time post- |  |  |  |  | Mean |
|  |  | dosing |  | Pre-dose | Post-dose | Difference between pre- | FECR |
|  | Drug | (weeks) | *n* | FEC (mean) | FEC (mean) | and post-dose FECs (%) | (%) |
|  | Ivm | 4 | 2 | 550 | 0 | 0 | 100 |
|  | Ivm | 5 | 2 | 713 | 125 | 18a | 82a |
|  | Ivm | 6 | 7 | 490 | 100 | 8 | 80c |
|  | Ivm | 7 | 6 | 441 | 100 | 23c | 93 |
|  | Ivm | 8 | 19 | 488 | 138 | 28b | 72b |
|  | Ivm | 9 | 4 | 544 | 275 | 51a | 49a |
|  | Ivm | 10 | 18 | 433 | 172 | 40b | 60b |
|  | Mox | 3 | 1 | 275 | 0 | 0 | 100 |
|  | Mox | 5 | 1 | 150 | 50 | 33a | 67a |
|  | Mox | 6 | 3 | 1613 | 250 | 16a | 85a |
|  | Mox | 7 | 1 | 325 | 50 | 15a | 85a |
|  | Mox | 8 | 1 | 1850 | 0 | 0 | 100 |
|  | Mox | 9 | 1 | 150 | 0 | 0 | 100 |
|  | Mox | 10 | 24 | 447 | 41 | 9 | 91 |
|  | Mox | 11 | 4 | 444 | 50 | 11a | 89a |
|  | Mox | 12 | 11 | 250 | 0 | 0 | 100 |
|  | Mox | 13 | 48 | 479 | 88 | 18b | 82b |

1. FEC, faecal egg count; FECR, faecal egg count reduction; Mox, moxidectin; Ivm, ivermectin
2. a Shortened egg reappearance calculated by FECR <90% and >10% difference between pre-
3. treatment and post treatment FEC from a small sample.
4. b Shortened egg reappearance agreed by both metrics from a larger sample.
5. c Early egg reappearance from one metric only.

**\*Highlights (for review)**

# Highlights

* + This study recorded ivermectin egg reappearance from 5 weeks post-dosing.
  + Moxidectin egg reappearance occurred from 5 weeks post-dosing.
  + Agreement using two metrics to measure egg reappearance warrants further investigation.

**\*Revision Note**

01/11/2016

Dear Editor

Further to your email 31st October 2016, ref YTVJL-D-16-00099R2, please find below a revision note addressing the reviewers comments. As per my previous correspondence it has not been possible to keep strictly to the word limit for a short communication while following the reviewers requests for further explanation throughout the manuscript. This has been kept as concise as possible while exploring the reviewers comments in full.

**Reviewer 5**

**Comment 5.1** There is just one remaining point left which authors can remedy in a heartbeat. In the abstract in L26-27 it is not readily clear that the individual horses were tested on other premises than the so-called moxidectin and ivermectin premises. Consequently, someone who starts reading the abstract may wonder why the shortest ERP on ivermectin premises was 5 weeks, while the shortest ERP for individual horses was 6 weeks. I suggest to change the term individual horses into on premises with only one horse (present or tested).

**Response** Lines 25-27 have been updated to reflect the reviewers comment, hopefully this is now explicitly clear to readers.

**Reviewer 6**

**Comment 6.1**. The descriptions of persistently positive/continually positive counts (lines 39-40 and 45-46) are still confusing. These terms imply that several serial samples were taken and all tested positive. Was all that was done was simply to look through the records to select instances where eggs were detected at times that would be considered within the normal ERPs? If so this needs to be described more accurately. What precisely is meant by "efficacy testing was included"? Does this simply mean that when eggs were detected earlier than usual, an efficacy calculation was performed?

**Response:** We have added clarification to the term persistently positive and the definition of efficacy testing in lines 45-51. While our analysis was on retrospective data the description of how the original data was collected in the manuscript reflects the process put in place by the commercial provider. Where FECs were persistently positive after anthelmintic treatment, the company started efficacy testing. This involved adding additional FECs and anthelmintic dosing to check the effectiveness of the drug. It was these records that were used in this study. We thank the reviewer for highlighting that this was not sufficiently clear, hopefully this revision now clarifies this.

**Comment 6.2**. The authors should state that the two parameters FECR and % difference pre and post, are essentially the same (% diff = 100-FECR). As used in their study, there thus appears little need to examine both! This is of some relevance to those planning to take a similar approach, based on this work.

**Response:** Clarity has been added to lines 110-116 that two of the metrics calculated from the same data use a similar approach and this instance are in agreement 99% of the time. As there are no universally accepted guideline for efficacy testing both of these metrics appear acceptable tools. Our suggestion is that these could be used together to provide a more robust measure for reduced efficacy. Hopefully this is now clear with further explanation.

I hope that these revisions are satisfactory, I look forward to your response.

Yours sincerely S Daniels Simon Daniels