ONE MEDICINE

Collaborative Research on Human & Animal Disease

in cooperation with
Missouri University's
College of Veterinary Medicine
& School of Medicine

MSMA member names in bold

ONE MEDICINE
Collaborative Research on Human & Animal Disease for the Betterment of Both

by Craig L. Franklin, DVM, PhD

MU is one of the few universities with a school of medicine, college of veterinary medicine, and several strong departments ranging from biology to engineering, whose outstanding research is devoted to the life sciences.

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"Between animal and human medicine there is no dividing line – nor should there be. The object is different but the experience obtained constitutes the basis of all medicine."

-Rudolf Virchow (1821-1902)

The theme of this issue of *Missouri Medicine* is animal modeling with an emphasis on ongoing activities and unique resources at the University of Missouri (MU).

MU is one of the few universities with a school of medicine, college of veterinary medicine, and several strong departments ranging from biology to engineering, whose outstanding research is devoted to the life sciences. As a result, MU is uniquely suited to advance 'One Medicine,' a concept recognized by Virchow and Sir William Osler, and now the lodestar of collaborative biomedical research.

Through its Office of Infrastructure Programs (within the Division of Program Coordination, Planning and Strategic Initiatives, Office of the Director), the National Institutes of Health funds several animal resources (http://dpcpsi.nih.gov/orip/cm/index.aspx). MU is home to three such resources: the Mutant Mouse Regional Resource Center (MU-MMRRC http://www.mu-mmrrc.com), the Rat Resource and Research Center (RRRC http://www.rrrc.us), and the National Swine Resource and Research Center (NSRRC http://www.nsrrc.missouri.edu).

Moreover, MU maintains a number of other animal model-based resources including, but not limited to the National Cancer Institute's Comparative Oncology Trials Consortium (COTC https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home), the Research Center for Human Animal Interaction (http://rechai.missouri.edu/), the Christopher S. Bond Life Sciences Center (http://bondlsc.missouri.edu), the Laboratory for Infectious Disease Research (http://rbl.missouri.edu), and the Comparative Medicine Training Program (http://cmp.missouri.edu).

In a multi-department collaboration we present a collection of manuscripts that exemplify the broad range of comparative medicine initiatives at MU. Authors include faculty and residents from MU's School of Medicine, College of Veterinary Medicine, and School of Nursing. For a history of animal models and a brief look into the

future, readers are referred to the manuscript by Ericsson et al. Bryda builds on the discussion of rodent models for disease and describes the MMRRC and RRRC, critical centralized repositories for the preservation and distribution of rodent models that serve biomedical researchers across the globe. Walters and Prather change species and describe the growing use of swine in biomedical research with a focus on how the NSRRC is using new genetic engineering tools to create swine models with distinct advantages over historical rodent models. Henry and Bryan introduce the concept of One Medicine which promotes the study of naturally occurring diseases in companion animals for the benefit of both animal and human patients. This is best exemplified by activities of the COTC, which facilitates nationwide studies of and therapeutic trials for cancer in domestic animals. The definition of One Medicine is broadened by Johnson to studies of the human-animal bond, which provides great benefit to both human and animal health.

A common buzzword in today's research is translational medicine. To this end, Choudhary and Ibdah discuss the translation of data from animal models to the human condition, which drives progress of research along the continuum from basic biomedical research findings into clinical practice. Finally, Alvarado and Dixon describe the role of laboratory animal veterinarians as a bridge between the humane use of laboratory animals and the advancement of scientific and medical knowledge. ◼MM◼

Human/Animal Interaction

Two recent resources for promoting pet owners' exercise with their companion animal are books co-authored/co-edited by the author of "Promoting One Health: MU Research Center for Human/Animal Interaction"
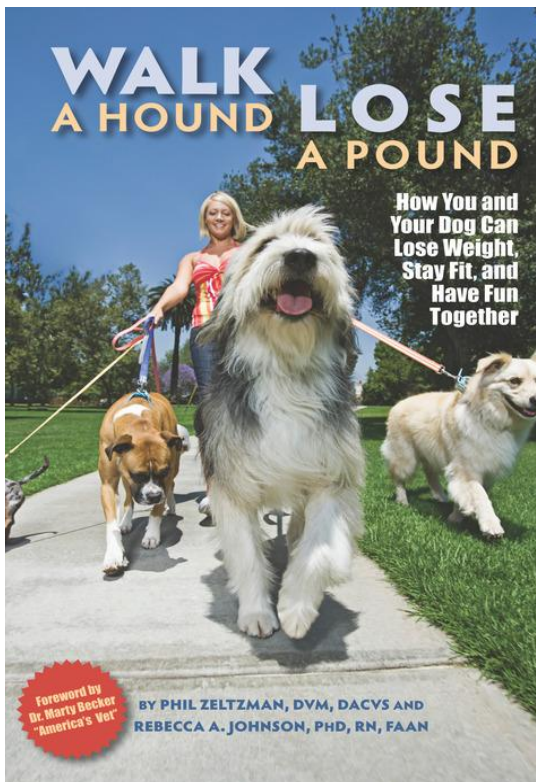
Zeltzman, P. & Johnson, R. A. Walk a hound, lose a pound: How you and your dog can lose weight, stay fit and have fun together. West Lafayette, IN: Purdue University Press; 2011. Available Amazon.com
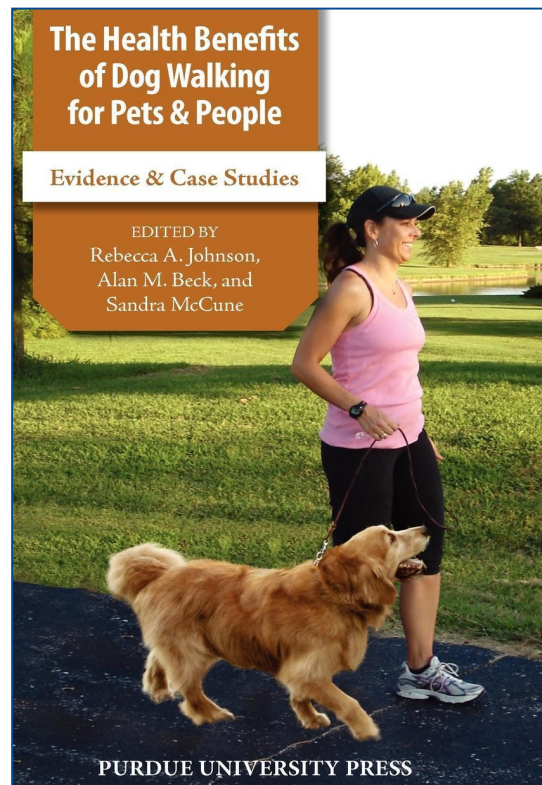
Johnson, R. A., Beck, A. M., & McCune, S. The health benefits of dog-walking. West Lafayette, IN: Purdue University Press; 2011. Available Amazon.com

Research Center for
Human-Animal Interaction

Promoting One Health:
The University of Missouri
Research Center
for Human/Animal Interaction

by Rebecca A. Johnson, PhD, RN

Pets, especially dogs, have been shown in rigorous studies to improve both the mental and physical health of most of their owners.

