POSTER PRESENTATIONS

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The use of Ultrasound imaging to refine the technique of tumour detection in Neuroblastoma mouse models

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Aim

Overall aim was to improve the outcome for children with solid tumours. Using the TH-MYCN transgenic Neuroblastoma mouse model, with targeted MYCN expression to develop spontaneous tumours.

With the use of the portable ultrasound the aim is to **Reduce** the number of animals used and to **Refine** our techniques for detecting tumours.

Using Portable Ultrasound to refine tumour detection and growth

Before using Ultrasound we would use palpation techniques to detect tumour growth in the abdomen.

Palpation
- May cause harm and distress
- Inaccurate
- Time consuming
- Possible internal injuries
- Loss of data
- Inconsistent measurements
- Rough estimations

→

Ultrasound
- Quick
- Less stress
- Accurate
- No lasting harm
- Image data collection
- Actual measurements
- Detects abnormalities
- Consistent

Portable ultrasound

The Ultrasound is small and easy to move around the facility. All animal information and images are saved on the ultrasound machine as ‘patients’.

All images can be transferred by USB.

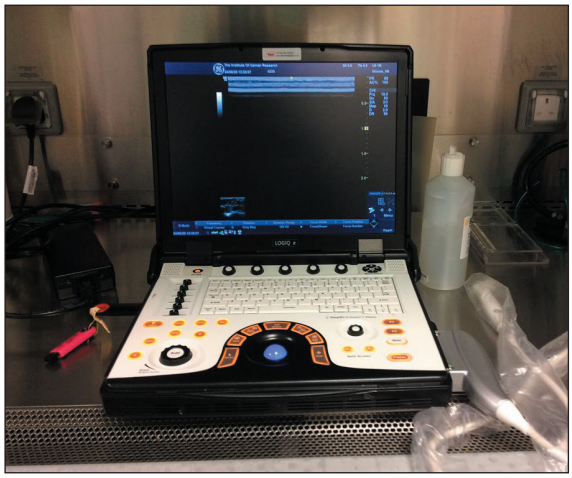

Figure 1.

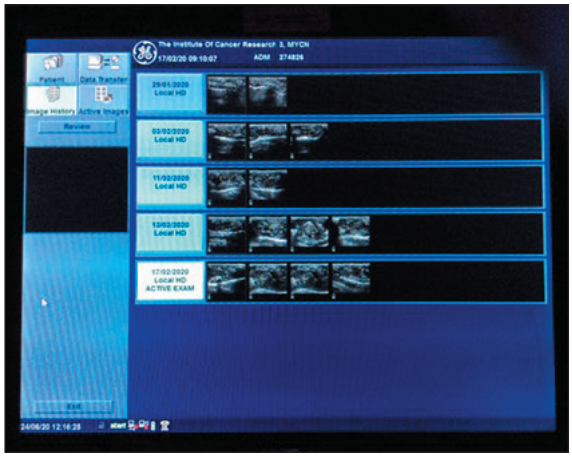

Figure 2.

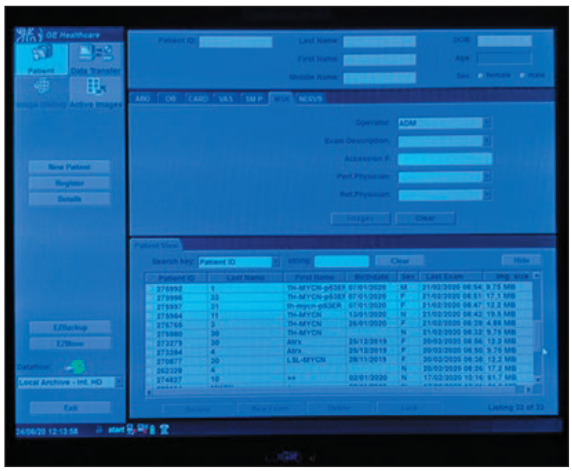

Figure 3.

Control imaging

Spontaneous tumours develop on thoracic region of the spinal column and between the kidneys. Control images help locate the kidneys and spinal column to detect the tumour more efficiently.

Lateral view of the abdomen

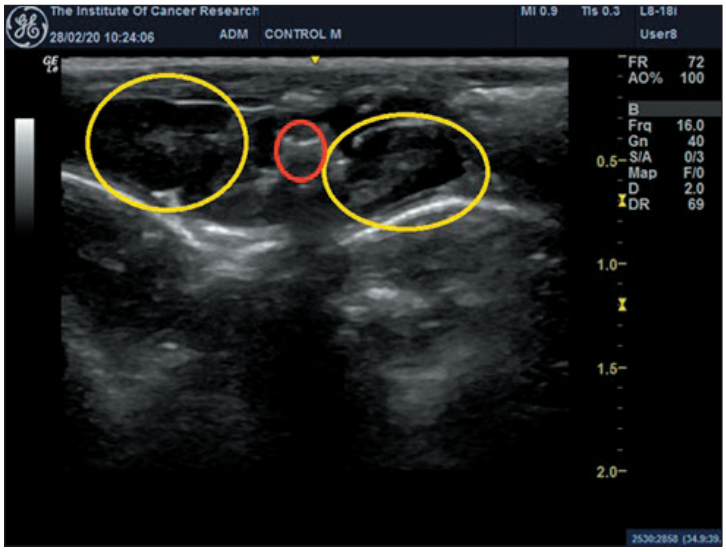

Figure 4. Kidneys and spinal column.

Medial view of the abdomen

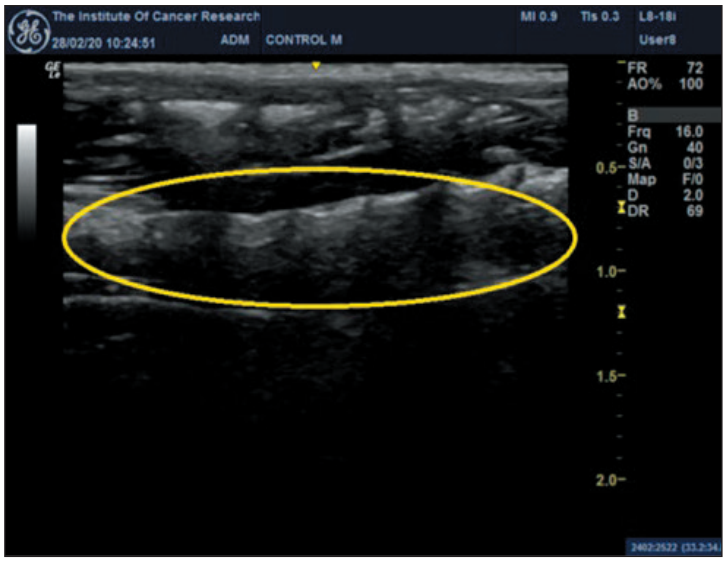

Figure 5. Spinal column.

