Comparative Medicine
Copyright 2020
by the American Association for Laboratory Animal Science

Vol 70, No 5
October 2020
Pages 368–370

Abstracts of Scientific Papers
AISAL XXVI Annual Meeting
Bologna, 24–25 October 2019

ORAL PRESENTATIONS

International guidelines.

Monica Forni*
Department of Veterinary Medical Sciences - University of Bologna
**Corresponding author. Email: monica.forni@unibo.it*

The reproducibility of animal experiments must be ensured for evident ethical, economic and scientific reasons.

ARRIVE (Animal Research Reporting In Vivo Experiments) guidelines intended to improve the reporting of research using animals – maximising information published and minimising unnecessary studies (NC3Rs). The ARRIVE guidelines were developed in consultation with the scientific community following a review of the quality of research using animals in the UK and US (PLoS One, 2009. 4 (11) e7824). The guidelines have been endorsed in the last ten years by over 1,000 scientific journals but surveys on their effects show some limitation (PLoS ONE, 2016. 11(12): e0165999; PLoS ONE, 2018. 13(5): e0197882).

The ARRIVE checklist is designed to be used when submitting manuscripts describing animal research, but, before making a good report, it is essential to be well prepared.

More recently, the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, based upon the collaboration between animal facilities experts and researchers have been published (Laboratory Animals, 2018, vol. 52(2):135–141). The PREPARE guidelines focus on a large number of factors which, although reported in scientific papers, can dramatically influence the validity and outcome of studies on animals, as well as the health and safety of all those concerned. They cover all stages of quality assurance, from the management of the animal facility to the individual Standard Operating Procedures which are part of a study (NORECOPA).

ARRIVE and PREPARE must be considered as complementary informational guidelines aimed to develop best practice in the field of research animal science and 3R-alternatives and to obtain reproducibility in animal experimentation.,

Formulating a clear study hypothesis, choosing the primary and secondary response variables

Flavia Chiarotti*
Reference Center for Behavioral Sciences and Mental Health; Istituto Superiore di Sanità, Rome, Italy
**Corresponding author. Email: flavia.chiarotti@iss.it*

In the lecture, the issues of an experimental study protocol are first described, namely the experimental design, the characteristics of the grouping factors and of the statistical units, the variables to be collected (response variables and covariates) and the statistical tests to be performed. Second, the primary and secondary response variables are defined, along with the main characteristics to be taken into account in choosing the most appropriate response, i.e. relevance, measurability, and variability. Third, the elements to be accounted for to determine the sample size are described: type I error probability (significance level alpha), power (1-beta), effect size, and variability of the response variable. Finally, an experimental study in oncology is used to demonstrate how the choice of the primary response variable(s) can influence the study sample size.

The benefits of preclinical systematic reviews

Merel Ritskes-Hoitinga*
SYRCLE (www.syrcle.nl), Department for Health Evidence, Radboud university medical center, Nijmegen, The Netherlands.
**Corresponding author. Email: Merel.Ritskes-Hoitinga@radboudumc.nl*

Systematic reviews have been routine methodology in medicine since 1992, when the Cochrane Collaboration was founded. The Cochrane Collaboration focusses on systematic reviews of clinical trials, in order to learn what evidence there is for certain treatment efficacies and safety, and also to evaluate the quality of the results underlying this evidence.

Because animal studies are often performed to deliver information on safe and effective treatments before exposing humans, it seems only logical to analyse the evidence from animal studies in similar ways, before going to clinical trials. SYRCLE was

officially founded in 2012 and has taken up this challenge by using the Cochrane methodology and developing this further for the preclinical field. SYRCLE provides education and research in this field, and has developed tools and guidelines. A systematic review is defined as follows: The process of systematically locating, appraising and synthesizing evidence from scientific studies in order to obtain a reliable overview. When possible, the results of individual studies are combined in an overall statistical analysis, the so-called meta-analysis. In my presentation I will highlight the benefits of preclinical systematic reviews, as they create transparency on the quality of publications and translational success and failure rates. Moreover, by doing systematic reviews more evidence-based choices of (animal) model systems can be done and the methodology 'automatically' contributes to the implementation of the 3Rs, Replacement, Reduction and Refinement. Examples of the aforementioned will be given during my presentation.

