

## Response to Reviewers' Comments:

### REVIEWER #1:

Interesting case report about the use of gastrografin in cases of enteric anisakis. I want to remark several questions before its publication.

1. The paragraph about in vitro study is little consistent: it's impossible to extract conclusions with 9 cases. I think this paragraph must be removed.

**Response:** Thank you for your comment. We have removed the paragraph about in vitro study in the manuscript as below:

- ~~we examined the effect of the Gastrografin solution on the Anisakis larvae. Early death was confirmed in the Anisakis larvae that were soaked in Gastrografin solution. ; Page 3, line12-14~~
- ~~In vitro experiments—In order to examine the anti-nematode effect of Gastrografin, we performed an in vitro experiment. We collected and used nine Anisakis larvae. We prepared three laboratory dishes with 5.0 mL of the Gastrografin solution (Group 1), 5.0 mL of half of the quantity of the Gastrografin and saline (Group 2), and 5.0 mL of saline (Group 3), respectively (Figure 4). Nematodes were placed into the solutions, and we measured the duration for the nematodes to stop moving. We determined that the higher the concentration of the Gastrografin solution, the shorter the duration for the Anisakis larvae to cease moving (Figure 5). This result shows that Gastrografin has not only a laxative effect, but also a direct effect on the Anisakis larvae.; Page 6, line12-21~~
- ~~In addition, in this case report, we confirmed the direct effect of Gastrografin on Anisakis larvae via in vitro experiments, and also demonstrated that it contributed to a shorter hospital stay.; Page 7, line26-Page 8, line1-2~~
- ~~Figure 4~~
- ~~Figure 5~~

We have also changed the sentence as below;

### Before

- ~~We confirmed the direct effect of Gastrografin on Anisakis larvae via in vitro experiments, and also demonstrated that this treatment contributed to a shorter hospital stay. performed ileography using Gastrografin to extract enteric Anisakis larvae after placing an ileus tube, which led to shortened hospital stay. ; Page 4, line1-3~~

After

- We performed ileography using Gastrografin to extract enteric Anisakis larvae after placing an ileus tube, which led to shortened hospital stay.

2. Table 1 is unuseful; I'd remove it too.

**Response:** Thank you for your comment. We have removed the Table 1.

3. Case report description is right. At the end of the discussion, I'd modify the conclusion: "Consequently, we suggest that the Gastrografin administration therapy could be a safe, convenient, and useful method to extract enteric Anisakis larvae, although more studies are necessary to confirm our results.

**Response:** Thank you for your comment. We have changed the paragraph as below:

Before

- ~~In conclusion, based on this case, abdominal CT is useful for the diagnosis of enteric anisakiasis, and Gastrografin administration therapy is useful for the extraction of enteric Anisakis larvae.~~ Since Anisakis larvae die over time, enteric anisakiasis is generally alleviated through conservative therapy. For example, Amano et al. suggested that retrieving Anisakis larvae through endoscopic extraction using double-balloon enteroscopy via the anal approach is useful for treating enteric anisakiasis[11]. However, double-balloon enteroscopy still requires a high-level of expertise and is not routinely performed. Using an antiallergic drug such as Stronger Neo-Minophagen C and steroids is useful; however, it is just one of many conservative therapies and is not a radical treatment[12,13]. Kasuya et al. examined the killing effect of foods such as *Perilla frutescens viridis* Makino, *Zingiber officinale*, *Wasabia japonica*, *Allium sativum*, and ethanol to find the most effective form of prophylaxis, and confirmed that these foods were effective in stopping the motion of worms in vitro[14]. However, these foods would need to be consumed in too high of a volume to be practical as an effective prophylaxis. Thus, of the available known treatment options, Gastrografin administration is the most convenient and useful therapy.

~~In conclusion, based on this case, abdominal CT is useful for the diagnosis of enteric anisakiasis, and Gastrografin administration therapy is useful for the extraction of enteric Anisakis larvae. Because most patients with intestinal anisakiasis cause intestinal obstruction and ileus, an ileus tube is indwelled. Our Gastrografin administration therapy is only two times of ileography using~~

*Gastrografin after placing an ileus tube, which is less in burdens on a patient. However more studies are necessary to confirm our results. ; Page8, line3-19*

After

- *Since Anisakis larvae die over time, enteric anisakiasis is generally alleviated through conservative therapy. For example, Amano et al. suggested that retrieving Anisakis larvae through endoscopic extraction using double-balloon enteroscopy via the anal approach is useful for treating enteric anisakiasis[11]. However, double-balloon enteroscopy still requires a high-level of expertise and is not routinely performed. Using an antiallergic drug such as Stronger Neo-Minophagen C and steroids is useful; however, it is just one of many conservative therapies and is not a radical treatment[12,13]. Kasuya et al. examined the killing effect of foods such as Perilla frutescens viridis Makino, Zingiber officinale, Wasabia japonica, Allium sativum, and ethanol to find the most effective form of prophylaxis, and confirmed that these foods were effective in stopping the motion of worms in vitro[14]. However, these foods would need to be consumed in too high of a volume to be practical as an effective prophylaxis. Thus, of the available known treatment options, Gastrografin administration is the most convenient and useful therapy. In conclusion, based on this case, abdominal CT is useful for the diagnosis of enteric anisakiasis, and Gastrografin administration therapy is useful for the extraction of enteric Anisakis larvae. Because most patients with intestinal anisakiasis cause intestinal obstruction and ileus, an ileus tube is indwelled. Our Gastrografin administration therapy is only two times of ileography using Gastrografin after placing an ileus tube, which is less in burdens on a patient. However more studies are necessary to confirm our results.*

**REVIEWER #2:**

Thank you for submitting an interesting case report regarding gastric and enteric anisakiasis successfully treated with Gastrografin therapy! It is challenging that you performed in vitro experiment to explain Gastrografin effect in this case study. It might be a first report treated with enteric anisakiasis successfully treated with Gastrografin therapy. However, enteric anisakiasis can generally be treated with conservative treatment. It is questionable that Gastrografin therapy is really necessary treatment for enteric anisakiasis. In addition, it can be uncomfortable treatment for the patients with enteric anisakiasis. There are some comments for revision. Mention about these comments in your discussion.

***Response:*** Thank you for sharing these concerns. I have added “***Because most patients with intestinal anisakiasis cause intestinal obstruction and ileus, an ileus tube is indwelled. Our Gastrografin administration therapy is only two times of ileography using Gastrografin after placing an ileus tube, which is less in burdens on a patient.*** ” in the revised manuscript. (Page 8, line 4-10)