Detection and monitoring of tumour images

Lateral view of the abdomen 14 days apart

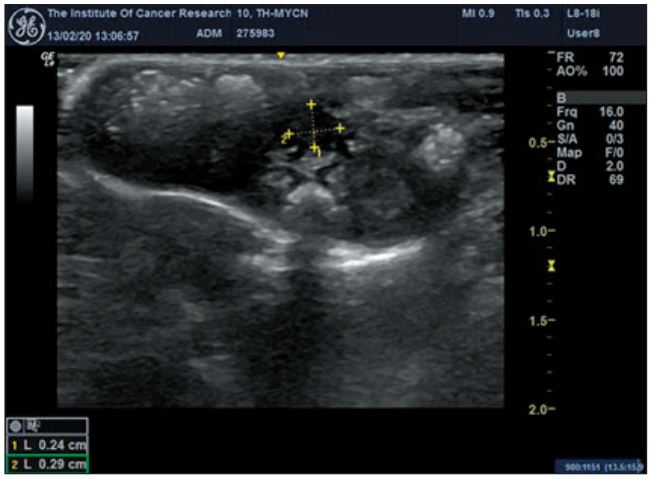

Figure 6. Day of tumour detection.

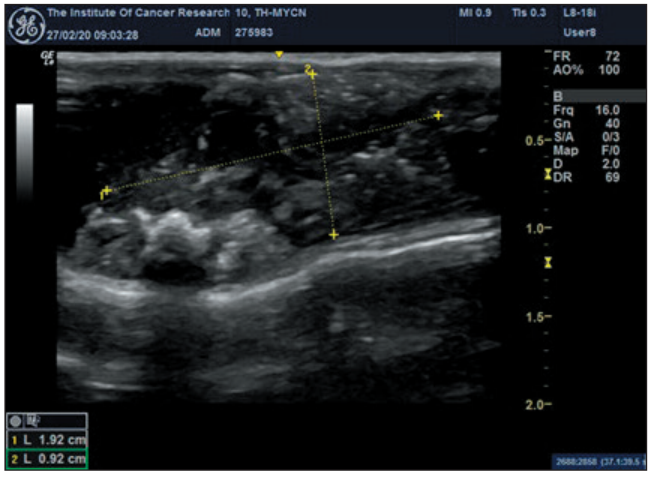

Figure 7. Day mouse was culled.

Medial view of the abdomen 14 days apart

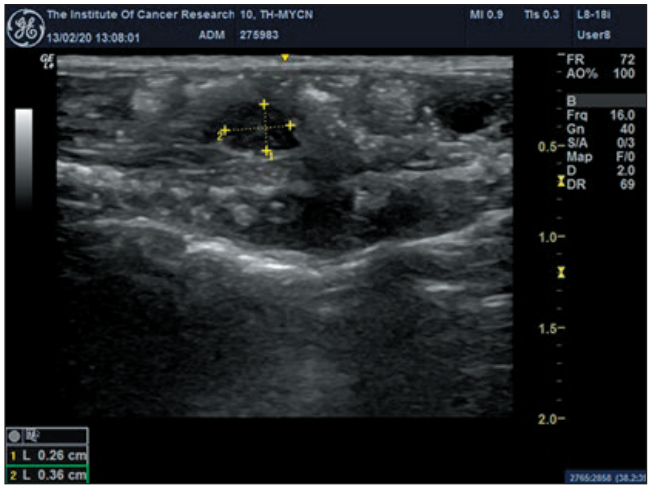

Figure 8. Day of tumour detection.

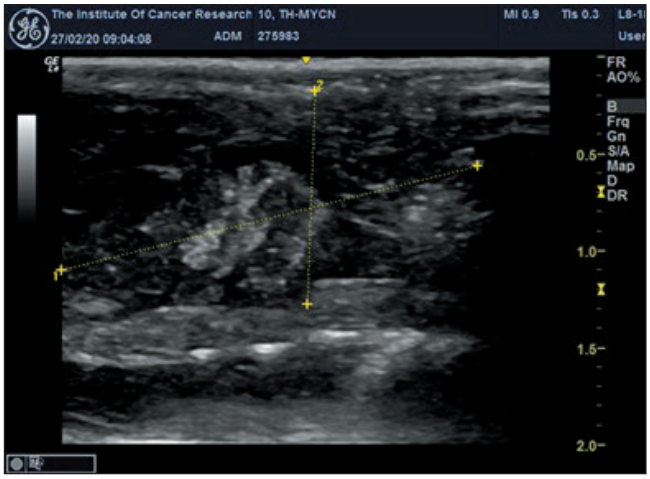

Figure 9. Day mouse was culled.

Palpation v Ultrasound measurements

Ultrasound measurements are significantly more accurate than palpations. With the ultrasound we are able to generate a number of dimensions, measurements and volumes of one tumour. With palpation we are only able to estimate one dimension and measurement with large variations between different people.

Measured on	Type	X	Y	Volume
13/02/2020 00:00	Palpation	2	0	2
17/02/2020 00:00	Palpation	4	0	4
20/02/2020 00:00	Palpation	6	0	6
24/02/2020 00:00	Palpation	7	0	7
04/03/2022 00:00	Palpation	10	0	10

Measured on	Type	X	Y	Volume	Type	X	Y	Volume
13/02/2020 00:00	Lateral	0.24	0.29	0.0084	Medial	0.26	0.36	0.0122
17/02/2020 00:00	Lateral	2.68	3.01	10.8095	Medial	3.98	2.69	21.3053
20/02/2020 00:00	Lateral	6.78	5.36	123.1953	Medial	10.56	6.89	384.1644
24/02/2020 00:00	Lateral	8.94	7.29	291.3215	Medial	15.39	7.45	882.2741
27/02/2020 00:00	Lateral	10.92	9.92	591.4621	Medial	20.21	10.1	2062.6427

Acknowledgements

Allan Thornhill, Head of Biological Services.