Scientometric analysis as decision tool in designing experimental research.

Nicola Bernabò*
University of Teramo, Teramo, Italy
*Corresponding author. Email: nbernabo@unite.it

Experimental research, especially on animal models, requires always increasing amount of economic resources and is becoming a very competitive field. As a consequence, scientist are called to adopt new approaches in designing successful project that requires multidisciplinary competencies. In particular, the ability to manage the prior knowledge and to get new information from already published data (the so called hidden knowledge) could play a key role in increasing the chances to be funded for applicants in competitive project calls. In addition, it is becoming more and more important to be able to face with an amazingly increasing number of published data that have reached an unprecedented amount and are intended to have a further fast increase (big data problem).

In this context, it has been developed a branch of science, the scientometrics, with the purpose to collect large data sets from bibliographic data-bases (i.e. PubMed, Scopus, Web of Science, etc…) and to analyse them by using sophisticated techniques based on computational statistics, mathematics and data visualization tools.

As a result, for scientists, it is available a high-value resource, able to increase their ability to write project, to explore scientific communities, to identify emerging trends in different disciplines, as well as the topic that are more suitable to be funded by funding Agencies. In conclusion, scientometric analysis could be a valuable tool in strategical design of research.

Defining the relevance of scientific work for publication

Jan-Bas Prins*
Chair of Laboratory Animals Ltd.
*Corresponding author. Email: j.b.prins@lumc.nl

In the process of scientific publishing defining the relevance of scientific work is regarded important. For a topical journal, like Laboratory Animals, defining the relevance appears to be easier than for a journal with a broader scope. Having said that, the process of impartially reviewing 'relevance' and quality of submitted manuscripts is labour intensive, time consuming and under pressure. Who decides what is relevant for which audience? The Editor in Chief (EIC) is the first and the last post to pass. The submission has to fall within the scope of the journal, it has to be a 'relevant' addition to the already existing corpus of literature and the science needs to be sound and properly referenced. The EIC has quite a responsibility. The peer review process, which is under increasing pressure, is still critical for delivering a quality product without a proper alternative. Emerging platforms like F1000 use peer-review as a cornerstone of their 'open science platform' albeit in a modified operational model compared to the more traditional journals. Furthermore, F1000's scope appears to give priority to originality of articles over 'relevance' when they state: 'Articles must be original (not duplications). All research is suitable irrespective of the perceived level of interest or novelty; …'. Does that mean that 'relevance' is just in the eye of the beholder in the changing science publishing world?

Biological and Statistical Relevance

Alessandro Giuliani*
Istituto Superiore di Sanità, Rome, Italy
*Corresponding author. Email: alessandro.giuliani@iss.it

Scientific community widely recognizes the existence of a reproducibility crisis menacing the entire research system with particularly serious consequences on biomedical sciences.

One of the causes of this crisis is by no doubt the progressive shift of meaning of the 'statistical significance' concept (*P*-value) from 'worthy-of-a-second-look' (its original goal when it was definitively formalized more or less one hundred years ago) to 'definitive-truth' mark.

This shift is evident in day-to-day research work and many scholars (among them American Statistical Society that recently diffused an appeal to biomedical scientists[1]) published didactical papers pushing a correct use of statistical significance. This is for sure important but statistical thinking is not only 'sensible use of *P*-value'. Methodological issues pervade the entire scientific work influencing crucial choices going from the most suited measurement(s) (in the case of –omics they can be in the order of thousands) scale of analysis, kind of effect (interactive, additive, change in mean, variability or correlation properties). We skip any deep thinking about what we consider a 'finding' only concentrating on the probability it is there by chance.

