Peer Community In Ecotoxicology & Environmental Chemistry

Low potential of arthropod species to aquire resistance to invermectin drug could induce a risk of extinction in contaminated pastures

Christian Mougin *based on peer reviews by* ***Marcel Amichot*** *and 2 anonymous reviewers*

Daniel Gonzalez Tokman, Antonio Arellano Torres, Fernanda Baena-Diaz, Carlos Bustos, Imelda Martinez M (2024) Ivermectin resistance in dung beetles exposed for multiple generations. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry.
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For many decades, the macrocyclic lactone drug ivermectin is extensively used in veterinary medicine and agriculture, as well as human medicine. Residues of ivermectin excreted in cattle dung remain persistent in soils (Mougin et al., 2003), biologically active and threaten non-target soil and coprophagous organisms such as dung flies and beetles (Lumaret et al., 2012). Ivermectin affects highly beneficial and taxonomically diverse groups inhabiting dung pats, including flies, parasitic wasps, as well as coprophilus and predatory dung beetles (Villar et al., 2022). Ivermectin resistance is well document in insects, but it seems to take longer and to be less effective than resistance to insecticides or other antiparasitic drugs, because of different physiological mechanisms involved in resistance (Seaman et al., 2015).

In that context, Gonzalez-Tokman et al. (2024) compared the reproductive success of a line of dung beetles (Euoniticellus intermedius, Scarabaeinae) exposed to a moderate concentration of invermectin during 18 generations, and a control line of beatles that was maintained free of antiparasitic drug. They carried-out toxicity experiments with increasing ivermectin concentrations to determine if sensitivity to ivermectin was reduced after some generations of exposure, possibly by acquiring resistance by means of transgenerational

effects. Thus, dung beetles did not generate resistance to ivermectin after 18 generations of continuous exposure, and quantitative genetic analyses showed only low genetic variation in response to ivermectin.

The results published by Gonzalez-Tokman et al. (2024) indicated a low potential of beetles for adaptation to the drug, and suggest for non-target invertebrate groups a possible risk of extinction in ivermectin-contaminated pastures. These effects can greatly impact grassland ecology, lower their quality and reduce the area available and palatable to livestock.

References:

Mougin, C., Kollmann, A., Dubroca, J., Ducrot, P.-H., Alvinerie, M., Galtier, P., 2003. Fate of the veterinary medicine ivermectin in soil. Environ. Chem. Letters 1, 131-134. https://doi.org/10.1007/s10311-003-0032-9

Lumaret, JP., Errouissi, F., Floate, K., Römbke, J., Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Current Pharmaceutical Biotechnology 13(6), 1004-60. https://doi.org/10.2174/138920112800399257

Villar, D., & Schaeffer, D.J., 2022. Ivermectin use on pastured livestock in Colombia: parasite resistance and impacts on the dung community. Revista Colombiana De Ciencias Pecuarias, 36(1), 3-12. https://doi.org/10.17533/udea.rccp.v36n1a2

Seaman, J.A., Alout, H., Meyers, J.I., Stenglein, M.D., Dabiré, R.K., Lozano-Fuentes, S., Burton, T.A., 471 Kuklinski, W.S., Black, W.C., Foy, B.D., 2015. Age and prior blood feeding of Anopheles gambiae influences their susceptibility and gene expression patterns to ivermectin-containing blood meals. BMC Genomics 16, 797. https://doi.org/10.1186/s12864-015-2029-8

González-Tokman, D., Arellano-Torres, A., Baena-Díaz, F., Bustos, C., Martínez M., I., 2024. Ivermectin resistance in dung beetles exposed for multiple generations, bioRxiv ver. 3 peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2023.05.08.539900

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.05.08.539900
Version of the preprint: 2

Authors' reply, 16 February 2024

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Decision by Christian Mougin, posted 14 February 2024, validated 14 February 2024

Minor revision needed

Dear colleague
Please take into account the remark of referee 1.
Best regards
Christian

Reviewed by anonymous reviewer 1, 31 January 2024

The authors have taken into account most of the comments and have made corrections. The manuscript looks fine to me, with the exception of one sentence:

Line 304 - 306 “We also cannot discard that the observed reductions in the number of emerged beetles in some of our studied generations has resulted from genetic drift, preventing the detection of adaptation to ivermectin.”

It is the large population reduction (i.e. bottleneck), possibly due to the deleterious effects of ivermectin, that can cause genetic drift, and not the other way round. Next, genetic drift can lead to an erosion of genetic diversity, reducing the capacity of populations to adapt to the pollutant.

Reviewed by Marcel Amichot, 02 February 2024

Dear authors,

I was glad to read your responses and the manuscript with the changes you made to it. I therefore withdraw my suggestion to rewrite the manuscript (last sentence of my conclusion). On the subject of resistance to ivermectin, I now fully understand your objectives, which are not to obtain a resistant population but to check whether resistance can develop with doses recorded in the field. From that point of view, I agree with my colleague who stated in his review ” In the context of ecotoxicology, the questions posed in this study seem very relevant to me ”. What’s more, the discussion clearly mentions the importance of the number of generations required to reach a significant level of resistance. In fact, given the identified effects of ivermectin on insect mortality and reproduction rates, would there not be a basis for modelling the demographic impact of ivermectin on Euoniticellus intermedius?