Exploring Treatments for Idiopathic Chronic Diarrhoea (ICD) In Rhesus Macaques *(Macaca mulatta)*: A Systematic Review

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Introduction

Idiopathic Chronic Diarrhoea (ICD) is an intestinal disorder prevalent in captive Non-Human primates (NHPs). It is characterised by recurrent loose stool in the absence of classically presented diarrhoeal causing pathogens (Westreich *et al*, 2019).[1] Annually, ICD affects upwards of 15% of NHPs in a colony, posing a serious clinical threat disturbing both research and stock (Blackwood *et al,* 2008).[2] Monkey's experiencing ICD are likely to suffer from severe dehydration, weight loss, and poor body condition. Often leading to a rapid decline in individual welfare accounting for 33% of euthanasia unrelated to research.[2]

The Pathogenesis of ICD is poorly understood. It is complex and seemingly multifactorial. However, an inflammatory component is strongly implicated. Histopathologic analysis of colonic tissue specimens from primates suffering from ICD, indicate surface gut epithelium changes such as goblet cell depletion (Ardeshir *et al.*, 2013).[3] A compromised mucosal barrier allows for increased bacterial attachment leading to dysbiosis of the mucosal microbiota (Broadhurst *et al* 2012).[4] A decreased microbial diversity causes an imbalance in normal gut flora (Figure 1). Commensal bacteria may become pathogenic and trigger an immune response that leads to inflammation.

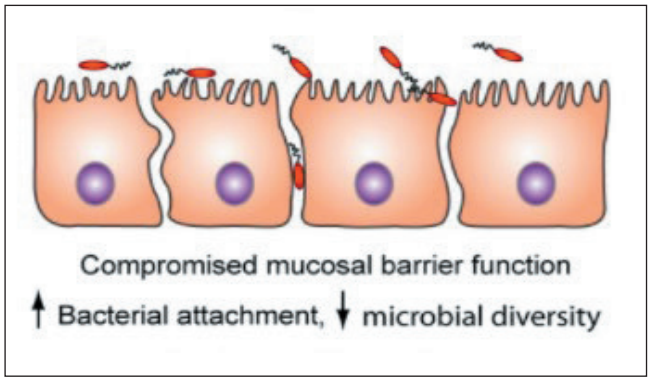


Figure 1. Diagram depicting bacterial attachment to gut epithelium causing a decreased microbial diversity.[4]

Method

A systematic review was conducted on three different databases Research Gate, Web of Science, and Sematic Scholar. A carefully directed search query was utilised to retrieve the most relevant articles. (Figure 2)

(Idiopathic Chronic Diarrhea OR ICD) captive 'Rhesus macaques' + treatment

Figure 2. Search string used.

The articles yielded were screened. All duplicates and irrelevant papers were removed. A strict inclusion and exclusion criteria was developed to select articles based on their relevance to the topic and their novel implications for use in an animal research centre. All randomised clinical trials exploring potential treatments that worked on stool consistency charts were selected. (Figure 3)

The data collected was grouped in order of the most efficient and practical way of managing ICD in a research centre.

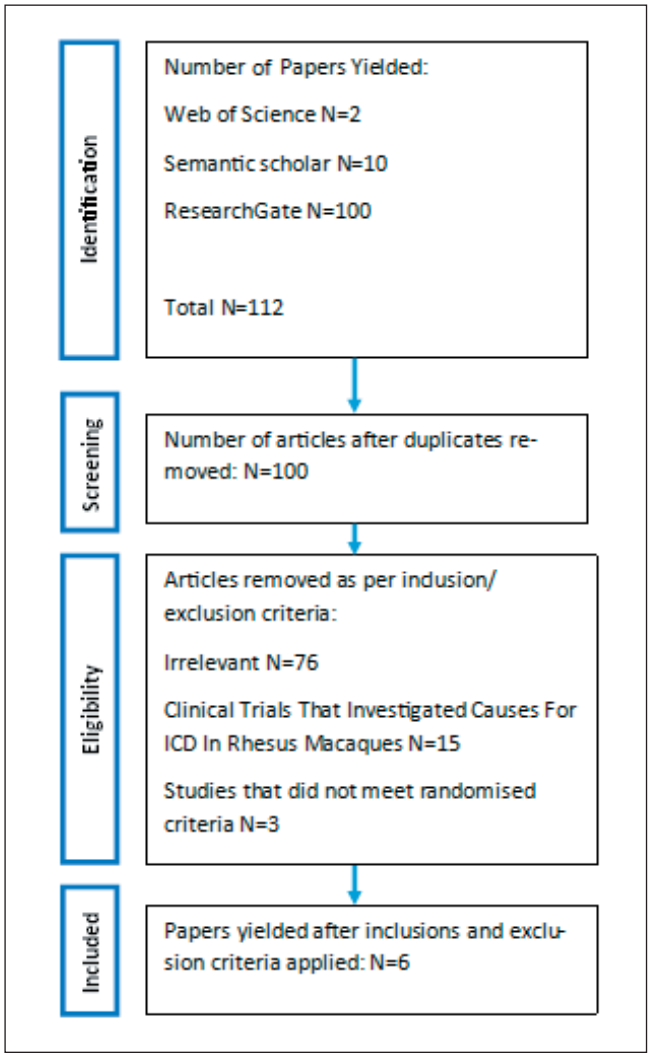
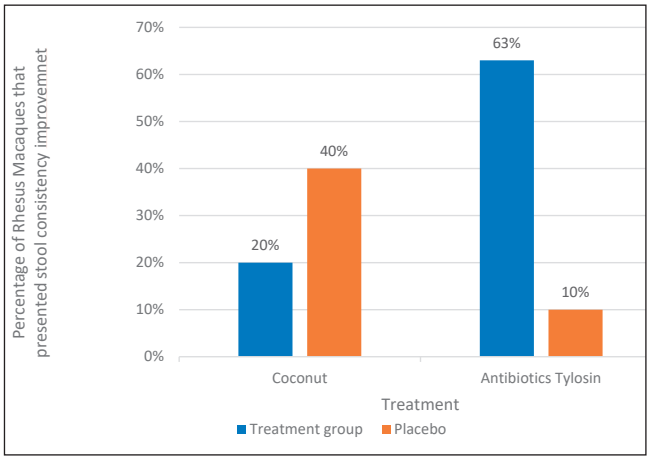


Figure 3. Identification of eligible papers.



Graph 1. Graph depicting the percentage of primates that had improved stool consistency for both treatment groups. Coconut (N=10), Antibiotic (N=21).

Results

Six randomised clinical trials (RCT), that included the use of a stool sample consistency charts, met the criteria and were included in the analysis. The RCT's were separated into treatment categories and presented in the table below (Table 1).