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Abstract

The University of Missouri's College of Veterinary Medicine is home to the Research Center for Human-Animal Interaction. This center uniquely addresses a growing area of research that focuses on how the human-animal bond impacts health in people and animals. This article highlights the One Health basis for the center, several research projects, and future goals for the center.

Introduction:
One Health Defined

There is considerable evidence that there has been an interrelationship between humans and animals since well before the domestication of animals. In varying degrees, the health of humans and of animals was then, and continues to be, interdependent. In particular, humans relied on animals for food, protection, and warmth. The concept of One Health is based on this premise and has resulted in a movement between human and veterinary medicine promoting cross-disciplinary collaboration to address not only illnesses common across species, but also interventions to facilitate health as more than just the absence of illness.

The American Veterinary Medical Association has arrived at the following definition:

"One Health is the collaborative effort of multiple disciplines - working locally, nationally, and globally - to attain optimal health for people, animals, and our environment. Together, the three make up the One Health triad, and the health of each is inextricably connected to the others in the triad. Understanding and addressing the health issues created at this intersection is the foundation for the concept of One Health."[1]

In recognition of the importance of long-standing cross-species interconnectedness, in recent years there has been considerable growth in human-animal interaction research. One specific area in this field is human-companion animal interaction. This interaction commonly takes place in four contexts: pet ownership; animal assisted activity in which specially trained and registered therapy animals and their handlers visit patients in acute and long term care settings; animal assisted therapy in which an animal is incorporated by a licensed professional as part of a patient-specific treatment plan; and service animals commonly dogs trained to perform specific tasks to facilitate independent living of their owner.[2]

Health Benefits Abound, Especially for Dog Owners

Pet ownership benefits human health in a number of ways, and to greater degrees in particular populations. In adults, research demonstrated that the presence of a pet has been associated with better performance on mental tasks.[3] Pets are commonly identified as nonjudgmental members of families.[4] Pet ownership has been associated with greater exercise levels (particularly dogs and in older adults); dog walking was associated with sustained physical functioning over a three-year period,[5] and fewer patient-initiated physician visits.[6] Dog ownership has been associated with better lipid panel outcomes[7,8] and pet owners have been found to be more likely to survive one year after myocardial infarction than non-pet owners controlling for age, severity of illness and comorbidities.[9] Older pet owners were found to be less depressed than non-pet owners.[10] This outcome is important for preventing the downward spiral in functional ability, mood, and social engagement that may lead to nursing home placement.

Human animal interaction and pet ownership have been found to be beneficial in relation to obesity prevention and treatment. Obesity is now a major public health problem given that there are over 79 million obese adults in the U.S. and 12.5 million children and adolescents. This condition is ranked as the leading cause of preventable death and resulted in annual national health care expenditures of over $147 billion in 2008. Missouri is one of nine states with an adult obesity rate of over 30% (http://www.cdc.gov/vitalsigns/AdultObesity/index.html#). The problem also occurs in companion animals. An estimated 34% of dogs are overweight or obese.[11] Existence of this problem on both ends of the leash suggests an opportunity for health care providers to engage the human-dog relationship to promote physical activity and one health across the two species.

Commitment to owning a dog has been found to promote exercise through dog walking as owners meet the needs of their dog for exercise and in doing so, benefit themselves. Australian dog owners walked 18 minutes per week more than non-owners and met physical activity recommendations of 150 minutes per week.[12] In the United States, adults who walked dogs accumulated a minimum of 30 minutes of walking per day in bouts of at least 10 minutes.[13] One trial found that obese adults with pets increased their moderate physical activity over those who did not have a pet.[14] Older adults who walked shelter dogs

The goal of PALS (Pet Assisted Love and Support for Seniors) is to improve the quality of life, and to bring healing to the team: the volunteer, the animals, and the older adults who benefit from this program.

were found to have significantly increased walking speeds when compared with those who walked with a human companion.[15] Stress reduction commonly associated with exercise was demonstrated in one study in which older Japanese adults experienced increased parasympathetic neural activity. This activity was sustained while dog-walking and was cumulative over succeeding walks.[16] These findings suggest that dog ownership may be one way to facilitate healthy physical activity patterns benefitting both owners and their dogs. Two recent resources for promoting pet owners' exercise with their companion animal are books co-authored/co-edited by the author of this paper: (See sidbar on page 196.)

Animal Assisted Activity

Animal assisted activity appears beneficial in anxiety-inducing disease states or treatment protocols. It is thought to provide distraction and stress relief, particularly in settings in which patients may feel isolated and need additional interaction and companionship (e.g. in hospitals and nursing homes).[17] Nursing home residents who had dog visits were significantly less lonely than those not having visits.[18] Companion animals have been found to facilitate social interaction. People are more likely to interact positively with others when an animal is present.[19] Increased positive social interaction occurred between nursing home staff, residents and staff, and residents with other residents when a dog was present.[20] Indirect interaction with animals has also been found to be of benefit for nursing home residents. Patients with Alzheimer's disease sat for longer periods and ate significantly more food when watching

fish swim in an aquarium placed in front of them during meals.[21] Hospitalized patients reported less pain and used fewer analgesics during and after an animal visit.[22] In patients with heart failure, anxiety, and epinephrine levels decreased during and after an animal visit.[23]

Animal Assisted Therapy

Animals are used in rehabilitation settings to facilitate patients' progress with specific therapy goals. In this and other therapeutic settings (e.g. psychotherapy), licensed professionals (e.g. psychologists, physical and occupational therapists and social workers) include interaction with animals in therapy protocols. Children with autism were better able to stay on task with their therapist with a therapy dog present.[24] In other modalities such as in therapeutic horseback riding, patients increased their balance, and improved their gait through specific activities while riding specially selected and trained horses.[25] Animal Assisted Therapy has increased; however, in order to ensure that such programs persist and continue to be adopted, randomized clinical trials are needed to define less equivocal and more replicable outcomes.

Service dogs perform specific tasks to assist their owners such as opening doors, turning on lights, or retrieving objects.

Service Animals

In contrast to pets, service animals live and work with their owners to facilitate independent living. These animals (generally dogs) are federally protected by the Americans with Disabilities Act and are permitted to accompany their owner wherever that person goes as long as the dog does not create a disturbance or unsafe situation through unruly or aggressive behavior. The dogs perform specific tasks to assist their owners such as opening doors, turning on lights, or retrieving objects. Presently, service dog roles are being expanded to alleviate post-traumatic distress symptoms (PTSD, e.g. in military veterans) through tasks such as alerting their owner to someone coming up from behind, going ahead around blind corners to signal the owner that it is safe to proceed, and creating personal space by standing in front of the owner in a crowded situation. The Veterans

Administration has recently released a policy indicating that it will not provide financial support for veterans' PTSD service dogs due to a lack of published research demonstrating their efficacy http://www.stripes.com/va-says-no-service-dogs-benefits-warranted-for-ptsd-sufferers-1.188166. However, veterans continue to seek and obtain PTSD service dogs to assist them in daily life.