The figures are more explicit now with the additions you made to their captions.

In conclusion, it seems to me that the manuscript has been modified to make it compatible with the requirements of the PCI Journal.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.05.08.539900
Version of the preprint: 1

Authors’ reply, 26 January 2024

Download author’s reply
Download tracked changes file

Decision by Christian Mougin, posted 11 November 2023, validated 13 November 2023

Revision of the preprint

Dear auhtors

We apologise for our delay of response. Your preprint has been rewieved by three experts of the field, and ther reviews are now available. I agree with them and encourage you to improve the preprint during this first round of review, by considering all the comments and carefully responding to them.

I recommend a major revision.

Best regards

Reviewed by anonymous reviewer 1, 25 July 2023

The authors present a study in experimental evolution, in which one population of Euoniticellus intermedius were exposed or not to ivermectin for 18 generations. Moreover, authors carried out toxicity experiments in generation F1, F2, F3, F6 and F18 for both lines (exposed or not to ivermectin across multigenerational experiment).

In the context of ecotoxicology, the questions posed in this study seem very relevant to me, by studying the long-term responses to a pollutant. The authors rely on evolutionary biology to characterize the response of Euonicellus intermedius populations to chronic exposure to ivermectin, in particular through genetic adaptation.

This study is on the whole clear and well carried out, despite some technical problems related to a mortality of individuals regardless of treatment.

I particularly appreciated the good contextualization of the subject and the fact that the hypotheses were well posed. The article is not too long, allowing not to get lost in the information. In addition, the statistics seem to me rather well done and rigorous. Finally, I think it is important to emphasize that such a long multigenerational experiment (18 generations) on arthropods is rather rare and requires significant work.

I would like ask about the following points to improve the manuscript.

Line 24: it would be interesting to add the life history traits studied, for example "We compared reproductive success (total brood balls, emerged beetles, proportion emerged and days to emergence)".

Line 22: I was wondering if the term "line" was the most relevant. Basically, you have a line that you place in two different conditions.

Line 33: I don't understand why talking about "pre-adaptation" here, it seems outside the subject of the article. Moreover, even if the authors were not able to observe adaptation during their multigenerational experiment, nothing says that in natural conditions, populations are not able to respond, whether by phenotypic plasticity or longer-term adaptation. However, it is possible that I did not understand the meaning of the sentence, can the authors clarify this notion of pre-adaptation in this sentence?

Line 34: in keywords, "maternal effects" doesn't seem very appropriate. On the other hand, I would have added "evolution experimental" or "multigenerational experiment".

Line 47 – 49: I find the wording of the sentence a bit "utilitarian", where only the money a species brings in can decide whether or not to keep it. I would modify the sentence as follows:

Before modification:

"The economic value of dung beetles in cattle pastures is calculated in up to $423 USD per cow and, therefore, their conservation is urgent to maintain their ecosystem services"

After modification:

"In addition, the economic value of dung beetles in cattle pastures is calculated in up to $423 USD per cow and, therefore their conservation is urgent to preserve these ecosystems and associated services"

Line 55 – 56: clarify what the "reproductive disadvantages" are.

Line 71 – 74: the explanation on why "ivermectin resistance seems to take longer and be less effective than resistance to insecticides" is not clear, it seems to me necessary to clarify the sentence, perhaps by providing examples.

In the experimental lines and the toxicity experiment part, the number of couples (replicates) maintained during all the experiments lacks clarity. Ideally, without mortality, there were 20 couples (replicates) in each generation and each condition?

In view of the significant mortality problems, is it possible that a strong genetic drift could have prevented or slowed down the demonstration of an adaptive response (improved resistance)? This aspect could have been addressed in the discussion.

Line 95 – 100: given its rapid expansion, I was wondering whether Euoniticellus intermedius was considered an invasive species or not? Why not study a local species instead?

Line 108: it would be interesting to specify the generation time of Euoniticellus intermedius.

Line 118: were the male-female crosses totally random?

Line 121: I don't get the impression that this part is about "toxicity experiments", I think that can be removed from the title.

Line 146: it would be interesting to add if possible the cause of the high mortality of the control line in F13?

Line 164 – 165: it could have been interesting to add and discuss the results on the change in sex ratio mentioned in line 164 – 165.

Line 168 – 169: if this experiment was not considered in the analyses, it is perhaps preferable to delete this sentence: "As an additional experiment, five couples emerged from IVM62 in F18 were exposed to the same ivermectin concentration (62 µg of ivermectin per kg of fresh dung), but not a single individual emerged in the new generation, which was not considered for statistical analyses."

Line 173 – 174: it may be interesting to better detail the method for estimating heritability and to show the regression curves.

Overall this part seems good to me, I was just wondering if some insertion was not in the order of discussion. For example, "surprisingly" (line 238), "giving a potential indication of resistance" (line 239 240), "ivermectin resistance ratios (RR) indicated lack of resistance and only small tolerance to the contaminant in generation F3" (line 241 – 242) or "indicating some tolerance" (line 251).