The great Italian actor Alberto Sordi, when asked why he never married, answered '*Why I should accept a stranger at home?*'. This is the very root of the problem: what is lacking is a real interaction between methodological and content aspects, scientists do prefer a homemaker to call (and let her go when they get a satisfactory *P*-value) than engage a mature (and consequently problematic) life-long with methodological thinking. Here I will try and sketch some very preliminary points to foster such a serious relation hoping it could be instrumental to contrast 'thermal death' of biomedical sciences.

1) Wasserstein, R. L., & Lazar, N. A. (2016). The ASA's statement on p-values: context, process, and purpose. *The American Statistician*, 70(2), 129–133. doi.org/10.1080/00031305.2016.1154108.

Translational research in physiology: the challenge in going from rodents to larger mammals

Matteo Cerri,[1] Domenico Ventrella[2]
[1] Department of Biomedical and NeuroMotor Sciences; University of Bologna, Bologna, Italy
[2] Department of Veterinary Medical Sciences; University of Bologna, Bologna, Italy
*Corresponding author. Email matteo.cerri@unibo.it

Historically, research in the field of physiology has been mainly conducted in medium-size animals, such as dogs or cats. Measuring physiological parameters in vivo was in fact limited by the degree of the available miniaturization. With the advancement of technology, this limitation was lifted and physiologist moved first to rats, and later to mice as their default animal model. Mice can indeed offer the great advantage of a large genetic toolbox, that can well combine with physiological and behavioral experiments. Despite the numerous advantages that rodents offer, there is today an increasing demand to conduct studies in larger species. The main reason is that larger mammals can be a better model for complex conditions such as, for instance, septic shock, in which intensive care-like setups are necessary for a valid study, and that allow for easier and more frequent sampling of biological specimens. Larger mammals, such as the pig, are for this reason a better model of human physiology; pigs, in particular, share many relevant physiological characteristics with humans: they are omnivores, have hairless skin, comparable surface/volume ratio. Translating from rodents to pigs though, present some difficulties. The larger size itself requires more and better trained personnelles, larger facilities, more expensive pharmacology, less availability of genetic tools. All these difficulties require, for larger mammals physiology to be study, a dedicated center, with centralized and hospital-like facilities, that can make use of all the recent advancement in human medicine, and build better animal model that can furthermore improve the human medicine: a virtual cycle in which medicine and science reciprocally benefit.

Bioluminescence imaging in preclinical animal models to follow in vivo physio/pathological biological processes.

Giulia Piaggio*
IFO, Rome, Italy
**Corresponding author. Email: giulia.piaggio@ifo.gov.it*

Bioluminescence imaging (BLI) strategies have recently been incorporated into animal research protocols, including those involving the use of genetically engineered animals. These tools offer the advantage of noninvasive in vivo assessment of the molecular and cellular events that are often targets of therapy; as such, these events can be studied in individual animals over time. This reduces the number of animals required for a given study and improves data set, as temporal data allow for each animal to serve as its own internal control.

One update goal is to develop animal models useful for drug screening allowing measurement of proliferative status of all cells, hence of activity of the compound under investigation, on cell proliferation in all tissues, both normal and cancerous in longitudinal studies. Mouse and Zebrafish models represent a critical step to improve treatments of malignant disease, as an intermediate method of experimentation between cell culture based assays and human clinical trials. Several cellular pathways are highly conserved between human, mouse and zebrafish, thus rendering zebrafish model very attractive. Recently we have generated a transgenic reporter mouse, called MITO-Luc, in which luciferase reporter gene is transcribed by cyclinB2 minimal promoter containing three CCAAT boxes regulated by the transcription factor NF-Y. In these mice, BLI of NF-Y activity visualizes areas of cell proliferation and regeneration during response to injury.