One Health Human Animal Interaction Work

The Research Center for Human Animal Interaction (ReCHAI) was established in 2005 at the University of Missouri, College of Veterinary Medicine, as a collaborative center with the Sinclair School of Nursing. ReCHAI's mission is "Studying and promoting the health benefits of human-animal interaction (HAI)." ReCHAI plays a vital role in international initiatives to promote research, practice, and education in human animal interaction through the International Association of Human Animal Interaction Organizations (IAHAIO), a global umbrella association of over 40 organizations conducting varied work in HAI. The author of this paper is currently President of this Association. In July, 2013, IAHAIO will hold its triennial conference in tandem with the 150th annual convention of the American Veterinary Medical Association. This will create unprecedented opportunities for collaboration and information sharing across human health and veterinary medical fields.

ReCHAI conducts both research and community programs. One example of a community program, the TigerPlace Pet Initiative, aims to enhance pet ownership among older adult residents of TigerPlace, an aging-in-place retirement residence originated by the Sinclair School of Nursing. ReCHAI provides older adults with support needed to keep their pets via a pet care assistant. This assistant visits pet owners at least three times each week to walk dogs (for those who are unable to do this), clean cat litter boxes, and provide other assistance as needed (e.g. administering medications to pets). We maintain an on-site veterinary medical exam room which is used by

our faculty veterinarian who makes monthly house calls on the pets, and also gives presentations for the older adults and facility staff on matters pertaining to pet health. The program includes a fund for providing foster care for pets until their adoption when their owners pre-decease them. This program facilitates One Health by providing older adults who own pets support to help them keep their pets and benefit from the known positive outcomes of pet ownership. In addition to increasing TigerPlace residents' happiness, this program also provides early detection of health problems in the pets, which enables prompt intervention, facilitating health of the animals.

Similar programs could be implemented in other retirement facilities by linking the facility with a local veterinarian willing to engage in this type of primary and preventive care for older adults' pets. An auxiliary or volunteer corps could be formed to help older adults with the tasks done by the pet care assistant at TigerPlace. ReCHAI is called upon to advise other facilities wanting to establish similar pet-owner facilitating programs. This includes recommending procedures to address animal behavior problems (e.g. protective dog behavior toward staff working with older adults), pet overfeeding, and concerns of residents who do not like pets.

Since 2005, ReCHAI has conducted 13 studies including five which are currently underway. The center is a research training site for doctoral students across disciplines, professional veterinary medical students and undergraduate students completing capstone or practicum experiences. ReCHAI's research investigates topics that include: alleviating US military veterans' post traumatic stress through shelter dog obedience training, relieving anxiety of abused children during forensic interviews by placing a trained service dog with them during the experience, exploring the role of the family dog in families with children who have autism, and identifying the effect of training shelter dogs in basic obedience on prison inmates' rehabilitation. The studies are funded by grants from external sources including but not limited to the National Institutes of Health, the Missouri Foundation for Health and several other foundations.

Future Directions and Goals

Future goals for ReCHAI are to expand its funding base through endowments, gifts, and additional research grants; and, to enable additional research, community programming, and student learning opportunities. A further goal is to develop pre and post-doctoral training fellowships in HAI and One Health through cross-disciplinary collaboration. Any reader interested in finding more information about the research and programs of the MU Research Center for Human-Animal Interaction can find it on the webpage at http://www.rechai.missouri.edu.

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Disclosure

None reported.

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A Brief History of Animal Modeling

by Aaron C. Ericsson, DVM, PhD, Marcus J. Crim, DVM & Craig L. Franklin, DVM, PhD

Animal models play a critical role in translational research and advancement of human and animal health.

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Abstract

Comparative medicine is founded on the concept that other animal species share physiological, behavioral, or other characteristics with humans. Over 2,400 years ago it was recognized that by studying animals, we could learn much about ourselves. This technique has now developed to the point that animal models are employed in virtually all fields of biomedical research including, but not limited to, basic biology, immunology and infectious disease, oncology, and behavior.

"Ought we, for instance (to give an illustration of what I mean), to begin by discussing each separate species-man, lion, ox, and the like-taking each kind in hand independently of the rest, or ought we rather to deal first with the attributes which they have in common in virtue of some common element of their nature, and proceed from this as a basis for the consideration of them separately?"

-Aristotle (384 -322 BC)
"On the Parts of Animals"

Early History of Animal Modeling

The use of animals as models of human anatomy and physiology began in ancient Greece (see Table 1). These first recorded instances of comparative science were very observational, their purpose being to better understand human ontogeny and physiology. Fortunately, many of the findings of prominent thinkers like Aristotle were documented and conveyed to other countries via trade routes, and animal modeling soon became a research tool of both European and Arab physicians. While this early period saw great discoveries, there were still many misconceptions about the workings of the body, and it was not until the Renaissance (fourteenth through seventeenth centuries) that animal modeling contributed to a true paradigm shift in our understanding of human physiology.

During the mid-sixteenth century, a few astute physicians such as Servetus and Lusitano deduced that blood followed two connected but distinct circuits through the body, i.e. pulmonary and systemic circulation. In the late sixteenth and early seventeenth centuries, William Harvey (1578-1657) assiduously studied and compared the anatomic and functional properties of the heart and vasculature in multiple species including eels and other fish, chicks, and pigeons. Based on these investigations, he penned several seminal texts including *De Motu Cordis* in which he describes with great accuracy, and in great detail, the human circulatory system. He also pioneered

Table 1. Early Milestones in Animal Modeling

Years	Researcher(s)	Milestone
6th c. BCE	Alcmaeon of Croton	Determined that the brain is the seat of intelligence and sensory integration based on studies using dogs
4th c. BCE	Aristotle	Studied embryogenesis and ontogeny in chicks
3rd c. BCE	Erasistratus	Studied the cardiovascular system in live animals and deduced that the heart functions as a pump
2nd c. CE	Galen of Pergamum	Studied cardiovascular and neuroanatomy extensively using live animals
12th c.	Avenzoar	Practiced surgical techniques on animals before applying them to humans, e.g. tracheotomy
17th c.	William Harvey	Studied anatomy of several species of live animals and provided accurate and detailed descriptions of the function of the cardiovascular and other systems

the theory of epigenesis, i.e. that embryos originate and develop from a single cell, based on his observations of embryonic chicks (recommended for developmental studies by Aristotle in Book II of *The Generation of Animals*). Of note, Harvey was careful in his selection of model species, in order to exploit certain properties of the animal such as heart rate and poikilothermy ("cold-bloodedness").

