Efficacy of Medola's Blue Stain for the Assessment of *Syphacia muris* egg viability

Original thinking... applied

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INTRODUCTION

Syphacia muris (Nematoda: Oxyuridae); commonly known as the rat pinworm, is a parasite of rodents. This ubiquitous nematode commonly infects laboratory rodents; primarily rats, via direct and indirect transmission routes. Daily shedding of pinworm ova in the host results in ingestion of the ova and contamination of water, food and bedding. This provides a continual inoculum that results in frequent re-exposure of the host to the parasite making the control of pinworms difficult (Meade & Watson. 2014).

The assessment of *S. muris* ova viability following the disinfection of an infested facility with an effective ovicidal agent can be costly and time consuming. Both viable and non-viable ova will persist following treatment. It is not possible to visually assess the viability of ova using standard microscopy. Therefore, to test the viability of ova following treatment with an ovicidal agent, traditionally, ova are subjected to hatching analysis using a suitable hatching media.

Most hatching media have a short shelf life and to ensure that unhatched pinworm ova are truly non-viable, it is advisable to test the hatching media on viable pinworm ova before analysing treated ova. Further to this, using hatching media for the visual assessment of hatched ova is not ideal as juvenile nematodes are digested in the hatching media within 30 minutes of hatching. Therefore, assessment is purely based on ova being correctly orientated on a Sellotape slide to allow the break in the ova cell wall that the juvenile nematode has vacated to be visible.

Medola's blue stain has been used in the viability assessment of plant-parasitic nematode ova for many years. We propose that this stain is a useful tool in determining the viability of *S. muris* ova in environmental and rodent samples when determining treatment/decontamination efficacy in rodent facilities.

METHOD

- A total of 20 HS rats (Homozygous Scottish) of known health status with an established *S. muris* infection were sampled. The animals were group housed 3-4 rats with husbandry procedures carried out in accordance with ASPA (1986).

- Ova were collected by Sellotape impressions of the anal area. Samples were taken in the afternoon in order to optimise collection as demonstrated by Van de Gulden (1967). The Sellotape impressions were dissected at 40x magnification into sections containing 25 viable ova and placed sticky side up and fixed to glass slides using 10 mm acid free craft dots. Non-viable ova were identified by the presence of degradation of the lipids inside the infective juveniles, this indicated that the nematodes within the ova were unable to hatch. These ova were omitted from the tapes.

- Viable control: 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

- Non-viable control: 10 replicates of 25 viable ova were heat killed by immersing the sellotape slides in distilled water at 65°C for 5 minutes. Once air dried the 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

- Chemical control: 10 replicates of 25 viable ova were chemically killed by immersing the Sellotape slides in 2% Neopredisan solution for 2 hours. Once air dried the 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

- Hatching: The hatching medium used was prepared according to the method previously reported by Dix *et al*: 2004. Viable, non-viable and chemical control ova were covered in hatching medium and incubated in ambient air at 37°C overnight. Slides were scanned x60 magnification and the number of hatched and non-hatched ova recorded. Ova were considered nonviable if the operculum was intact or the ova contained larva. An ova without larva or those with an open operculum were considered viable. Data was recorded for each replicate.

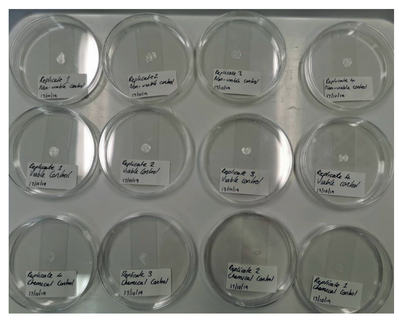

Figure 1. Anal tapes mounted on glue spot slides before staining. Non-viable control is heat control

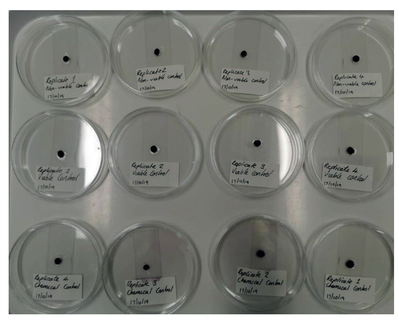

Figure 2. Anal tapes mounted on glue spot slides with Medola's blue stain. Non-viable control is heat control

RESULTS

The results can be found in table 1.