Potential Treatments	Number of randomised Clinical Trials Yielded	Article Author	Patient Cohort
Prebiotics Insulin	3	Ardeshir *et al* 2011	32 Total (placebo N=14) (Treatment N=18)
		Ardeshir *et al* 2012	32 Total (placebo N=16) (Treatment N=16)
		Ardeshir *et al* 2014	32 Total (placebo N=16) (Treatment N=16)
Coconut	1	Wilk, J.L. *et al.*, 2008	10 Total (placebo N=5) (Treated N=5)
Antibiotics 10-day course of Tylosin	2	Blackwood *et al.*, 2008	21 (placebo N=10) (Treated N=11)

Table 1. Characteristics of Randomised Clinical Trials.

Data was subtracted from the articles and presented in the form of a graph (Graph 1). Clinical success was measured by improvement in stool consistency. The percentage of Rhesus macaques that showed improvement was calculated and compared with the placebo group.

One RCT was eligible for coconut treatment. Only one primate out of five achieved a favourable response compared with two out of five for the placebo group. The outcome was not statistically significant.

One RCT was eligible for Antibiotic Treatment (Tylosin). Seven out of eleven primates displayed clinical improvement when compared with the placebo where one out of ten showed improvement. The outcome was statistically significant.

Discussion

Coconut

The benefits of coconut are attributable to its lauric acid component which has antimicrobial properties that supposedly contribute to a healthy gut microbiome. Although the article retrieved expressed a less than favourable outcome, the small sample size and the form coconut was delivered (coconut macaroons) may have impacted the results. Coconut oil has been proven to show clinical success in mouse-models with induced Colitis (Alok, 2013).[5] Perhaps, exploration into different methods of coconut consumptions and treatment quantities could hold more favourable findings. In any case coconut potentially provides an attractive option for treatment, it is palatable non-invasive and can easily be incorporated into enrichment.

Although all three RCT's for prebiotics were eligible, the full articles were not accessible. Retrieving specific data was unsuccessful. However, all three articles expressed statistical significance for treatment group when compared with the placebo group.

Antibiotics

Although the antibiotic Tylosin showed a significant improvement in faecal stools, 39% of primates suffered a relapse, suggesting continued use of treatment is necessary to maintain positive results. Long-term antibiotic use is associated with a host of disorders and the risk of developing antibiotic resistant bacteria. Furthermore, antibiotics may not be an ideal candidate for macaques on study as it may interfere with test compounds and have major impacts on results.

Prebiotics

Prebiotics are indigestible substances, usually a carbohydrate that promote the growth of healthy bacteria by supplying nutrition. Improved gut flora reduces inflammation and alleviates symptoms. All three articles retrieved, expressed clinical improvement in treatment groups when compared to the placebo. A major drawback in this review was the failure to have full access to the data presented. Prebiotics like Inulin have been reported to cause side effects such as nausea and stomach pain in humans. Access to more research is essential in evaluating prebiotics as a treatment plan.

Conclusions

Considering the scale of ICD in primate colonies, the gap in the literature is prevalent. Clinical management of the disease is often difficult and unrewarding largely due to the lack of knowledge surrounding pathogenesis.

Ideally, the best course action would be preventative care. A study by The National Primate Research Centre in California involving 1,930 *R.macaques* supported a relationship between stressors and the development of ICD. Particularly on primates that exhibit more gentle personality traits (Gottlieb et al, 2018).[6] Applying refinement techniques and providing a fully enriched environment may well negate the effects of ICD. However, within research stressors such as procedures as well as rehousing and disturbances in social hierarchy is often unavoidable. In these cases, the necessity of research into treatment may be the only viable option. Treatment is essential in providing healthy candidates for research as well as improving welfare standards and accuracy of results.

Future Work

Currently, the Biological Investigation Group (BIG) in Porton Down uses probiotics to treat primates with chronic diarrhoea. Unlike prebiotics, probiotics provide the body with live bacteria that support the normal gut flora to reduce inflammation. This systematic review did not yield any articles supporting the use of probiotics and outside research was scarce. Although it seemingly has a positive effect on primates housed in Porton Down UK Health Security Agency (UKHSA), more research is needed to provide an accurate evaluation of the current treatment being implemented.

In the meantime, reducing stress and providing enrichment is the main focus of the BIG group (Figure 2). Until a prominent resolution is bought forth.

Figure 4. Example of primate enrichment at the Biological Investigation Group, Porton Down.

References

1 **Westreich, S.T., Ardeshir, A., Alkan, Z., Kable, M.E., Korf, I. and Lemay, D.G.,** (2019). Fecal metatranscriptomics of macaques with idiopathic chronic diarrhoea reveals altered mucin degradation and fucose utilization. *Microbiome, 7*(1), pp.1-17.

2 **Blackwood, R.S., Tarara, R.P., Christe, K.L., Spinner, A. and Lerche, N.W**., (2008). Effects of the macrolide drug tylosin on chronic diarrhoea in rhesus macaques (Macaca mulatta). *Comparative medicine, 58*(1), pp.81-87.

3 **Ardeshir, A., Oslund, K.L., Ventimiglia, F., Yee, J., Lerche, N.W. and Hyde, D.M**., (2013). Idiopathic microscopic colitis of rhesus macaques: quantitative assessment of colonic mucosa. *The Anatomical Record, 296*(8), pp.1169-1179.

4 **Broadhurst, M.J., Ardeshir, A., Kanwar, B., Mirpuri, J., Gundra, U.M., Leung, J.M.**, *et al* (2012) Therapeutic Helminth Infection of Macaques with Idiopathic Chronic Diarrhea Alters the Inflammatory Signature and Mucosal Microbiota of the Colon. *PLOS Pathogens November 15, 2012.*

5 **Alok, Pranav Chandra**, (2013) Effect of Coconut Oil on Ulcerative Colitis in the Mouse Model. *Masters Theses & Specialist Projects.* Paper 1261. https://digitalcommons.wku.edu/theses/1261

6 **Gottlieb, D.H., Del Rosso, L., Sheikhi, F., Gottlieb, A., McCowan, B. and Capitanio, J.P.,** (2018). Personality, environmental stressors, and diarrhoea in Rhesus macaques: an interactionist perspective. *American Journal of Primatology, 80*(12), p.e22908.

Let us choose which food enrichment we prefer' – enrichment from a mouse perspective

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Introduction

This study focussed on rodents, specifically looking at food preferences as a source of rodent enrichment. The study method used was the preference test using two shapes of pasta and sunflower seeds.