The careful selection of the most informative species for an animal model is still very important, but it also presents a unique challenge for investigators. Scientists must consider not only financial feasibility and previous experiments utilizing a given species, but also the unusual biological characteristics of a species and the available palette of imaging and molecular techniques available for that species. The choice of a naturally occurring species model, sometimes called the comparative method, was perhaps most famously and succinctly stated by the 1920 winner of the Nobel Prize in Physiology and Medicine, August Krogh, in 1929, "*For a large number of problems there will be some animal of choice or a few such animals on which it can be [most] conveniently studied.*"[1] One recent example is the use of the nine-banded armadillo in studies of leprosy due to the armadillo's unique susceptibility to *M. leprae*.[2]

Animal Models in Modern Biomedical Research

By the beginning of the twentieth century, the use of animal modeling had increased dramatically and, while some individuals still questioned the ethics of their use, animal modeling, particularly in rodents, had become the *de rigeur* method of demonstrating biological significance. However, all research animals

at this time were outbred and as the use of animals became more experimental, rather than observational, researchers soon appreciated the confounding factor of genetic variability in their research. Through the efforts of many forward-thinking individuals such as William Castle, Clarence Little, Halsey Bagg, and Leonell Strong, this problem was addressed via inbreeding of mice to the point that genetically identical mice became available for experimental use (see Table 2). This provided a steady source of research subjects that bred to maturity very quickly and with limited variability from litter to litter and year to year. As more and more inbred strains of mice and rats were developed, it was soon appreciated that there were inherent differences between strains in basic biological parameters, as well as susceptibility to induced and spontaneously occurring diseases. Many of these were complementary strains bred in parallel providing susceptible and resistant strains that are otherwise genetically similar, such as the non-obese diabetic (NOD) and related strains.[3] Thus, strain selection is one of the most important considerations in animal modeling, particularly in rodents.

If natural models were not available or feasible, the ability to manipulate the genome of a model species allowed for the creation of animals uniquely susceptible or resistant to a certain model. So, as advances were made in the field of genetics, scientists became increasingly adept at manipulating the as yet unsequenced genome of mice. The 1980s saw an explosion in this technology with the advent of transgenic mice carrying additional genetic material, and knockout mice in which genetic material is deleted. Recently, our ability to manipulate the mouse genome has become increasingly refined with developments such as tissue-specific methods of

knocking out genes such as the Cre-Lox system,[4] methods of turning on or off gene transcription *in vivo* using tetracycline- or tamoxifen-induced systems,[5] and methods of identifying or removing entire cell lineages *in vivo*

Table 2. Recent Milestones in Animal Modeling

Years	Researcher(s)	Milestone
1902	William Castle	Begins breeding mice for genetic studies
1909	Clarence Little	Begins inbreeding mice to eliminate variation
1920s	Frederick Banting	Isolated canine insulin and effectively treated diabetic dogs
ca. 1930	Little and MacDowell	First fully inbred mouse (20 brother × sister matings) achieved
1940s	John Cade	Studied the use of lithium salts as an anticonvulsant in guinea pigs and translated his findings to treatments of depression
1976	Rudolf Jaenisch et al.	Developed first transgenic mouse
1980s	Several	Extensive testing of drug safety and dosing regimens for HIV performed in rhesus macaques
1987	Capecchi, Evans, and Smithies	Developed first knockout mouse
1997	Wilmut and Campbell	First animal cloned from an adult somatic cell, Dolly the sheep
2002	Several	Mouse genome sequenced
2004	Several	Rat genome sequenced
2009	Aron Geurts et al.	Developed first knockout rat

via fluorescent protein- and diphtheria-toxin receptor-knockin mice respectively.[6, 7] Additionally, researchers have used similar technologies to generate transgenic rats,[8] cats,[9] dogs,[10] rabbits, pigs, sheep,[11] goats, cattle, chickens,[12] zebrafish,[13] and non-human primates,[14] to name just a few. While the ability to generate targeted gene knockouts in other species has lagged behind, knockout rats were successfully created in 2009 using a zinc finger nuclease-based technique distinct from that used in mice.[15]

The mouse continues to be the powerhouse for biomedical research (see sidebar page 206). Undoubtedly, the most important change over the last 25 years is the spectacular escalation of the laboratory mouse in research, which stands in glaring contrast to the declining role of most non-rodent mammalian models (see Figure 1). By comparison, use of the rat has plateaued, as targeted genetic manipulations proved more difficult in this species. The creation of the first knockout rats may help to explain the very recent up-tick in rat model-based biomedical publications. However, with the rising capacity to modify the genomes of laboratory species other than the mouse, the face of biomedical research is now changing. Genetically malleable species such as swine and the zebrafish are increasingly out-competing once common model organisms like the guinea pig, rabbit, and ferret (see Figure 1). These important trends reveal both 1) the dramatically increasing utility of certain model species relative to others, and 2) the refinement of animal research via use of the lowest

ordered vertebrate possible to accomplish a given scientific objective.

Additionally, the recognition of the impact of the gastrointestinal and dermal microbiota led to the birth of an entirely new research era – gnotobiotics. Through the use of Caesarian birth, flexible-film isolator cages, and irradiated food, mice can now be maintained in completely germ-free conditions or colonized with one or more defined bacterial species. A combination of eight commensal aerobic and anaerobic bacteria called Altered Schaedler's Flora (ASF) is commonly used as the known intestinal microbiota.[16] However, with the recent development of robust methods of fingerprinting the entire gut microbial community such as Denaturing Gradient Gel Electrophoresis, Automated Ribosomal Intergenic Spacer Analysis, and deep sequencing, researchers are capable of quickly and reliably monitoring the composition of the gut microbiota and thus moving away from more reductionist models such as ASF. While the development of inbred rodent strains allowed for the control of host genetics, the development of research animals harboring complex but defined microbiota allows for control of microbial genetics known to impact host physiology. Moreover, gnotobiotics can also be applied to non-murine species, so this field is likely to continue to evolve.

Future of Animal Modeling

What does the future hold for animal models? As biomedical research funding agencies continue to
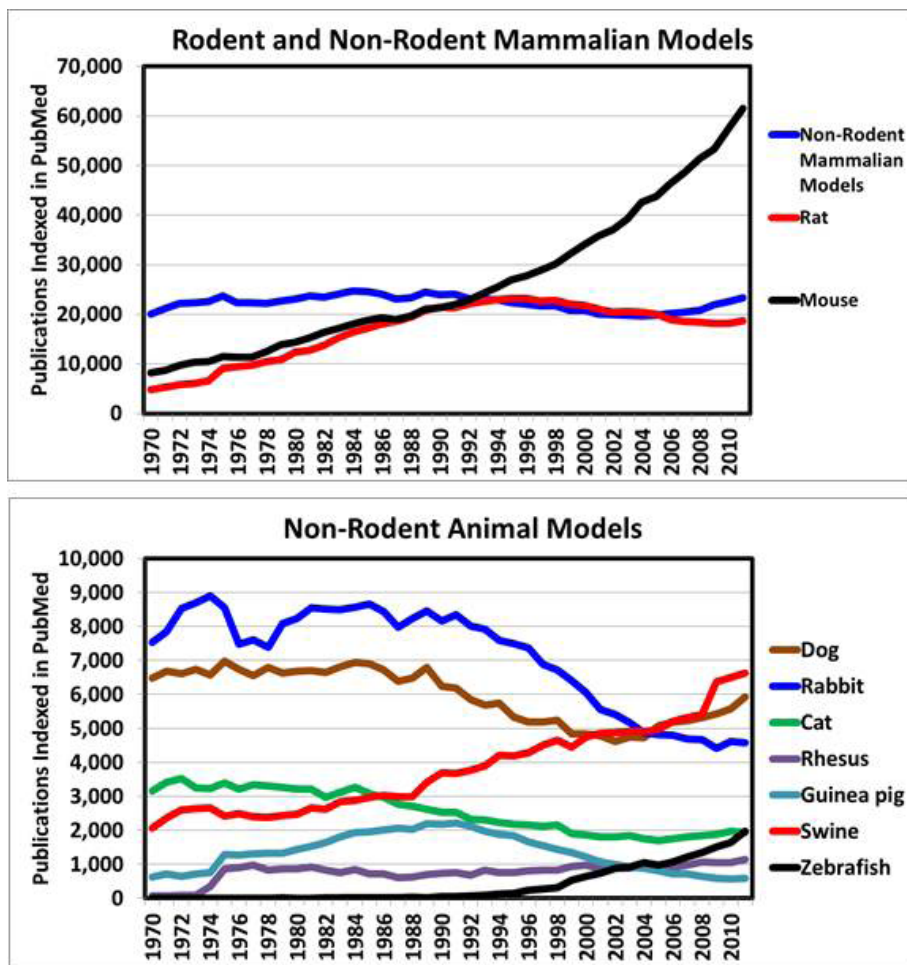
Figure 1
Pubmed search results by publication date, 1970 through 2011. Search terms for each species included the scientific name and the common name for each species; except that only the scientific name was used for mouse and rat. "Non-rodent mammalian models" includes the dog, rabbit, cat, rhesus macaque, guinea pig, swine, chimpanzee, and ferret.