Parallel we have developed bioluminescent Zebrafish models which allows us to visualize through BLI any proliferation events in the context of the entire alive animal during development and adult life.

Preclinical Development: How to select the appropriate specie.

Michela Pecoraro*
Aptuit
**Corresponding author. Email: Michela.Pecoraro@aptuit.com*

Before a potential new medicine can be administered to humans, it is essential that its safety is adequately assessed in animals as recommended by the regulatory guidelines.

Similar general concept is applied to any drug in development, but based on the differences between the new drugs. Different approaches in the development testing strategy, which includes the number of species used can be followed. It is important that these approaches remain always science-driven and predictive of relevant effects in humans.

For the new chemical entities (small molecules), the use of two species (a rodent and a nonrodent) for toxicological assessment is the gold standard approach and it is mandatory, based on the current regulatory guidance (eg, in ICHM3(R2), ICHS9). For new biological entities ("large molecules" or biologics), the use of two species is also mandatory; however, the species selection should be strictly correlated to its pharmacological relevance, and the nonhuman primate (NHP) is often the only species relevant, unless the large molecule demonstrates a cross-reactivity with multiple species.

In addition to the common two species (a rodent and a nonrodent) ie rat and dog, mouse, minipig, NHP can be used when these are the more relevant species (based on their pharmacological relevance, pharmacokinetic and/or metabolic profiles, and based on the literature data on class-related tolerability).

Recently, reviews of industry data and flexibility in drug development practices and regulations should identify a new ways of working which could create opportunities for replacement, refinement, or reduction in animal use within the drug development process.

The use of animals for clinical research end experimental purpose

Angelo Peli*, Mariana Roccaro
Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy
**Corresponding author. Email: angelo.peli@unibo.it*

The use of animals for scientific purposes is regulated under the Directive 2010/63/EU which, by defining the concept of "procedure"1, establishes a threshold above which the research activity must be part of a project to be authorized by the Competent Authority.

The Directive does not apply to some particular cases, including practices undertaken for the purposes of recognised animal husbandry, but also non-experimental clinical veterinary practices. However, the acquisition of data from healthy subjects or the study of spontaneous diseases are often essential to the progress of veterinary science. Therefore, animal care and the acquisition of new knowledge through research can be concurrent and to determine whether or not an investigation falls within the scope of the Directive can be challenging.

When trying to discriminate between clinical practice and research, three main questions should be answered: whether the intervention on the animal is a procedure or not, if it is done for the direct benefit of the animal and if it has a scientific purpose.

It should be underlined that serving the animal's interests and having a scientific purpose are not mutually exclusive.

Nonetheless, whether the research is experimental or clinical, it should always be subject to a formal ethical evaluation, which ensures the protection of the health and welfare of the animals used and the compliance with the current legislation, and it guarantees the quality and integrity of research.

---

[1] Procedure: <<any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.>>

Food quality and safety as an important element in the realization of in vivo studies

Musacchio F.
Mucedola SRL
*Corresponding author. Email: info@mucedola.it

Food safety is acquired as a consumer right and is one of the fundamental principles governing the entire food chain in its broadest sense, "from Farm to Fork".

The introduction of hazards (physical, biological and chemical) for food security can occur at any stage of the food supply chain, therefore adequate control is essential along the same and the joint effort of all the parts that contribute to it is necessary.

The wide regulatory framework in force on food safety guarantees a high degree of food and feed safety and establishes that each food business operator is responsible for the conformity of the products he places on the market.

Each company, in order to guarantee the food safety of its products, is required to adopt a system of hygienic and sanitary control which is, based on a clear legislative suggestion (Reg. 178/2002) and the Codex Alimentarius, based on the application of the HACCP method.

HACCP is a scientific and systematic method, based on hazard analysis and identification, within the production chain, of critical control phases (CCP).

