CAGE SIDE DETERMINATION OF POST-MORTEM INTERVAL IN MICE

S. Hopkins✉, M. Hitcham, L. Hughes-Hallett, C. Watson, O. Woolmer, C. Frost, D. Lafont, R. Buckmaster, E. Cambridge, A. Thomas.
Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.
✉ : sb37@sanger.ac.uk

INTRODUCTION

Unexpected death of animals within a research facility can indicate underlying welfare issues, cause the loss of scientific data and potentially represents a compliance breach under A(SP)A.

Currently there is no validated scoring system to estimate the post-mortem interval in mice beyond *rigor mortis*[1].

The aims of this preliminary study were to;

1. Document internal and external macroscopic post-mortem changes in wild-type mice between 0-48h;
2. Develop a simple visual cage-side scoring system, to help technicians estimate the time of death.
3. Inform and improve Home Office reporting of unexpected mouse deaths.

MATERIALS AND METHODS

30 male mice (C57BL/6$^{c-/c-}$; 15-18 weeks old) were used in this prospective, randomised and 'blind' study.

- The mice were euthanised in their home cage (CO_2, 20% cage volume.min^{-1}) and were randomly allocated to 1 of 5 groups: examination at 0, 3, 16, 24 and 48hr post-euthanasia (n=6 per group).
- The cadavers were maintained in ventral recumbency at stable temperature (22 ± 2^{o}C) and humidity (53 ± 5%), singly housed on aspen chips bedding within Techniplast GM500 cages until *post-mortem* examination.
- Rectal temperature was measured at the time of dissection.
- Post-mortem examinations and dissections were performed as previously described[2] by an operator unaware of the time of death of the animal. The operator scored the qualities of internal and external organs against a composite 3-points scoring system (Green, Amber, Red) developed during a pilot study.
- Organs scored included: eyes (EE); skin and extremities (SE); abdominal cavity (AC); liver and gallbladder (LG); spleen (SP); intestines (IN).

- Data was analysed using Fisher's Exact test for categorical data with pairwise comparisons (for organs) and one-way ANOVA with Tukey's multiple comparison (rectal temperatures) to give results for statistical significance between all the time-points.
- All analysis was performed using R version 3.6.0[3] with package R companion version 2.3.25[4].

RESULTS:

The appearance of the abdominal cavity; liver/gallbladder; spleen and intestines changed significantly between 0 and 48hr *post-mortem*. The changes observed on the skin, extremities and eyes were qualitatively identifiable but not statistically different by scoring.

Changes between 0-3hr: No significant change was identified in any of the organs scored. However, the rectal temperature decreased to 21.9 +/- 0.8 degree Celsius by 3h, in equilibrium with the room temperature.

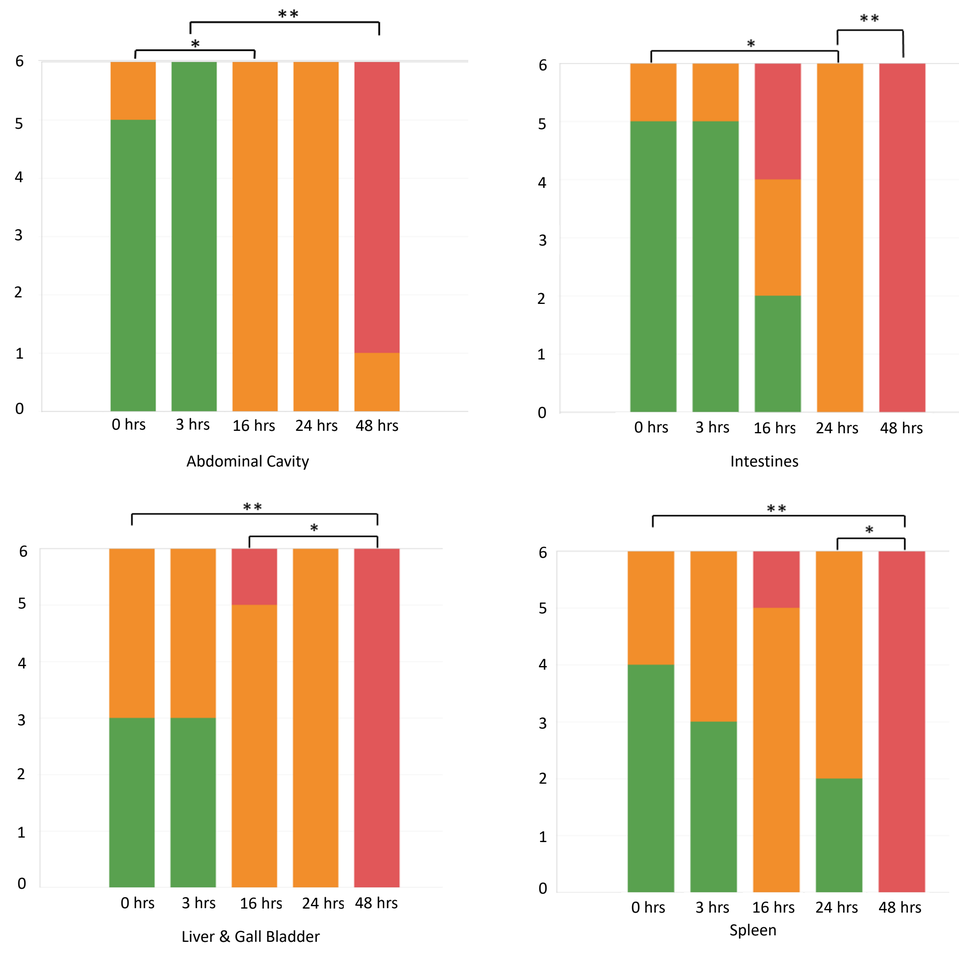
Changes between 0-16hr: the appearance of the abdominal cavity was the only statistically significant difference ($p<0.05$). Discolouration, the presence of free fluid, change in organs shape and odour were the 1st signs noted.