with this disease, but fail to develop the devastating pulmonary complications. To circumvent these deficiencies, a swine model was recently generated and early data suggest that the latter better replicates pulmonary disease.[17] Other examples include the study of naturally occurring diseases in domestic species that optimally mimic disease such as the study of osteosarcoma progression and response to therapy in dogs.[18] This concept, referred to as One Medicine, promotes the sharing of resources, knowledge, and effort toward the common goal of improving the health and well-being of all species and is proving to be a powerful adjunct to traditional laboratory animal models.

emphasize rapid and robust translatability of studies, it is likely that animal modeling will move more and more towards models that most appropriately mimic human conditions, using multiple models to ensure robustness of data and new genetic and metagenomic tools to develop and refine "humanized models." With advancements in genetic engineering in non-mouse species, we are also likely to see new models generated for diseases where mouse models have not adequately replicated the human condition. For example, genetically engineered mouse models of cystic fibrosis develop intestinal diseases similar to those seen in humans

Humanized models such as transgenic animals expressing human genes are also rising to the forefront. A classic example involves the insertion of the gene encoding the human major histocompatibility locus, HLA-B27 into rats.[19] Individuals with this MHC haplotype have increased susceptibility to several autoimmune conditions. Similarly, rats with this transgene are more susceptible to autoimmune disease and as a result, this model has proven indispensable to studies of MHC-related disease susceptibilities. This concept was expanded by coupling targeted mutations in endogenous murine genes with the introduction of

transgenes of mutated human genes. Newer models continue this process through combinations of multiple mutations that provide refined models that better recapitulate disease.

Humanization of models has also involved creating mice with entire human systems. To this end, mice with human "immune systems" were generated as early as 1988 by implanting either fetal lymphoid tissue or peripheral blood leukocytes into mice with spontaneous severe combined immunodeficiency. These mice, along with several refined versions have demonstrated their usefulness in studies of hematopoiesis, basic immunology, infectious disease, and autoimmunity.[20] The concept of creating human "organs" in mice has also made its way into other systems such as the liver, where humanized mice are proving invaluable in studies of drug metabolism and viral hepatitis.[21]

Taking concepts of gnotobiology one step further, researchers have recently begun reconstituting germ-free mice and rats with microbiota isolated from human fecal samples.[22, 23] These and other studies have yielded surprising discoveries regarding the role of microbiota in host physiology and well-being, in the gastrointestinal tract as well as other less intuitive disease models.[24, 25] These studies at the forefront of animal modeling take into account not only the variability present within the individual model organism but also the variability present within the superorganism, i.e. the host and its associated microbiota, allowing for control of important variables that were once often overlooked.

The combination of these concepts will likely lead to increased genetic engineering and humanization of non-rodent species, and coupling of this data with one medicine-based studies of domestic animals and human clinical trials. Thus it is likely that animal models will continue play a critical role in translational research and advancement of human and animal health.

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None reported.

New York Times Article Misleads on the Value of Mouse Models

by Catherine E. Hagan, DVM, PhD

In February 2013, the *New York Times*[1] covered a study published in a prestigious research journal, the *Proceedings of the National Academy of Sciences* (PNAS).[2] The sensational headline was "Mice Fall Short as Test Subjects for Humans' Deadly Ills." The study compared gene expression data in white blood cells from both humans and mice under conditions of trauma, burn, or sepsis.

A detailed response to the *Times* article was posted by Mark Wanner[3] on a blog at the Jackson Laboratory, a non-profit organization that conducts mammalian genetics research to advance human health. His rebuttal highlights the main problem with the PNAS study - it compares heterogenous human data to a single mouse strain, which is the equivalent of one mouse, given that animals within an inbred strain are genetically identical. Furthermore, the strain used has long been known to be resistant to bacterial infections.

The general public may be left with the impression that research using mice has been a waste of years of research and billions of dollars of funding. Headline scanners and casual readers may miss a key sentence: "The study's findings do not mean that mice are useless models for all human diseases."[1]

The hyperbole in the *Times*' article underscores the importance in distinguishing the various opinions of scientists from conclusions that are supported by actual data. In the original PNAS article, the study conclusions are more conservative, suggesting that genomic studies in the dawn of this new era of molecular medicine could complement or decrease the need for mouse models in drug discovery and development. That is a reasonable statement. The authors then claim their data support prioritization of genomic studies such as theirs over mouse studies. They set up a "straw mouse," using a model that one would not expect to recapitulate human sepsis - an infection-resistant strain - and then knock it down, citing the lack of correlation of its gene expression data compared to humans.

Though the media overstated the study's findings, it revitalized an important conversation about the strengths and limitations of mouse models. For understanding the role of genes in whole animals, the mouse is the best model. The *Times* article ultimately conceded the potential contributions of mice, stating "Researchers said that if they could figure out why mice were so resistant, they might be able to use that discovery to find something to make people resistant."[1] Funding agencies clearly recognize that medical progress will most likely occur through diverse funding portfolios that include both basic research using animal models and clinical research in human patients.

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The Mighty Mouse: The Impact of Rodents on Advances in Biomedical Research

by Elizabeth C. Bryda, PhD

What is not always appreciated is the extraordinary impact that laboratory mice and rats have on biomedical research.

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Abstract

Mice and rats have long served as the preferred species for biomedical research animal models due to their anatomical, physiological, and genetic similarity to humans. Advantages of rodents include their small size, ease of maintenance, short life cycle, and abundant genetic resources. The Rat Resource and Research Center (RRRC) and the MU Mutant Mouse Regional Resource Center (MMRRC) serve as centralized repositories for the preservation and distribution of the ever increasing number of rodent models.