All heat killed ova and all Neopredisan killed ova took up the Medola's blue stain. There was a minor difference in the intensity of staining observed between the heat killed and Neopredisan killed ova. Heat killed ova presented with a blue colour whereas Neopredisan killed ova presented as a blue/purple colour. None of the 250 untreated ova took up the Medola's blue stain.

The heat treated and Neopredisan treated ova could not be successfully hatched demonstrating that the stained ova were non-viable as expected. All untreated ova were successfully hatched demonstrating that the unstained ova remained viable.

The Medola's blue stain did not have any effect in the ability of viable ova to hatch.

Table 1. Syphacia muris *Medola's blue assessment results*

Chemical Control 2% Neopredisan for 2 hours.												
Replicate number	1	2	3	4	5	6	7	8	9	10	Average	Total
Number of *S. muris* ova stained with 0.05% Medola's blue	25	25	25	25	25	25	25	25	25	25	25	250
Number of *S. muris* ova stained after destaining for 30 mins	25	25	25	25	25	25	25	25	25	25	25	250
Number of *S. muris* ova hatched in hatching media	0	0	0	0	0	0	0	0	0	0	0	0
Heat Control 65°C for 5 mins in a water bath.												
Replicate number	1	2	3	4	5	6	7	8	9	10	Average	Total
Number of *S. muris* ova stained with 0.05% Medola's blue	25	25	25	25	25	25	25	25	25	25	25	250
Number of *S. muris* ova stained after destaining for 30 mins	25	25	25	25	25	25	25	25	25	25	25	250
Number of *S. muris* ova hatched in hatching media	0	0	0	0	0	0	0	0	0	0	0	0
Untreated ova												
Replicate number	1	2	3	4	5	6	7	8	9	10	Average	Total
Number of *S. muris* ova stained with 0.05% Medola's blue	0	0	0	0	0	0	0	0	0	0	0	0
Number of *S. muris* ova stained after destaining for 30 mins	0	0	0	0	0	0	0	0	0	0	0	0
Number of *S. muris* ova hatched in hatching media	25	25	25	25	25	25	25	25	25	25	25	250

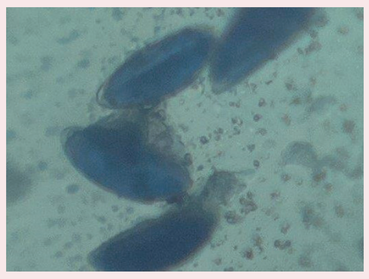

Figure 3. Syphacia muris *non-viable ova stained with Medola's blue after Neopredisan treatment*

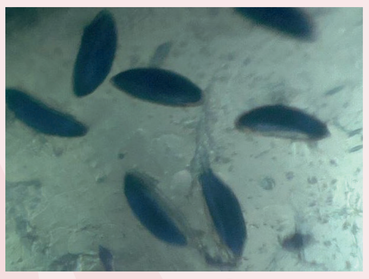

Figure 4. Syphacia muris *non-viable ova stained with Medola's blue after heat treatment*

Figure 5. Syphacia muris *viable ova, stained with Medola's blue*

CONCLUSION

The exact mechanism of uptake of this stain is not known for this organism. However, we observed that viable nematode ova with an intact operculum did not allow the stain to pass the outer cuticle of the infective juvenile. In non-viable ova or those without an intact operculum, the stain was readily observed in the juveniles. It is possible that upon death, the permeability of the infective juvenile nematode cuticle may have changed allowing the stain to enter and permanently bind with the dead nematodes.

Irrespective of the mechanism of action, this study confirms that Medola's blue stain is a useful tool in determining the viability of *Syphacia muris* ova, which has historically been impossible without the use of hatching media. This is a more cost-effective, technically simplified and less time-consuming method of determining ova viability which could be adopted by animal facility personnel.

This method could be used in facilities to confirm effective decontamination where ova may still be present in the environment after chemical treatment. This stain could also provide a useful tool to determine the efficacy of disinfectants for use in animal facilities.

REFERENCES

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