Changes between 0-24hr: In addition to the abdominal cavity ($p<0.05$), the appearance of the intestines changed ($p<0.05$), with gaseous distension and occasional free fluid bathing the abdominal cavity.

Changes between 0-48hr: In addition to changes to the abdominal cavity and the intestines, the appearance of the liver/gallbladder ($p<0.01$) and the spleen ($p<0.01$) changed, with gravitational blood pooling (*livor mortis*), swollen appearance and crumbly texture. The gallbladder was autolysed or broken.

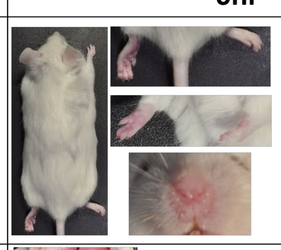
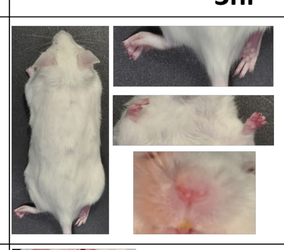
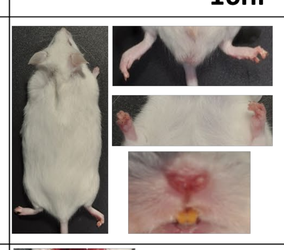
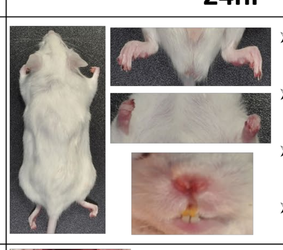
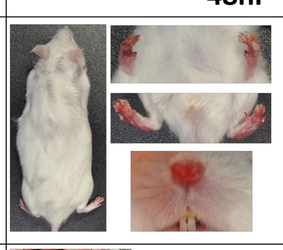
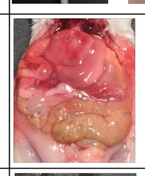
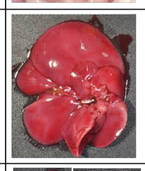
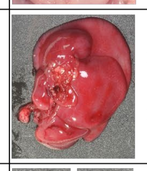
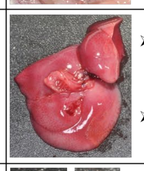
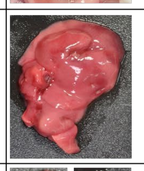
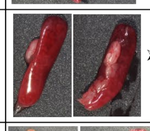
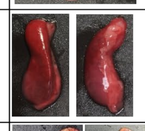
Figure 1: Number of mice scoring Green, Amber or Red at each time point (0, 3, 16, 24 & 48hr) for the major organs: abdominal cavity; intestines; liver and gallbladder; spleen. * $p<0.05$, ** $p<0.01$. Due to the number of comparisons, significant comparisons are only shown for:

- 0hr and the earliest significant following timepoint
- 48hr and the closest significant timepoint



PROPOSED VISUAL POST-MORTEM SCORING SYSTEM

Based on the experimental results outlined above, the proposed scoring tools aim to support technicians in their estimation of the *post-mortem* interval in mice. The tool also includes additional macroscopic trends that were not statistically significant, but may assist determination of timing.