Perceptions of Mice and Rats: An Introduction

Mention mice and rats to most people and images of unsanitary conditions and urban decay come to mind. Rats have been vilified as the carriers of the infected fleas that led to the dreaded Black Plague that decimated Europe, North Africa and Central Asia in the fourteenth century. It has been suggested recently that an apology is in order and that other influences, not rodents, were to blame.[1] More recently, infected mice have resulted in Hantavirus outbreaks including the recent scare in Yosemite National Park where many campers contracted the deadly virus from mice living in the cabins.[2]

For many people, exposure to rodents consists of seeing them used as food for snakes or birds of prey at the zoo, or having to deal with unwanted rodents invading their dwellings. A kinder image of rats and mice sometimes appears in literature – think of the rodents in E.B. White's beloved books: the adventurous mouse Stuart in *Stuart Little* or the gluttonous rat, Templeton, of *Charlotte's Web* fame. Recall the heroic cartoon character Mighty Mouse who always "saved the day" and of course, the best known mouse of all, Disney's Mickey Mouse.

What is not always appreciated is the extraordinary impact that laboratory mice and rats have on biomedical research. They

are often the preferred animal model for studies of human disease and the standard species of choice for pre-clinical trials.

Comparative Medicine Research

Comparative medicine is built on the ability to use information from one species to understand the same processes in other species. Basic biomedical research involves the characterization of genes/proteins, the study of anatomical and physiological functions and the characterization of normal and pathological states in a variety of animal species. This knowledge is then applied to understanding these same processes in humans. Likewise, information gained in the field of human medicine can be mined to advance veterinary medicine because of the commonalities among species that form the basis of comparative medicine.

Laboratory rats and mice provide ideal animal models for biomedical research and comparative medicine studies because they have many similarities to humans in terms of anatomy and physiology. Likewise, rats, mice, and humans each have approximately 30,000 genes of which approximately 95% are shared by all three species.[3-6] The use of rodents for research purposes has economic advantages: mice and rats are relatively small and require little space or resources to maintain, have short gestation times but relatively large numbers of offspring, and have fairly rapid development to adulthood and relatively short life spans. For example, mice have a gestation period of approximately 19-21 days; can be weaned at three to four weeks of age, and reach sexual maturity by five to six weeks of age, allowing large numbers of mice to be generated for studies fairly quickly.

The use of rodents also provides advantages related to the wealth of genetic information available to scientists. The human genome was sequenced in 2001,[4, 5] with those of the mouse and rat following in 2002 and 2004 respectively.[3, 6] The availability of the complete nucleotide sequences for all three species has enabled genome-wide comparisons across species which have been critical for the identification

and characterization of genes. The ability to use sophisticated molecular genetic techniques to manipulate the genes in mice and more recently rats, allows genes to be "knocked out" (no expression) or expressed at designated times of development or in select tissues in order to better understand their normal function and/or role in disease.

Mice as Models to Study Human Disease: Hereditary Deafness

The identification of genes responsible for hereditary deafness provides an excellent example of the utility of mice for studying human disorders. In humans, hearing loss is the most common sensory deficit, with congenital deafness occurring in one per every 1,200 to 2,000 live births.[7] Over a hundred different genes are involved in non-syndromic hearing loss.[8] The mouse has been instrumental in identifying and characterizing a large number of these genes.

Traditionally, it has been difficult to study the genetics of hearing loss and deafness in humans due in part to the lack of large families or large numbers of affected individuals for studies, the issue of genetic heterogeneity (the phenomenon in which a single genetic disorder, in this case deafness, can be due to mutations or defects in a number of different genes), and the general inability to perform detailed anatomical analysis on human ears, particularly inner ears. Especially problematic is the difficulty in ascertaining what damage is due to inherited factors versus environmental factors such as exposure to loud noise or infections.

The mouse allows investigators to circumvent all of these problems. Genetically identical inbred strains of mice carrying a single mutated gene are particularly valuable in identifying gene function. Many mice carrying mutations related to hearing loss have been identified over the years because of the often obvious phenotype (physical characteristics) of a deaf mouse: failure to respond to noise as indicated by the lack of Preyer's reflex (ear flick), unusual head tossing and circling (literally running around in a circle) behavior. This latter characteristic is the manifestation of the vestibular defects that are

common in mouse deafness mutants. Unlike with humans, mice can be purposely bred to specific mates in order to generate many offspring with the desired genetic make-up (genotype). Because the mice are raised in controlled, pathogen-free environments, the effect of environmental factors can be highly controlled. Thus, we can attribute any hearing deficits completely to the genetic mutation. Because of the identical anatomical structures, findings in the mouse can be directly correlated to the expected pathology in humans. Lastly, using comparative genomic techniques, identification of a gene responsible for deafness in the mouse allows the equivalent (orthologous) gene to be identified in people. Beyond these studies to identify genes important in auditory development and function, the mouse strain can serve as a model to further explore the biological function of the gene and better understand its role in the auditory system. To date, over 55 non-syndromic human deafness genes have been identified[9] and in every case, a corresponding mouse mutant serves as a model for that particular form of deafness (http://hearingimpairment.jax.org/models).

Advantages of Rats in Biomedical Research

Since mice are small in size and generally cost less to maintain, and because the tools to genetically manipulate their genomes have been available since the 1980s, mice are often the first choice as a rodent model. However, there are many areas of investigation where rats are preferred, including cardiovascular research, behavioral studies and toxicology.[10]

Cardiovascular disease is the leading cause of death and morbidity in developed countries (http://www.who.int/cardiovascular_diseases) and it is typically multifactorial (caused by combinations of genetic and environmental factors).[11] Rats are often the preferred rodent model for cardiovascular research where their larger size is an advantage, especially for facilitating surgical procedures and other types of testing. Many unique strains of rats have been generated that model the complex nature of human obesity, diabetes, and cardiovascular disease and therefore in this case, rats provide excellent animal models for the study of these diseases.[12,13]

Rats are commonly used for behavioral studies because they are much more social than mice and their behavior better mimics behavior seen in humans. For example, expansion of a three-base pair sequence in the FMR1 gene is responsible for Fragile X syndrome, the most common cause of inherited intellectual disability in humans.[14] This expansion leads to methylation of the FMR1 gene, essentially shutting it down such that the gene is not expressed.[15] Defects in this gene are a known genetic cause of autism.[16] When the FMR1 gene is knocked out in mice, they exhibit elevated social interactions.[17] However when the same gene is knocked out in rats, they become less engaged in social play and emit fewer vocalizations during play sessions. These social impairments more closely parallel social behavior symptoms seen in humans with FMR1 mutations. Affected rats also display compulsive chewing behavior. Compulsive and repetitive behaviors are core symptoms in autism spectrum disorders (http://www.sageresearchmodels.com). Thus, in this case, the rat is the more appropriate rodent model.