- The rodent preference test was monitored by visual observation to record the amount of mice interacting with each type of enrichment.
- The mice were weighed and their teeth checked weekly to assess any impact on behaviour, health and the body condition score.
- The benefit of giving the animals food enrichment may allow the animals to express natural behaviours of storing food within their nest and reduce stress and stereotypic behaviours. The enrichment may also be seen as a reward after handling and also assists technicians and researchers train the animals in our care.

Figure 1. Cage containing food enrichment.

- Prior to deciding on this trial, the idea of giving food enrichment was discussed with the Named Animal Care Welfare Officer (NACWO) and the Named Veterinary Surgeon (NVS), both agreed that this would be something worth trialling.

Methodology

Two cages of appropriate mice were selected, these were two cages of 5 female mice. This was some of our older wild type stock, DOB: 20/5/21. These mice were made identifiable using tail marks.

Figure 2. Study wild type mice.

Prior to the introduction of the food enrichment, the mice were weighed and given a thorough health check. Using this information, we could compare further weight gain/loss. Weighing would be performed on each following Monday until the end of the study.

These two cages were both given access to each form of enrichment, this included: penne pasta, macaroni pasta and some sunflower seeds.

This was given to the mice twice a week, on a Tuesday and Friday for four weeks.

Figure 3 and 4. Mice interacting with the food enrichment.

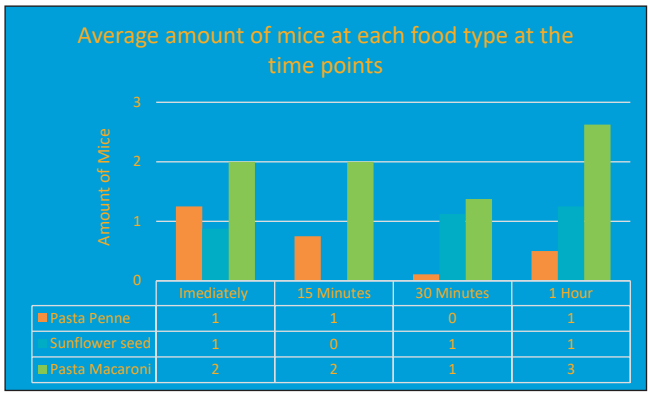
To avoid any data bias in the study, the location of each type of enrichment was moved to a different area of the cage.

The observation stage was performed for both cages and the amount of mice interacting with each enrichment was recorded.

I observed both cages for the first 5 minutes after introduction of the enrichment, then again after 15 mins, 30 mins and an hour following the introduction of the enrichment.

During observations I would record how many mice from the top cage were at each enrichment at that time.

Data



Graph 1. Average number of mice interacting with different food types at study time points.

Cage/ Animal Number	Weights				
S122/21	22/22/2021	29/11/2021	06/12/2021	13/12/2021	20/12/2021
_1 (1 DOT)	29.1	28.4	28.0	27.9	28.6
_2 (2 DOTS)	32.6	31.3	32.0	31.8	32.2
_3 (3 DOTS)	38.6	37.8	38.8	39.2	39.4
_4 (1 LINE)	28.3	27.7	28.8	29	28.7
_5 (NO DOTS)	23.6	23.5	23.4	23.6	23.1
S127/21					
_1 (1 DOT)	35.2	34.1	33.1	33.0	33.3
_2 (2 DOTS)	30.9	30.4	30.3	30.1	30.4
_3 (3 DOTS)	28.1	27.5	27.2	27.3	27.8
_4 (1 LINE)	27.8	26.8	27.1	27.3	27.1
_5 (NO DOTS)	30.1	29.0	29.7	29.9	29.1

Chart 1. Mouse weights during study period.

Discussion

Benefits of using food as enrichment for mice:

- Maintains healthy teeth.
- Simulation of foraging behaviour.
- Stimulates their brains to be more active, handling the food with forelimbs.

- Reduces distress and stereotypies by learning that a treat would be given after handling / procedural work.
- Eases handling difficulties and improves interaction with technologists.
- May help mice increase weight if they have lost any during procedural work.

Potential limitations or drawbacks of using food enrichment for mice:

- Weight gain.
- Reduced intake of the 'complete diet' provided in the cage, may lead to deficiencies if the mice would rather eat enrichment than their food.
- Interference with experimental design.
- Creates more mess in the cages, may require more work to clean the cages afterwards.

Conclusion

Although weight gain would be a potential worry for the health of the animals, the weight of the mice in this trial only varied slightly week to week.

On the second weighing occasion, the mice all seemed to have lost a small amount of weight.

An observation made during this trial was that the mess in the cages was slightly more which was almost completely due to the shells of the sunflower seeds.

As can be seen in our graph, the average number of mice tended to be higher with the macaroni.

The graph also shows a slight preference towards the macaroni but the mice did appear to eat the sunflower seeds quite a bit as well. Whereas the large pieces of pasta were often nibbled but rarely fully eaten.

Our data shows the macaroni had slightly higher interaction observations and the fact that the seeds created more mess would mean the macaroni was a better overall enrichment for the mice.

Acknowledgements

Thanks to the full BSF team at York for help and support.

References

1 **Rabl, R., Horvath, A., Breitschaedel, C., Flunkert, S., Roemer, H. and Hutter-Paier, B.,** (2016). Quantitative evaluation of orofacial motor function in mice: The pasta gnawing test, a voluntary and stress-free behaviour test. *Journal of Neuroscience Methods*, 274, pp.125-130.

2 **Ratuski, A. and Weary, D.**, (2022). Environmental Enrichment for Rats and Mice Housed in Laboratories: A Metareview. *Animals*, 12(4), p.414.

Medicated jelly as a replacement for injectables and the use of Maropitant to manage itchy skin in mice

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Background

It is desirable to administer drugs by the least invasive route to (1) optimise welfare, (2) reduce the risk of injury to animal handlers and (3) minimise the impact of restraint and transient pain on physiological and experimental readouts.

- Provision of medications in a palatable form to be consumed voluntarily is ideal for drugs that need to be given repeatedly e.g. for post-surgical pain relief.
- Bio-Serv® Electro-Gel™ is an electrolyte-balanced hydration gel that has thermal reversing properties to enable preparation of medicated versions.
- We tested whether commonly used drugs provided in Electro-Gel™ would be effective and tolerated by mice as a replacement to injectable versions, and to reduce the need for handling of mice.