	0hr	3hr	16hr	24hr	48hr
SE	 ➢ Good skin elasticity. ➢ Pale paws and digits. ➢ No presence of blood under nail bed. ➢ Pale nose.	 ➢ Good skin elasticity. ➢ Pale paws and digits. ➢ Some accumulation of blood under nail bed. ➢ Pale nose.	 ➢ Reduced skin elasticity. ➢ Base of paws and digits darker in colour. ➢ Accumulation of blood under nail bed. ➢ Nose starting to show signs of blood pooling.	 ➢ Marked reduction of skin elasticity ➢ Base of paws and digits show blood pooling ➢ Marked accumulation of blood under nail bed ➢ Clear signs of blood pooling in nose	 ➢ No skin elasticity ➢ Paws and digits show marked blood pooling and dehydration ➢ Marked blood pooling in nose
AC	 ➢ Organs: shape, colour and position normal. ➢ Free fluid and odour: None. ➢ Inner abdominal wall normal.	 ➢ Organs: shape, colour and position normal. ➢ Free fluid and odour: None. ➢ Inner abdominal wall normal.	 ➢ Organs: abnormal shape, mild discolouration, easily blanched. ➢ Odour: present. ➢ Inner abdominal wall normal.	 ➢ Organs: distended, discolouration, easily blanched. ➢ Free fluid and odour: present. ➢ Inner abdominal wall thick and fragile.	 ➢ Organs: distended, discolouration, easily blanched. ➢ Free fluid and odour: present. ➢ Inner abdominal wall: marked discolouration.
LG	 ➢ Liver: Shiny, plump, normal colour size and texture. ➢ Gall bladder present and normal.	 ➢ Liver: Shiny, plump, normal size, texture. Mild colour gradient from blood pooling. ➢ Gall bladder: present but empty.	 ➢ Liver: Shiny, plump, abnormal shape, texture. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken.	 ➢ Liver: Shiny, plump, abnormal shape, texture. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken.	 ➢ Liver: Shiny, distended, marked abnormal shape, texture friable. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken.
Sp	 ➢ Shiny, plump, normal colour size and texture.	 ➢ Plump, normal colour size and texture. Surface dull.	 ➢ Abnormal shape, colour gradient from blood pooling. Surface dull.	 ➢ Distended, colour gradient from blood pooling. Surface dull.	 ➢ Distended and abnormal shape. Marked colour gradient from blood pooling. Surface dull.
In	 ➢ Normal colour and shape. Easily manipulated without breakage. Mesenteric intact (in situ). No discolouration.	 ➢ Normal colour and shape. Easily manipulated without breakage. Mesenteric intact (in situ). No discolouration.	 ➢ Mild discolouration. Presence of gas bubbles. Difficult to manipulated without breakage.	 ➢ Mild discolouration and distention. Presence of gas bubbles. Easily broken when manipulated. Fluid filled.	 ➢ Marked distention and presence of gas bubbles. Loss of definition. Easily broken when manipulated. Fluid filled.

DISCUSSION AND CONCLUSION:

- This poster provides the foundation for a macroscopic scoring system for the determination of post-mortem interval in male mice.
- Key tissues for examination include the abdominal cavity, intestines, liver, gallbladder and spleen.
- However, this method was not sensitive enough to detect early changes (0-3h *post-mortem*).
- External macroscopic changes of the skin, paws and extremities may be helpful indicators of the time of death, but would require further investigation.
- To increase generalisability, this study can be expanded upon by varying the sex and strain of the mouse, the method of euthanasia and the animal's *post-mortem* positioning.

REFERENCES:

1. Capas-Peneda C, Goncalves-Monterio S, Oliveira B, Duarte-Arajo M,. (2016) 'How do you tell how long a mouse has been dead? Rigor mortis as a tool to estimate mice time of death in animal house facilities'
2. Scudamore CL, Busk N and Vowell. K., Laboratory Animals 2014; 48: 342-344; 'A simplified necropsy technique for mice'
3. R Core Team, 2019 'R: A language and environment for statistical computing'
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