The Hygienic sanitary check system made mandatory by the mandatory regulation is subjected to periodic checks by the Competent Authority and can also be certified by the relevant Bodies according to voluntary sector regulations such as, ISO 22000, BRC, IFS, etc.

In particular, the ISO 22000 Standard is an international standard that defines the requirements of a management system for food safety and hygiene, based on the Risk Assessment, definition of the Standard Prerequisites (PRP), of the Specific Operating Prerequisites (oPRP) and of HACCP control plans.

ISO 22000 is also strongly based on:

- definition of an HACCP team
- tracking and tracing of raw materials and finished products, which allow rapid recall of the product from the market in the case of health warnings or findings of non-compliance
- validation, verification and continuous improvement of the food safety management system
- communication and transparency regarding food safety aspects, both within the organization and towards the customer

POSTER SESSION

Comparison between treatments inhibiting β3-integrin activities in B16 subcutaneous tumor: synthetic peptide vs intracellular silencing

Barboni C.,[2] Sanapo M.,[1] Campadelli-Fiume G.,[1] Gianni T.,[1] Leoni V.,[1] Zaghini A.,[2]
[1]Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Alma Mater Studiorum-Università di Bologna, Bologna, Italy;
[2]Dipartimento di Scienze Mediche Veterinarie, Alma Mater studiorum-Università di Bologna, Bologna, Italy
*Corresponding author. Email: catia.barboni@unibo.it

Tumors utilize a number of effective strategies, including the programmed death 1/PD ligand 1 (PD- 1/PD-L1) axis, to evade immune-mediated control of their growth. PD-L1 expression occurs in human and murine cancerous and non-cancerous cells. αvβ3-integrin activity positively regulates PD- L1 expression and the anticancer immune response.

To study the role of integrin αvβ3 in modulating the intratumoral immune response two alternative strategies can be adopted and compared: functional inhibition of integrin at the transcriptional/gene level or inhibition of post-translational integrin. The first strategy consists in the in vitro generation of tumor cells in which the integrin has been silenced in a transient or stable manner, either by siRNA or shRNA. The obtained cells are then inoculated in the mouse. The second strategy consists in the intratumoral treatment with a peptide (cilengitide, *cln*) after implantation of cells expressing integrin αvβ3 in the mouse. *Cln* is a synthetic cyclic peptide that acts as an inhibitor of integrin blocking integrin-mediated adhesion, migration and signaling.

About the second strategy we carried out two different experiments: the main purpose of the first was to evaluate PD-L1 expression, while the purpose of the second one was to evaluate late times therapeutic efficacy of the peptide. In this study we used B16 melanoma (DIMES laboratory), a murine tumor cell line used as a model for human skin cancers. To induce tumor, B16 cells were subcutaneously implanted in immune competent C57BL/6 mice (wild type), bred at the DIMEVET facility.

Comparing the two tested strategies a decrease in primary tumors and a regulation of PD-L1 expression were observed in both cases. But the intracellular β3-integrin silencing led to a dramatic decrease in the growth of primary tumors and a durable immunotherapeutic protection while the *cln* treatment led to a transient and limited decrease.

Refinement and Reduction approach in small rodents subjected to experimental surgery: analgesia strategies and possible non-invasive tools.

Calvillo L.
IRCCS Istituto Auxologico Italiano, Milan, Italy
*Corresponding author. Email: l.calvillo@auxologico.it