Pilot testing

- During preliminary testing, control C57BL/6 mice were provided with placebo versions of both strawberry and orange Electro-Gel™ as enrichment. *Mice consumed both flavours but preferred the strawberry version.*
- During a pilot study, which involved surgical implantation of radiotelemetric devices, we successfully trialled carprofen Electro-Gel™ Strawberry for post-surgical pain relief.
- All mice were comfortable in the week post-surgery but typically started to scratch at surgical wounds during later stages of healing (around day 10) as scabs formed. This increased the risk of wound opening and infection.
- 70% of mice in the pilot study were observed showing pruritus (itchy skin) after surgery which led to wound opening and mice having to be culled via Scheule 1 method.
- A previous study (Williams-Fritz *et al*, 2011),[1] showed that the anti-emetic maropitant citrate was an effective anti-pruritic for mice suffering with ulcerative dermatitis.
- We decided to trial Maropitant and (antibiotic) versions of strawberry Electro-Gel™ for managing post-surgical pruritus and infection in implanted mice.

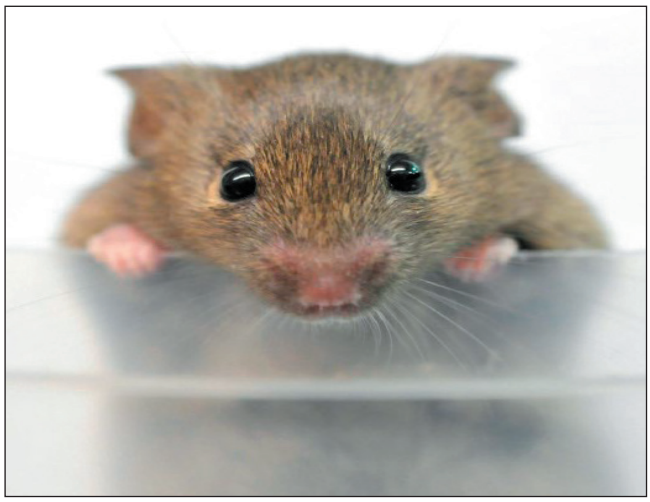
Extended study trial

49 mice (C57BL/6 background) underwent several days of training with placebo strawberry Electro-Gel™ before surgical implantation.

- During the 14-day surgical recovery period mice were singly housed in divided GM900 rat cages.
- Mice received a single dose of Carprofen strawberry Electro-Gel™ on days 1,2,3,5,7,9,10,11 and 13.

– Maropitant Electro-Gel™ was provided as soon as scratching was observed as single daily doses for up to 3 consecutive days until the condition resolved.

– Enrofloxacin Electro-Gel™ was provided as a single daily dose for 3-5 days in incidences where there was a suspected high risk of infection (e.g. self-trauma requiring minor wound repair).



Electro-Gel™ preparation

Each 1oz pot of Electro-Gel™ contains 27ml.

Step 1 – place sealed tub in hot water for 5 min to liquify.

Step 2 – using sterile insulin syringe, add drug of choice as follows by injection through lid.
0.16ml carprofen (50mg/ml Rimadyl solution)
0.32ml maropitant (10mg/ml Cerenia solution)
0.65ml (25mg/ml Baytril solution)

Step 3 – seal needle puncture hole with tape and label/ date.

Step 4 – shake gel mixture to ensure even distribution.

Step 5 – the liquid can now be dispensed into smaller volumes or left in the original container. Place in fridge for at least 20 min to return to gel state.

Step 6 – provide gel dose to mice as follows:

Mouse weight (g)	ElectroGel Dose (g) – max once daily
21	0.35
24	0.4
27	0.45
30	0.5
33	0.55
36	0.6

Electro-Gel™ should be covered to prevent evaporation and may be kept refrigerated for up to 1 week.

Each dose should be offered in a 35mm petri dish lid. Unconsumed ElectroGel™ should be removed from cages after 24h and replaced if necessary.

Results

– 47/49 implanted mice readily ate medicated strawberry Electro-Gel™; the remaining 2 mice ate medicated orange Electro-Gel™, especially if Bio-Serv® Rainbow Foraging Bits were mixed in.

– 44/49 mice were treated with Maropitant for pruritus within the 14-day recovery period.

– 3/49 mice were euthanised during the 14-day recovery period; these were females with late, self-trauma induced wound infection that did not resolve with Enrofloxacin.

– We found that recovery ranged between 6 – 17 days, with 27% of mice fully recovered within 10 days of treatment.

Summary

– The use of Electro-Gel™ over Injectables was **a refinement** and **reduced** the number of needle pricks a mouse received and **reduced** the need to handle the mice.

– Mice freely ate medicated Electro-Gel™, and once acclimatised would freely eat it if offered months later.

– Electro-Gel™, was an effective for providing 3 different drugs in a pain and stress-free way for singly housed mice.

– **Maropitant Electro-Gel™ appears to be successful for managing pruritus related to later stages of wound healing. We observed that 94% of mice treated with Maropitant recovered.**

– We found that the optimum treatment regime was to offer 3 days of Maropitant Electro-Gel™ followed by a 1 day break repeated 2 times.

Further Use

– We have since used Maropitant Electro-Gel™ for mice, undergoing different surgeries, that also scratched at their wounds post-surgery. This helped to prevent further wound deterioration.

– Our Named Veterinary Surgeon (NVS) now advises on the use of medicated jellies for our stock mice that have wounds caused by over grooming or fighting.

– **We plan to investigate if Maropitant provides a successful treatment for mice suffering from Ulcerative Dermatitis who show signs of pruritus. Observations will be made to see if Maropitant improves this condition.**

– Our vasectomised stud males are now provided with carprofen Electro-Gel™ for 3 days post-surgery.

– We plan to conduct a randomised blinded study to compare the effect of carprofen Electro-Gel™ versus carprofen injection on post-surgical outcomes including comfort, behaviour, wound healing and recovery time.

Acknowledgements

Kevin O'Connell, Keith Mayes, Jessica Banbury, Lara Few, Katie Higginson, Eleni Vioumidi, Nina Rzechozek, MRC Ares.

References

1 **Williams-Fritz *et al***. (2011). Maropitant citrate for treatment of ulcerative dermatitis in mice with a C57BL/6 background. *J Am Assoc Lab Anim Sci. 2011 Mar;* 50(2):221-6.

2 **Rzechorzek *et al.*** (unpublished). O'Neill Lab Mouse Radiotelemetry HHEP and EGP.

3 Bio-Serv protocol for preparation of carprofen Electro-Gel™.

The evolution of how Guinea pigs are housed at high containment in the Biological Investigations Group

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Introduction

Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis*. It usually attacks the lungs; however, TB can affect any part of the body such as spine, kidney, and brain. New interventions against TB are desperately needed globally to treat humans and animal models are important to progress new treatments to the clinic.

