

TECH-2-TECH

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GA Passports Workshop Report – IAT/LAVA Congress 2013

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Introduction

The RSPCA GA Passports Working Group (GAPWG) was set up in 2008 to progress and develop the idea of 'mouse passports'¹ and 'welfare databases' as tools for ensuring consistent standards of care throughout the lifetime of all genetically altered (GA) animals. The idea of communicating animal care information when animals are transferred between establishments is not a new one. Indeed, in 2001 an APC report on Biotechnology suggested the development of databases to contain information with welfare implications for all strains of GA animals and recommended that "data relating to any adverse welfare effects should be made available to any

potential user of a new GA strain"². This idea re-surfaces in the 2003 JWGR report recommending that "The nature of the phenotype and any specialist care required should be discussed prior to the transport of the mice and detailed in written information accompanying the GM mice"³. Three years later, a GA mouse working group set up to progress many of the recommendations contained within the 2001 APC report, published their 2006 report¹ recommending that "information on any welfare concerns should be documented" and "should form the basis of a welfare profile that is.....also (used) to create a 'mouse passport' ". This report also provides a template 'mouse passport' for people to use when transferring

GA mice between establishments. A FELASA working group report a year later⁴ recommends that “*for every transgenic strain, a data sheet should be prepared at the first opportunity, which collates all available information relating to its breeding, husbandry and care. This data sheet, or a pointer to enable the information to be accessed via the web, should be sent in advance to the receiving laboratory or central repository and a further copy should accompany the animals*”. The FELASA paper also contains a list of the minimum level of information that FELASA working group members consider should be recorded within a data sheet.

By 2008 no significant progress appeared to have been made within the UK in terms of the recommendations becoming common practice, so the GA Passports Working Group (GAPWG) was formed. The group comprises representatives from a range of UK commercial, academic and private organisations with an interest in the use of GA animals for research purposes and who had knowledge of the published recommendations and/or experience of putting these recommendations into practice. During discussions the group were unanimous that there is not one passport/data sheet/database entry format that works for all establishments, nor is there a reason to limit the initiative to just GA mice. With this in mind, in 2009 the group published an easy-to-read, quick reference booklet of recommendations with the aim of establishing a minimum standard of information to be shared between establishments when sending or receiving a GA animal of any species⁵. Since then group members have been raising awareness of GA passports through poster and oral presentations at meetings and conferences both within the UK and internationally. The latest of these presentations was the introductory talk during the GA Passports workshop at the IAT/LAVA Joint Congress 2013. Below is a summary of the talks and discussions from this workshop.

GA passports – the story so far.....

First to speak was Nikki Osborne, convenor of the RSPCA GAPWG, who gave a brief history of the background to the formation of the working group (see above) before introducing participants to the ‘GA Passports: the key to consistent animal care’ booklet⁵. The booklet defines a GA Passport as a record containing all the information that any staff caring for the GA animal can use to minimise the potential for pain, suffering or distress and improve welfare. The format of the information does not matter in terms of whether it is a paper document, an electronic file, an entry on a spreadsheet or welfare database, so this can be varied according to resource. It does however need to be intuitive to use, as well as quick and easy to complete in order for it to be a practical addition to routine practice.

There are many reasons to use GA passports; to reduce the incidence of adverse events such as breeding failure, disease outbreaks and health or welfare problems; to minimise the need to duplicate or replicate research by sharing all known screening results or phenotypic data (positive and negative); to disseminate good practice around the world by sharing information on housing, husbandry, enrichments and procedural refinements. These are just three reasons and whilst the value of specific pieces of information contained within a GA passport will vary between individuals in terms of what they consider to be of major or minor importance, the crucial point is that the passport package of information serves to improve animal welfare and the quality of the scientific data. There is an argument that the data contained within a GA passport should be recorded and shared for all animals regardless of species and genetic status but the ‘passport’ is specifically relevant when an animal (or indeed embryos or gametes) are transferred between locations where their journey ends with a new set of carers. In this situation a copy of the passport should always remain with or accompany the animal or materials whilst being transported but when sending live animals it becomes vital that the information is also sent in advance so that the new set of carers can prepare in advance for the new arrival.