Several studies performed on laboratory animals, demonstrated that pain is associated with profound changes in the physiology of the immune and nervous systems (1)(2), moreover, routine laboratory mice handling has profound effects on their anxiety and stress responses (3). Despite these issues might affect experimental results, there is not yet a thorough knowledge about pain severity and its prevention in small rodents, subjected to different surgical procedures, and about possible new handling methods able to improve animal wellbeing. In the last years our group devoted efforts to Refine procedures

involving small rodents, using a Reduction approach at the same time. In particular, we supported researchers who use laboratory animals by filming the animals themselves during procedures without interfering with the main experiment, and we avoided recruiting animals on purpose, by using animals already scheduled for biomedical studies. Video analysis allowed to verify the actual level of suffering experienced by rodents when treated with the commonly used analgesic drug and to test different analgesic strategies, thus Refining the experimental procedure. At the same time we tested new non-invasive procedures describing the physiological and welfare state of the animal, such as Infrared Thermography. Finally, we are testing Non-Aversive Handling methods (NAH), like Tunnel or Mechanoceptive Handling, to quantify possible anxiolytic effect in order to evaluate NAH usefulness during experimental procedures. Experiments are in progress, but it seems that, after a stress injury, NAH can improve recovery of wellbeing more than what seen in aversive handled animals. Stress-related neuro-inflammation will be also analysed in NAH vs AH treated animals.

Expression of NUCB2/NEFASTIN-1 in the brain and gut of the teleostean model for aging research nothobranchius furzeri

De Felice E.,[1] D'Angelo L.,[2] Montesano A.,[2,3] Leggieri A.,[2] Dall'Aglio C.,[4] de Girolamo P.,[2] Scocco P.[1]
[1]Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria
[2]Università degli Studi di Napoli Federico II, Dipartimento di Medicina Veterinaria e Produzioni Animali.
[3]Leibniz-Institute on Aging - Fritz Lipmann Institute (FLI).
[4]Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria.
**Corresponding author: Email:*
Elena De Felice elena.defelice@unicam.it

The mechanisms that regulate food behavior are the result of a complex neuroendocrine integration. In 2006 a new regulating molecule of eating behavior called nesfatin–1 (Nesf-1) was identified. Nesf-1 and its precursor NEFA/nucleobindin 2 (NUCB2) showed a highly conserved structure between mammalian and non-mammalian species, where they were detected both in appetite-control hypothalamic nuclei and in peripheral tissues involved in energy metabolism. Although tissue distribution is known in Vertebrates, there are few reports on expression during ontogenesis. In this study we examine for the first time the age-related central and peripheral expression of NUCB2/Nesf-1 in a model organism for aging research the teleost Nothobranchius furzeri. Experiments were performed on fishes at 5 weeks post hatching (wph) (young-adult) and 27 wph (old) belonging to MZM 04/10 strain provided by the FLI Leibniz Institute. Our analysis were directed on brain areas involved in food intake and on rostral intestinal bulb, which is analogous of mammalian stomach. Sequence analysis evidenced that in N. furzeri gene structure was well conserved and protein sequence showed an overall identity of 78% with medaka, the closest related species. Real-Time PCR in old fishes compared to young revealed an increase in brain and a decrease in rostral intestinal bulb in NUCB2 expression levels. Either in young and aged brains both NUCB2 mRNA transcript and Nesf-1 immunoreactivity cells were detected not only in the hypothalamic area but also in non diencephalic regions, specifically telencephalon, optic tectum and semicircular tori. Western blot on brain revealed a band of about 40 kDa, confirming the specificity of antibody employed. Either in young and aged rostral intestinal bulb NUCB2 mRNA transcript was detected mainly in the lining epithelium while Nesf-1 immunoreactive cells were distributed in the submucosae. Our results represent a step for understanding the regulation of NUCB2/Nesf-1 during vertebrate aging processes.

Teaching the 3Rs to VETs will improve science-to-society communication and science quality

Forni M., Gandini G., Barbarossa A., Bettini G.
Department of Veterinary Medical Science - University of Bologna
**Corresponding author. Email: monica.forni@unibo.it*

Students starting a biomedical degree should be aware of the relevance that animal testing still has in the progresses of scientific research and of the efforts spent to implement the 3Rs (Replacement, Reduction and Refinement) approach around the world.