Many different animal species are susceptible to infection with this organism. However, the most used experimental animal models are Guinea pigs, Non-Human Primates (NHPs) and mice. The Guinea pig model has been used for more than 100 years as a research tool. The disadvantage of TB studies in animals is that the disease progression is naturally slow and therefore the animals require need to be housed for longer periods of time.

When housing any species within the facility, we always consider the 3Rs – namely Reduction, Replacement and Refinement. Improving the caging system has allowed us to promote improved animal welfare and observe the 3Rs.

Improvements to the caging system will allow the animals to express their natural behaviours such as foraging, which keeps them active, alert and makes them feel happier in their environment.

Historically TB studies are carried out in flexible film isolators which allow us to house a large number of Guinea pigs in pairs. Due to changes in housing requirements within the Code of Practice, we reassessed how Guinea pigs are housed for long term studies due to their increased size as they get older. We took this opportunity to explore changes that will positively influence the welfare of the Guinea pigs.

Flexible film isolators can be difficult for technologists to work in, due to challenges with the need to move heavy cages, basic husbandry, cleaning out and removal of waste. Another major point is that the dexterity of staff is restricted when in the isolator due to the suit/gloves needing to be a standard size to fit all and layers of gloves you must wear when working makes it harder for basic tasks and procedures.

Methods

Previously, we housed Guinea pigs in pairs, where study design allowed, using RC2R cages (NKP-Isotec). (Figure 1).

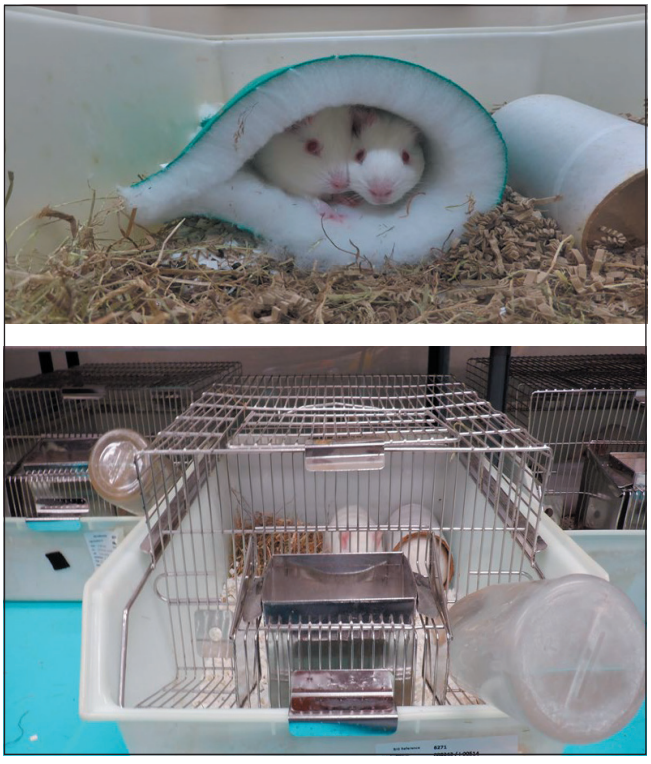

Figure 1. Pair housed Guinea pigs and RC2R cage (NKP-Isotec).

Our change was to house the Guinea pigs in bigger groups with more space. This resulted in moving the Guinea pigs into a floor pen, which allowed them more space and to be housed into groups of 8. This was done within Containment level 2 (CL2) to trial. (Figure 2)

Figure 2. Floor pen used to house group housed Guinea pigs.

We noticed that when the Guinea pigs were housed in the floor pen, they became more active and alert and more interactive with the technicians entering the room each day. It also allowed us to make and provide more enrichment for them which they enjoyed thoroughly. As well as this, they were able to express their natural behaviour, due to having more room and places to hide and forage.

Although this floor pen was an improvement, it was impractical for Containment Level 3 (CL3). Some of the considerations taken into account was the volume and velocity of the air required at floor level (in order to create a laminar flow away from the operator) would have a detrimental effect on the animal's welfare i.e. significant draughts. It would also be extremely challenging to maintain environmental conditions in line with ASPA. Also, the use of suitable substrate for bedding would be limited, ruling out the use of sawdust, nesting material etc.

Even if this were mechanically possible, the containment boundary would be breached each time the animals needed to be handled, i.e the operator would have to enter the 'contaminated' zone upon each intervention.

The final idea was to use our double tier caging system as we have used these previously at CL3 with different animal species. The system was first trialled with naïve Guinea pigs within the directional airflow CL3 suite.

Results

The test trial of the Guinea pigs in the double tier caging proved successful. (Figure 3). Since then, we have completed two CL3 studies using this caging system. We have noticed the change of behaviour since they have been housed in these cages. The Guinea pigs now show more natural behaviours such as foraging, this is aided by being able to use a deeper substrate.

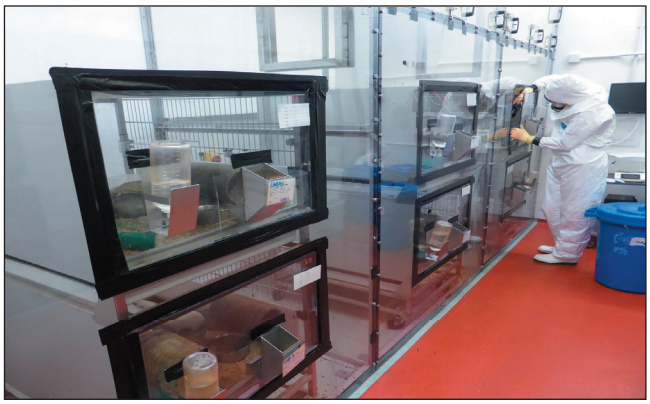

Figure 3. Shows CL3 housing and a technologist taking an air reading in the CL3 suite.

In these cages at CL3, the maximum number of Guinea pigs housed in one cage was six. The other groups of cage mates that we housed was four and two. The groups which housed six Guinea pigs showed that they were more active and more sociable with one another. (Figure 4). Grooming between cage mates was observed within certain individuals where they licked and nibbled at each other's coats. This behaviour helps with group bonding.