Line 266 – 267: there appears to be an amplification of adverse effects of ivermectin on traits measured. These results were not discussed.

Reviewed by anonymous reviewer 2, 05 November 2023

The work carried out by Daniel González-Tokman and colleagues seeks to detect the possible occurrence of resistance to ivermectin in a line of Euoniticellus intermedius fed with droppings containing the famous antiparasitic. The experiment was conducted over 18 generations (+1 with a complementary test). Several biological parameters are taken into account, and the line is compared with a parallel-bred line fed without ivermectin. The choice of E. intermedius is an interesting one, as it is a species that seems to show great ecological plasticity. It is an abundant and frequent species in pastoral systems in Africa, America and Australia (it has been introduced in the latter 2 areas).

Maintaining a breeding program over such a large number of generations is not without its risks, and the authors have encountered a few difficulties. But these appear to be minor and do not detract from the study. Consequently it seems to be a nice work, well designed and well described. I must point out that I am not fully competent to judge the relevance of the analyses carried out. In any case, they seem appropriate to me.

The main result is the non-appearance of resistance. Given the potential capacities of this generalist species and the rearing conditions (systematic feeding with IVM), it is reasonable to assume that the absence of resistance is representative of the process at work within dung beetles under real-life conditions.

This result is of main importance. I think this study deserves to be published. I have no conceptual or methodological criticisms to make. I have only a few minor remarks:

a) in Materials and Methods:

- line 172: you should precise "Figure 3a".
- line 218: it is written "were significant for most analyzed variables" but in Table1 1 ALL the results are in bold (even with P=0.051). Either you consider P=0.051 is signficant and change your text in "were significant for all analyzed variables" or you apply a strict interpretation of P=0.05 treshold and, in the Table 1, this result must be unbold.

b) in results :

- table S1 (announced line 259) is lacking.
- the titles of tables 1 to 3 may be improved : they are too similar (for example Table 3 presents the results for 5 generations among 18, it could be precised) and I think that it is not correct to write "18 generations of exposure" beacuse the line "control" is out of IVM exposure. Same remark for the legend of figures 2 and 3.

c) references :

- Messina (1993), Price & Schluter (1991), Ritz et al. (2016) and Young et al. (2003) do not figure in the text.
- conversely: Hlina 2020 is cited line 199-200 but is not listed in the references.

Reviewed by Marcel Amichot, 10 November 2023

Dear Authors, I spent a lot of time making sure I understood everything and, in fact, I didn't understand everything. I have two major concerns about this manuscript, the first one is the selection protocol for resistance and the second one is the concept of insecticide resistance as used by the Authors.

First the the selection protocole.

-Why use such a low dose when it is known that ivermectin concentrations in dung are higher in livestock facilities ? In addition, when resistance to insecticide needs to be selected for, the selection pressure (i.e. the dose/concentration of insecticide) applied to the insects must be gradually increased throughout the generations.

-Secondly, it is not clear whether the treatments were applied to pairs (20?) placed in separate containers or whether the pairs were all placed in a single container. In the latter condition, it seems difficult to be sure of avoiding inbreeding.

-Third, at lines 140-141, the Authors state " In generations F6 and F11-F17 we were not able to register emerged beetles in the IVM lines ". So my question : if you have no progeny, how were you able to pursue the selection process ?

Second, the resistance to insecticides

All along the manuscript, the Authors, from my point of view, mix the insecticide resistance concept with the effects that ivermectin may have on life traits as fecundity for instance. In brackets, I mention the definition of insecticide resistance as proposed by the IRAC (irac-online.org) " When insect population can no longer be controlled by a dose of insecticide which used to provide control of them this is termed as insecticide resistance. However, for it to be considered true resistance, the resistant insects must be able to pass on the ability to resist the insecticide to their offspring". In the text of the manuscript, the Authors refer to insecticide resistance although they describe effects on larvae or adult emergence from brood balls or developmental time. I would like the Authors to be more selective in their wording.

Additional comments

-The difference between figure 2 and figure 3 is difficult to address. Does the figure 2 present the result of tests done with the parents submitted to 10µg of ivermectine/kg of dung and does the figure 3 present the results of tests done with the progeny submitted to other ivermectine concentrations (10 ; 31 or 62 µg/kg) ? Please clarify.

-There is a wide variability for the number/proportion of emerged insects in figure 3 as compared to figure 2, please discuss that point.

-The Authors collected insects and dung from two different ranches in which ivermectin is not used. What about other antiparasitic, antibiotic or other drugs ? And why two ranches ?

Conclusion

In fact, this manuscript provides interesting results as far as I understand them in the current version of the manuscript: the reproductive capacities or development times of control and treated beetles are not so different in several situations. Having said that, the interest of the results also depends on the condition that the dose of ivermectin used here, 10 µg/kg of excreta, can be realistic under certain environmental conditions. I suggest that the authors completely reconsider the structure of the manuscript and build it around these physiological characteristics.