In Italy, if present, LAS teaching is not compulsory. At the University of Bologna, LAS has been taught within the biotechnology degree since 2006, with a very positive students' feedback. Starting from 2016, LAS teaching was also offered as optional subject to the students of the Veterinary Medicine degree programme, whose feedback was not positive.

We organized a survey among veterinary students aiming to better explain the reasons for student's low interest in LAS and also to compare their opinion on animal research with the public opinion evaluated by Ipsos in 2011 and 2014. A total of 1,000 people in Ipsos' research and 154 veterinary students from the I to the V year were surveyed.

Possible explanations of the low interest include the scarce awareness of the importance of the topic, and the students' attention focused mainly on traditional clinical subjects. The comparison public/vet opinion shows an higher support given from students to medical research but a considerably lower belief that animal tests are still the safest way to conduct them.

At present, Replace, Reduce and Refine principles are, in most establishments, offered interspersed in different curricular subjects (including statistics, physiology, ethology and legislation). Authors believe that competencies on LAS should be provided to students in a more integrated and clear way. A specifically designed course would offer the possibility to acquire structured knowledge and competences to approach, without stereotypes and prejudices, the ethical and scientific issues of LAS.

Health Monitoring and Welfare Assessment of Laboratory Gerbils

Fuochi S., Galasso M.E.
Charles River Laboratories Italia SRL.

Gerbils have unique characteristics which make them appropriate for a number of animal models and are still used in research involving stroke, parasitology, infectious diseases, epilepsy, brain development, behavior, and hearing. Although their use is still relevant in specific research fields, the low numbers of individuals of this species used and the erroneous tendency to consider them very similar to other rodents have led to a limited availability of dedicated publications.

The main objective of this work was to review health management and best welfare practices based on data recorded and clinical and behavioral observations performed by authors between 2012 and 2019 on the Mongolian Gerbils (Meriones

unguiculatus) bred at the CRL Italy site of Calco - since 2012 CRL Italy is the only CRL site breeding Mongolian Gerbils in Europe.

The review takes into account three milestones of health and welfare programs: clinical findings, screening for infectious agents and behavioral management.

According to authors' experience, gerbils tend to be healthy and resistant animals, showing a significant smaller and less severe range of clinical conditions compared to what relevant bibliography describes. To build a solid health monitoring program it is crucial to target type of samples, frequency and relevant infectious agents, taking into account that, agents being equal, susceptibility and clinical presentation can be extremely different between gerbils and other rodents. Finally, the set up of a species-specific behavioral management can significantly improve animal welfare, reduce unwanted behaviors - like aggressions and stereotypes - and improve production.

Tackling differences in health monitoring among Italian animal facilities

Galligioni V.,[1] Zarattini P.,[2,*]
[1] Trinity College Dublin, Dublino, Irlanda
[2] Università degli Studi di Trieste, Trieste, Italy
Corresponding author. Email: pzarattini@units.it

Defining health status of animal facilities is important not only for importing/exporting animals, but also for research reproducibility. As already shown by PREPARE guidelines, health monitoring (HM) is one of the parameters affecting quality of the study. However, different facilities apply different methods (sentinels, sampling animals of the colony, environmental monitoring) and type of protocols (pathogens and number of animals tested for).

Proper information on health status of animals housed in the facility helps researchers to properly design experiments: unknown infections and infestations affect data, that can be misinterpreted and, on the long run, not reproducible.

During September 2018, we run an anonymous survey (16 questions in total on Survey Monkey platform) among AISAL (Italian Association of Lab Animal Science) members regarding housing systems and HM of their facility.

The survey was intended to identify i) how HM is performed (animals and microbiological agents tested, frequency, *Helicobacter* spp. identification, screening of biological monitoring; ii) if there is any correlation between capacity of facility, HM methodology and research fields; iii) how HM results are shared with researchers; iv) critical bottlenecks in standardization of housing, HM and data reproducibility.