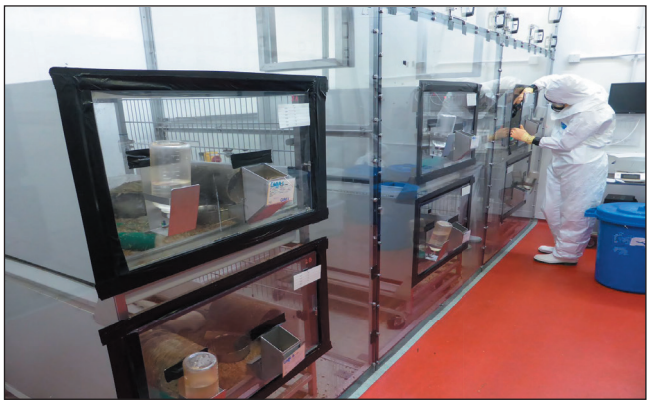

Figure 4. The Guinea pigs housed within the CL3 suite.

In these larger cages we were able to assess and health check animals more efficiently as we could see behaviours more clearly in the home cage rather than placing the Guinea pig into a separate holding box – which can cause them stress. As they are prey animals, they are easily scared. Therefore, when individuals are picked up their respiratory rate may quicken which will

affect the clinical scoring system as each study used its own scoring system applicable to the study.

The animals have also shown more positive behaviours which suggest that they are happy; behaviours include 'popcorning' and vocalisations such as 'wheeking' – this is a high-pitched sound which denotes happiness and excitement. 'Popcorning' is the behaviour where a Guinea pig leaps and jumps in the air, a head shake can also be seen. Using this caging, we have also seen that Guinea pigs like hides that are higher up and off the floor. Through using different levels, it allows us to give the animals more exercise such as jumping – which promotes good muscle tone.

The study director analysed the weights of the Guinea pigs in the different housing systems comparing the old caging to the new caging and plotted it on a graph. (Figure 5). The data showed that the rate of growth of Guinea pigs is the same in both the old and new caging system. However, the Guinea pigs in the new caging are consistently lower in weight compared to those in the old caging system – this difference may be because the animals in the new caging system are getting more exercise and animal to animal interaction, due to more space in the new cage system compared to the older caging system.

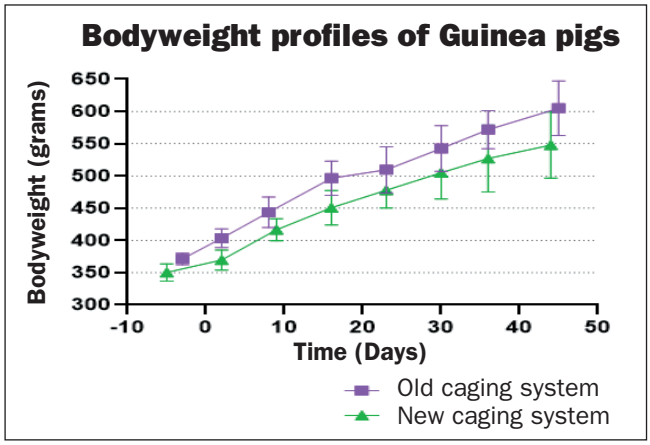

Figure 5. Shows the weight change between the new and old cage systems.

Discussion

The caging which housed pairs of Guinea pigs, is ideal for short-term studies such as diphtheria and anthrax because the Guinea pigs are not in there for a long time, so they do not grow to full size. The caging also works well for our studies when they are housed within CL3 isolators (Figure 6). These cages are easy to use as they are made from polypropylene which ensures that they are sturdy and robust. Using the material allows for the cages to be cleaned easily and as polypropylene is a non-porous material cages can be fumigated and put into an autoclave for sterilisation and then cleaning.

However these cages are not ideal for long-term studies as there is a limit to what enrichment we can give them although a play tunnel and an aspen brick to chew on is provided. Whilst using the RC2R cages for the TB studies we found that as the animals are in there for a long time, the bedding became soiled easily and cages needed clean outs every other day. These cages also have potential to cause health issues for the Guinea pigs such as urine burns on their feet causing discomfort and infections.

The floor pens which we trialled for a few weeks worked well, we could house up to 8 Guinea pigs in there, still leaving plenty of space for animals to move and grow. These floor pens are easy to assemble and clean – however, due to having plastic on the side panels and door they cannot be autoclaved due to it melting, so they could only be used within CL2.

Although, the caging is suitable for the Guinea pigs, it is not compatible with the directional flow CL3 system. However, the caging is ideal for making enough levels and sufficient spaces for animals to hide. The cleaning routine was easier and less stressful for the Guinea pigs due to us only cleaning them out once a week with 'spot' cleaning when needed, unlike when in the smaller housing which required cleaning out every other day.

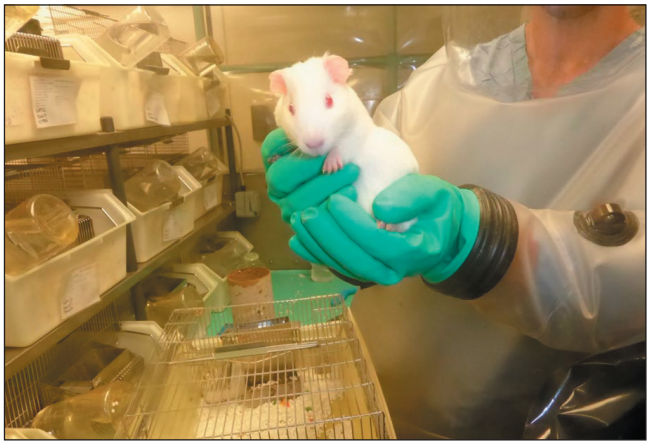

Figure 6. Guinea pigs housed in a CL3 flexible film isolator.

Future work

With future studies, when housing animals within the RC2R cages the overall weight of both Guinea pigs must be under 250g. If the weight is over 250g only one Guinea pig can be housed in a RC2R cage.

We would like to make bespoke cages for the directional flow suite that will make better use of the width of the bay and reduce the depth. This will be more ergonomic for the technicians.

Also, in the future, we aim to make more durable and practicable enrichment that can be decontaminated

and reused across other Guinea pig studies. We want to be able to make different levels for Guinea pigs to explore and more interactive enrichment. We will need to evaluate the benefits of this new system by looking at the enrichment and comparing data from Guinea pigs housed in isolators. We are always working towards the 3RS and to improve the animals environment. Working towards the 3Rs allows for higher welfare standards which results in better science.

Acknowledgements

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References

1 **Clark, S., Hall, Y., Williams, A.** (2014). Animal models of tuberculosis: Guinea pigs. *Cold Spring Harb Perspect Med. 2014 Dec 18.5(5) a018572*.

2 **NKP-Isotec. 2022**. ***Laboratory Rat Housing***. https://nkpisotec.com

3 **Vet Help Direct 2022**. Why does my Guinea pig jump? What is 'popcorning'?' https://vethelpdirect.com