Choosing the Best Species

In the past, the use of the mouse often eclipsed that of the rat because of the availability of better molecular techniques to manipulate the mouse genome. Recent advances in genetic tools to create knockout rat models promise to eliminate these barriers and may lead to an increase in the use of rats for a wider variety of biomedical research. Ultimately, the choice of rodent model depends on which species more closely recapitulates the symptoms and disease process seen in humans. It is clear that rats are not simply huge mice and that each species has advantages and disadvantages that often depend on the particular process or gene being studied. From a translational medicine standpoint, it is particularly critical to choose the appropriate model because a tremendous amount of money is

Figure 1
Example of fluorescence seen in EGFP rat strains. Pictures of organs from SDTg(GFP)2BalRrrc (RRRC:0065) animals (images on right of each panel) and wild type controls (images on left of each panel). Upper images for each panel are under bright light, bottom images for each panel are under fluorescent light. Panel A: kidney; Panel B: eye; Panel C: lung; Panel D: heart.
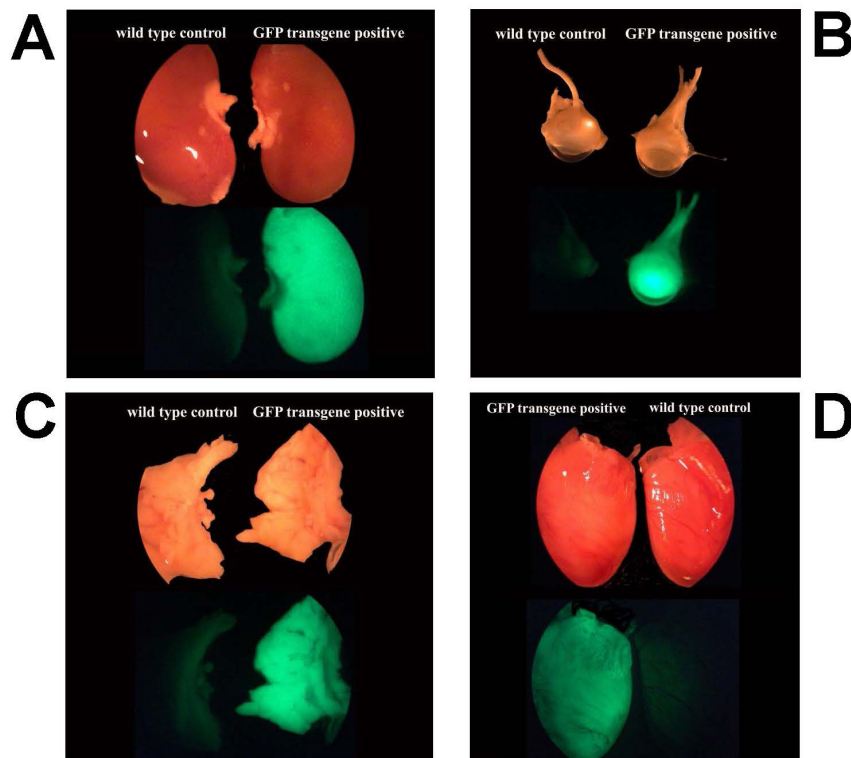
of several best selling drugs found that the mouse knock out phenotypes of the targets of these drugs correlated well with known drug efficacy. The therapeutic effect observed in the knock out model was often a good indicator of success in the clinic.[19] This supports the notion that establishing a more specific and sensitive preclinical trial paradigm based on the best animal models will reduce drug development costs and more importantly, reduce the risk to human subjects in clinical trials.

Rodent Resource Centers

The use of rodents in biomedical research continues to rise and the number of unique strains and models is increasing tremendously as individual investigators and large federally funded multi-group projects generate increasing numbers of genetically engineered mice and rats.

The University of Missouri has the unique distinction of being the home of three National Institute of Health (NIH)-funded animal resource centers: The Rat Resource and Research Center (RRRC), the MU Mutant Mouse Regional Resource Center (MMRRC) and the National Swine Resource and Research Center (NSRRC). The RRRC and NSRRC are the only Centers of their kind in the United States. The MMRRC is part of a consortium of four regional centers located throughout the U.S. The purpose of the two rodent-centric Resource Centers is to serve as repositories for rat and mouse strains/stocks that are important for use in biomedical research. The Centers 1) import these important animal models, 2) cryopreserve

spent testing drugs and therapies that ultimately fail at various stages of pre-clinical and clinical trials. One reason for this is that results obtained in animal trials do not always accurately reflect outcomes in humans.

It has been estimated that new drugs take an average of 15 years to go from discovery to market at an average cost of $900 million.[18] Based on a report from the Tufts Center in 2001, it is estimated that of 5,000-10,000 compounds that enter the development pipeline, 250 will make it to preclinical trials and of those, only five will move into human clinical trials. Of those five, on average, only one will actually make it to market. The investment losses of money and time associated with the four failed drugs is huge. Interestingly, a retrospective study

embryos and/or sperm as a means to store and bank the models, and 3) serve as distribution centers to send the animals or cryopreserved materials to investigators worldwide who use these models in their research.

These repositories came into existence as a means to centralize the storage and distribution of important rodent animal models. The time and money used to create genetically modified animal models and characterize them is quite large; it is therefore important for them to be readily available to the scientific community for further research. The Resource Centers take the burden from the individual investigators of preserving the strains and shipping the animals to other researchers. Importantly, the Resource Centers use strict quality control measures to ensure that the genetics and clean health status of these models are monitored and maintained at the highest standards.

Currently, the most highly requested rats that are distributed by the RRRC are strains that contain a genetic modification consisting of the addition of a fluorescent reporter gene (enhanced green fluorescent protein or EGFP) within their genome. The consequence of this genetic addition is that every cell in these rats expresses the green fluorescent protein, allowing them to be readily observed by fluorescence microscopy (see Figure 1). These strains are particularly useful for transplantation and immunological research. Regenerative medicine, adoptive cell transfer and identification of genetically modified cells after gene therapy in vivo require the ability to track donor cells in host tissues and the EGFP rat strains facilitate these types of studies. In 2012, the RRRC received requests for EGFP rat strains from 24 academic institutions, research institutes, and for profit companies located not only in the United States but worldwide.

Future of Rodent Models in Biomedical Research

Rats and mice will continue to play a central role in biomedical research. Increasingly sophisticated manipulations of rodent models, including the creation of so called "humanized" rodents that carry human genes, cells, tissues, or organs may lead to improved and refined models for developing therapeutics for human disease. The power of comparative medicine and the use of mice and rats will continue to provide a powerful tool for advancing the understanding of both normal and disease processes across species and facilitate the transition of research from "bench to bedside" to improve human health.

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Disclosure

None reported.

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Advancing Swine Models for Human Health and Diseases

by Eric M. Walters, PhD & Randall S. Prather, PhD

Although the pig is the relative 'new kid' on the block, pig models of human disease will continue to have a significant impact on the scientific community.

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Abstract

Swine models are relatively new kids on the block for modeling human health and diseases when compared to rodents and dogs. Because of the similarity to humans in size, physiology, and genetics, the pig has made significant strides in advancing the understanding of the human condition, and is thus an excellent choice for an animal model. Recent technological advances to genetic engineering of the swine genome enhance the utility of swine as models of human genetic diseases.