We got only 20 responses, which are not clearly representative of the Italian situation, but still they can provide inputs on which limitations veterinarians, facility managers and researchers are facing when dealing with health monitoring.

Furthermore, we aim to run another survey with a Delphi approach, to address more specifically some of the limitations of the first survey.

References: Smith A. et al., Laboratory Animals, 2018

The bodies in charge of the welfare of animals used for scientific purposes

Redaelli V, Luzi F.*
Università degli Studi di Milano
Corresponding author. Email: fabio.luzi@unimi.it

The coming into force of the 2010/63/EU regarding the protection of animals used for scientific purposes, has made mandatory to have an Animal Welfare Body (AWB) for all establishments involved in the use of such kind of animals.

The AWB is built on the 3 Rs principle, and believes that most of the effective safeguard of animal welfare depends on the professional skills of personnel in charge of their care and use. The European Community legislators therefore identify a body, inside the institution that houses the animals, to support the practical implementation of the 3 Rs and the subsequent training and follow-up training of personnel.

More, the AWB has the responsibility to define and review internal procedures regarding the welfare of the housed animals and to follow the outcome of research projects. Some critical aspects, such as the monitoring of the procedures, the retrospective assessment of the suffering degree, the management of research work, constitutes a challenge for every organization working with animals.

Much can be done to optimize this process, guarantee the maximum level of safeguarding animals' welfare, improve the full implementation of the concept behind the 3 Rs, suiting it to the needs of research at the same time.

The consolidation of the role of the AWBs and the possibility to effectively interact both inside the establishments and with control authorities and the integration with pre-existing entities, such as the CESA, constitute crucial steps in the implementation of Legislative Decree 26/2014.

Acknowledgements
(The whole research was published in Front. Physiol., 17 April 2018).

Mus musculus Endoparassitosys innovative Health Monitoring protocol in a conventional animal facility as a new component of a good laboratory practice

Valeri C., Capezzone C
Plaisant Srl Via di Castel Romano, 100 Roma
Corresponding author. Email: c.capezzone@plaisant.eu

The animal model is an important way to study many of the human diseases and it is irreplaceable with *in vitro* techniques.

Thanks to the standardization of these animal models, in terms of genetic and biological knowledge, it is possible to maintain an uniformity of the experimental data, and guarantee their reproducibility. The animal Health Monitoring (HM) is important to supervise some biological characteristics, as environmental and genetic factors, because their interactions can influence the suitability of an animal in the experimental protocols. [1;2]

From our preliminary data, although the HM includes almost all of the pathogens, still few measures are adopted in terms of control for the opportunistic agents. For example some infections, attributable to various species of protozoa, are often tolerated for a conventional sanitary state. The aim of our work is to propose a new protocol for monitoring the endoparasitosys in laboratory rodents, through an innovative method (Mini-FLOTAC® kit) already used in human and in veterinary medicine for farm and pet animals. [3;4] The advantages of this procedure are multiple: the low costs, the diagnostic accuracy, the fast and easy of the execution and furthermore develop the principles of the 3Rs. [5;6]

Our results show that the examination of the cecal content, performed by direct microscopy, confirms the data obtained with Mini-FLOTAC® technique, even at low concentrations of

oocysts. We hope that this new procedure can become a routine practice for the monitoring of parasites in conventional animal facility, reducing the number of animals used for regular FELASA panel analysis.

Bibliography

1. Behnke J.M, et al. J Helminthol 2003;77:99–110.
2. McNair DM & Timmons E.H. Lab Anim Sci 1977;27:38–42.
3. Cringoli G., et al. Nat Protoc. 2010 Mar;5(3):503–15.
4. Cringoli G. et al. Nat Protoc. 2017 Sep;12(9):1723–1732.
5. Snyder P.W. et al. J Wildl Dis. 2015 Oct;51(4):843–8.
6. Lima V.F. et al. Parasitol Res. 2015 Sep;114(9):3529–33.