Deciding what details should be included within the GA passport proved very straightforward with ten fields of information featuring within all the passport or database systems that group members had developed and implemented. These top ten information fields were; name (using formal nomenclature and local name if applicable), general information (such as colour, diet, housing, enrichment, behaviour), phenotypic abnormalities and observable traits with welfare implications, remedial actions for all adverse effects, breeding strategy/performance, method of supply (fresh/frozen protocols), origin (who, when and where created), background (strain or stock and the back-cross/inter-cross generation of animals supplied if appropriate), contact details (person(s) at supplying establishment name, number, email, postal address), and other (including references/websites, additional contact details for the originator or carer/NVS, extra scientific information such as PCR protocol, stem cell info etc, and a list of phenotypic screening undertaken to date with statement of positive/negative result). The final factor to consider when creating or updating passports is the language, which needs to be clear, easy to understand and defined, such as the terms found at www.mousewelfareterms.org or nomenclature according to current rules, to name just two examples.

GA Passports – Building a Birmingham passport.....

Second to present was Caroline Chadwick from

University of Birmingham. Birmingham was one of the first establishments to develop a 'mouse passport' as early as 1999, so Caroline began by using the Turning Point system of handsets to get the participants to vote what level of importance they attribute to different fields of 'passport' information. Strain name, contact details, origin and background came top of the essential list with 88% of votes, closely followed by phenotype and welfare information with 76% of the vote. Next came breeding performance with 60% of the vote and phenotypic screening history with 53% of the vote. Bedding and enrichment came top of the list for useful information with 60% of the vote, followed by PCR protocols at 50%. A photograph or references took joint third place in terms of useful information with 47% of the vote, closely followed by diet and water with 41% of the vote. The field of information considered least important of the options provided was cage type with 60% of the vote.

Caroline went on to discuss where 'passports' and the information they contain should be kept. The assumption is that the information arrives at the new establishment with the GA animals, in this case mice, and is stored on a computer somewhere or filed in the boss's office. If the point of having a passport of information is to ensure consistent animal care, then it does raise the question – why isn't the information with the mice? Each establishment will handle and disseminate information in different ways depending on the resources available but at Birmingham they have introduced cage side welfare cards. These cards are coloured so they are easy for staff to identify when entering a room and contain key information. For example '*Name – Mickey. This strain can experience seizures. Do not disturb. Close visual check only.*'; '*Name – Minnie. The homozygotes (-/-) do not develop teeth. Provide soft diet daily.*'. The aim of these welfare cards is to ensure relevant husbandry/welfare information is available to all staff and to highlight strains where there is an adverse phenotype. This cage side approach is supported by the inclusion of the full passport record of information on a central database and means that problems getting hold of the correct nomenclature strain name and full history of the respective animals, do not prevent staff access to information on the cage side welfare cards.

GA Passports for multiple species.....

Next to present was Mary-Ann Haskings from the London Research Institute (LRI) of Cancer Research UK. To provide some context for her talk on developing GA Passports for multiple species Mary-Ann provided some background information on mouse cages and zebrafish tanks within her establishment. This highlighted the fact that the import/export activity is predominantly mice and zebrafish and accounted for

approximately 200 movements last year. These movements were overseen by an import/export co-ordinator with around 80% of the GA mice sent as live mice and the remaining 20% being embryos or sperm. Zebrafish are transported as embryos.

At the LRI the primary challenge in relation to GA passports is providing/gathering the complete information. As mentioned by previous speakers the process and resulting information needs to be user friendly, something that can be more complicated than it may first appear. In the past importing and exporting has entailed two different forms because some of the details can vary, but the aim was to have one format for both that can be completed for either species. The chosen format also needed to be understandable by people both within CR-UK and from external institutes. The solution for the LRI was to ensure that all the fields recommended in the GAPWG good practice booklet were included within the internal import/export database. This development enabled them to incorporate drop down lists wherever possible to speed up the time taken to enter information and enable irrelevant fields to be greyed out. The use of a database also facilitates the automatic generation of electronic and/or paper documentation when exporting and enables the automatic upload of information on imported animals once the relevant fields are completed and submitted. It has also enabled the inclusion of statements at relevant points within the process to remind individuals of current good practice. For example the material sections states 'live animal transfers should be requested only when receiving tissue, gametes or embryos is not practical'.

In terms of welfare related information, the LRI encompasses it all within the husbandry information section of the passport/database. The general section includes a description of the supplying facility in terms of whether it's a closed facility or allows introduction with quarantine or direct introduction from commercial or non-commercial sources, description of barrier, caging/tank system, dietary regime, standard environmental enrichment and/or special husbandry requirements. The description of the mouse or zebrafish includes strain name, type, genotype, background, colour and originator details. Phenotype information is expanded to include a description, % with phenotype, age phenotype first detected, pre-weaning/early mortality, age when moribund or endpoint and breeding performance. This level of information is also included when entering the details of zebrafish they hold within the Zebrafish International Resource (ZIR) center database (www.zebrafish.org).

Like the ongoing work at other establishments the LRI passport system is still quite new and is continuing to be developed and refined over time. Mary-Ann discussed the ongoing challenges in terms of

perfecting how to store what will become a growing body of 'passport' information so that it is both secure and accessible to all who need it. There is also work to be done in relation to how the information will be used, for example flags are being added to the data to make sure that important information is noted. Another challenge for the future faced by all establishments is that each export will require a new passport to be generated with the most up to date information, something which brings with it the question of who should be responsible for compiling and updating passport data and how duplicated effort can be avoided.

GA Passports as 'living' records.....

This question was progressed further by the fourth speaker, Jenny Salisbury from the Wellcome Trust Sanger Institute (WTSI). The WTSI research support facility is one of the largest within the UK, contributing to international efforts to generate and characterise targeted knock-out mouse and zebrafish models through initiatives such as International Mouse Phenotyping Consortium (IMPC), Baylor College of Medicine, Sanger, Harwell (BaSH) consortium, Knock Out Mouse Project (KOMP) and European Conditional Mouse Mutagenesis (EUCOMM), as well as generating huge amounts of phenotyping data through high throughput screening. One of the key measures of success at the WTSI is the early production and sharing of mouse models with requests from the scientific community accepted as soon as one live heterozygous individual with the confirmed genotype is identified. Sharing details of GA animals at such an early stage brings with it some novel challenges, as well as some more common issues associated with the gathering and sharing of information with individuals and facilities on all continents and in multiple languages. Both of these issues mean that the value of using passports as a communication tool when importing and/or exporting GA animals was quickly recognised and the recommendations of the GAPWG adopted from the outset.

Like the LRI, the WTSI has linked their 'passport' to an in-house database that incorporates welfare observation data. The database has been quite labour intensive to set up and continues to develop to meet the facility's growing needs but led to the early realisation that to facilitate searching, filtering and collation of data a controlled vocabulary would be required. This marked the beginning of an ongoing collaborative effort with MRC Harwell to develop a controlled list of welfare terms to ensure accurate and consistent reporting of phenotypes and welfare observations. The whole scientific and animal care community are encouraged to contribute to this initiative through the website address www.mousewelfareterms.org. Jenny illustrated the

importance of using controlled vocabulary and recording welfare observation data within the database to demonstrate that phenotypes and welfare concerns can and do occur throughout the life of a colony, not just during the time that the line is undergoing specific phenotypic screening. For example, analysing data for 386 models with ≥ 28 progeny within the database enabled them to look at the percentage of offspring resulting from heterozygous inter-crossing at postnatal day 14 that were lethal (0% homozygous), sub-viable ($0 \leq 13\%$ homozygous) or viable ($>13\%$ homozygous). This analysis showed that 110 lines were homozygous lethal, 66 lines were sub-viable and 210 lines were viable. Thus differentiating between these models enabled staff to improve their colony management by tailoring breeding strategies to the needs of individual lines identified as lethal or sub-viable.

Having demonstrated the benefits of in-house databases for aiding GA colony management and recording phenotypic and welfare observations, Jenny went on to talk about how the WTSI share the data they record with the scientific community through their Mouse Resources Portal. Using a search for Spns2 as an example, Jenny showed workshop participants where to look to download a spreadsheet heat map of phenotyping information by assay which contains links to phenotyping data. She also drew attention to a link that individuals can click to subscribe to a mailing list for the line of interest to be kept up to date with information as it is generated and recorded. Jenny highlighted the importance of keeping a two-way channel of communication open with facilities that they have sent lines to, because many of their lines are sent out at a very early stage when there is very little information to accompany them so they are very happy to receive and collate information back from their collaborators.

GA Passports for legacy lines.....

Last to speak was Amanda Pickard from the MRC Frozen Embryo and Sperm Archive (FESA) who gave us an insight into how the meaning of information changes over time and what remains useful as time passes. This presentation is informed by the FESA experience of rederiving lines that were frozen 10 or more years previously. FESA has been in existence since the early 1970's and it is the UK's central archiving centre with approximately 1,500 stocks of transgenics, mutants, chromosome anomalies & inbred strains. They also have over 10,000 individual ENU mutagenised males, with an associated DNA library for mutation detection and so are well established as a worldwide resource.

Most, if not all, GA passports or database entries begin with the strain name. This can include a local name, but should also contain the full name consistent with existing nomenclature rules. It is interesting to note

that the rules and naming conventions have changed slightly over time, and strain names are also updated as more information becomes known. For example GENA251, became Junbo, then Jbo, Evi1^{bo} and is now Mecom^{bo} (MGI 2158381). Knowing when a strain name was assigned gives an insight into the original meaning if protocols have changed and gives a starting point from which to determine what the current name would be. General information is also a recommended part of any passport, with some details that shouldn't change unless there is a problem, for example colour. Other details can change over time, for example diets, even standard ones can and do change over time, housing changes as technology and species specific knowledge develops, likewise environmental enrichment varies with knowledge & trends. All of these details can affect the phenotype & behaviour or breeding so it remains important to include these details.

When building passports for the future, an obvious challenge, as highlighted by previous speakers will be how to handle the growing body of data relating to phenotypic anomalies and observable traits. Undoubtedly a lot of data is far more accessible now than it has been in the past, when it was only to be found in a lab book or library somewhere. However each establishment keeps their own records in their own format, giving rise to questions such as what do these records tell us, how meaningful are they to others and how do you decide what to share, or link to a passport? Increased accessibility to data brings with it a responsibility to ensure it is meaningful and presented in a form such that it cannot be misinterpreted. This can be greatly improved by the use of standardised vocabulary, such as the mouse welfare terms already described. Amanda went on to show participants an example of a record on their in-house database system and how it relates to the information that gets sent out with animals from FESA. She also provided a number of links to some other resources that are useful sources of information on mouse strains such as Mouse Genome Informatics (www.informatics.jax.org), Mousebook (www.mousebook.org), European Mutant Mouse Archive (www.emmanet.org), Welfare Terms (www.mousewelfareterms.org), International Mouse Phenotyping Consortium (www.mousephenotype.org) and Europhenome (www.europhenome.org). This led on to a broader discussion of whether in the future it could, or should, be possible for the scientific and animal care community to have access to an online depository of GA passport information that is collated, updated and maintained such that the entire community and animal welfare can reap the benefits of individual efforts and minimise the potential for duplicated effort. Only time will tell what the future will hold, but if you have any thoughts, comments or opinions on anything contained within this report the RSPCA GAPWG would like to hear from you at GA@rspca.org.uk

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