Introduction

Pigs are an ideal animal model for human health and diseases because their anatomy and physiology are similar to humans and because the porcine genome is three times closer than the mouse genome to that of the human.[1] Moreover, the recently completed swine genome sequence (Sus scrofa Build 10) will help advance the pig as an animal model[2] by facilitating the manipulation of genes. The pig has previously gained acceptance from the medical community for studies in organ transplantation, surgical training of medical students, and the development of novel techniques and medical devices.

More recently, naturally occurring and genetically modified pig models have gained acceptance from the biomedical community as animal models for human health and diseases. This shift from the classical rodent model to the pig model for certain diseases has been largely due to the failure of several rodent models to recapitulate the physiology of the disease. One prominent example is cystic fibrosis. When the mutation that causes cystic fibrosis in humans is introduced into mice, the mouse does not recapitulate any of the human disease symptoms; however the recently developed pig model appears to fully mimic the human condition.[3-6]

Domestic swine have been selectively bred for centuries to produce animals with desired characteristics. Altering these characteristics to be more useful to researchers can be done quickly with genetic engineering, or altering the DNA sequence. The integration of foreign DNA into the genome provides a method to rapidly produce animals with a desired characteristic. In 1985, Hammer produced the first transgenic pig through microinjection of the foreign DNA into the pronuclei of the pig zygote.[7] In addition to this method there are several other methods that can be used to produce transgenic pigs including the current standard method of somatic cell nuclear transfer

(SCNT).[8] SCNT can be used in combination with simple transgenesis, as well as advanced genetic manipulations to create knock-ins and knock-outs. More recently, there have been advances in genetic manipulation of cells that will allow more cell-based transgenesis such as Zinc Finger Nucleases and Transcription Activator-Like Effector Nucleases (TALENs) that will enhance the production of swine models for human health and diseases.

The Pig: An Animal Model

The classical rodent models have proved to be very important for understanding the basic biology of genes and proteins involved in human health and disease, but with limited ability to recapitulate some human diseases, their usefulness is restricted. In addition to cystic fibrosis, murine models of diseases such as Spinal Muscular Atrophy (SMA)[9] and Parkinson's disease[10] are inadequate and swine models may prove to be superior.

The pig has many advantages as a non-rodent biomedical model because of the wealth of information about husbandry, logistical support, and reproductive management. Pigs reach sexual maturity at about six to eight months of age (domestic) and have a relatively short gestation period (∼four months) resulting in multiple births. Pigs are also not seasonal breeders so mating can occur year round. There are several genetic backgrounds of both domestic and miniature pigs that thrive in a variety of environmental conditions. The anatomical structure and function of the cardiovascular system, the gastrointestinal tract, and the pancreas of the pig are very similar to humans making them ideal models for investigating diseases related to these systems. Pigs have some notable difference when compared to humans. The pig lymph node is inverted compared to the human lymph node with follicles central to a peripheral medulla.[11] Besides anatomy, genetic analysis has identified naturally occurring swine models for a variety of human health conditions such as hypercholesterolemia, puerperal psychosis, human melanocytic proliferation, and polycystic ovarian syndrome.[12-16] In addition to the spontaneous pig models, genetically engineering the pig genome has allowed us to advance the understanding of genes, proteins, pharmacological "pharming," and to develop organs for xenotransplantation.

Another potential use of the pig as an animal model is in the field of toxicology and drug discovery. With the physiologic similarities between pigs and human, the pig is becoming the non-rodent species of choice for toxicological studies. Examples are assessment of the effects of putative endocrine-disrupting compounds and drug discovery. Advantages of the pig to toxicology studies include: 1) they have a similar response to several varieties of drugs; 2) tissue

Currently there are 29 different lines of pigs (wild-type and genetically engineered) available for request from the NSRRC.

and body fluid samples can be collected during the dosing period prior to euthanasia; 3) oral and parenteral dosing is similar to humans; and 4) the use of pigs eases some of the increasing societal pressure about using primates and dogs as animal models. The challenges to using swine as a model for toxicological studies include requirements for more experimental reagent, higher costs of purchase, and the requirement for more space which increases per diem costs and is not possible in some animal facilities. Some of these concerns can be addressed with the use of miniature pigs, and development and utilization of novel techniques such as xenografting procedures, proteomics, and metabalomics.

Genome and Transgenesis

The swine genome is estimated to be ∼2.7 GB (about 7% smaller than the human genome), and is comprised of 18 autosomes and two sex chromosomes. The lineage of the pig and human genome (in comparison to the human and mouse) has a lower rate of nucleotide substitution with the exonic sequences showing the slowest evolution followed by the 5' untranslated regions and 3' untranslated regions, respectively.[1] Having the sequenced pig genome will be advantageous in developing biomedical models where differences exist between the pig and human. An example of this is Spinal Muscular Atrophy (SMA). Humans have two survival motor neuron genes (SMN1 and SMN2), while the pig only has a single gene for SMN. With this knowledge of the pig and human genomes, investigators made appropriate adaptations to best recapitulate SMA in the pig.[9] This was achieved by adding a transgene for human SMN2 and subsequently disrupting swine SMN to simulate a disrupted SMN1 in humans with SMA.

Table 1
NSRRC Swine Availability

Strain Name	Modification(s)	Gene(s)	Application(s)
Truline® Hampshire	none	wildtype	background for genetic modification
Truline® Duroc	none	wildtype	background for genetic modification
Truline® Landrace	none	wildtype	background for genetic modification
Truline® Large White	none	wildtype	background for genetic modification
Minnesota Mini	none	wildtype	immune system ontogeny and regulation, xenotransplantation, xenoimmunotherapy of cancer
Ossabaw	none	wildtype	background for genetic modification, obesity, diabetes, cardiovascular, Polycystic ovarian syndrome
GGTA1KO/hDAF	transgenic targeted knock-out	α-1,3-galactosyltransferase knockout; human decay-accelerating factor	xenotransplantation research
GFP NT5	transgenic	enhanced green fluorescent protein	cell tracking
FAT-1	transgenic	express a humanized *Caenorhabditis elegans* gene, fat-1, encoding an n-3 fatty acid desaturase	research in the areas of reproduction, cardiovascular, immune system, depression and cancer
Yucatan	none	Wildtype	small porcine model as background for genetic modification
NIH Mini g/g	none	MHC SLA $^{g/g}$	research in the areas of immune system, and xenotransplantation
NIH Mini c/c	none	MHC SLA $^{c/c}$	research in the areas of immune system, and xenotransplantation
NIH Mini a/a	none	MHC SLA $^{a/a}$	research in the areas of immune system, and xenotransplantation
GFP NT92	transgenic	enhanced green fluorescent protein	cell tracking
Rhodopsin	transgenic	rhodopsin P23H substitution	retinitis pigmentosa research
FVIII	tri-transgenic	human coagulation factor VIII; human alpha-antitrypsin; propeptide cleavage enzyme (PACE)	hemophilia research
FIX	tri-transgenic	human coagulation factor IX; human alpha-antitrypsin; von Willebrand factor	hemophilia research
GFP NT92-Yucatan	transgenic	enhanced green fluorescent protein	cell tracking
Tie2-eNOS	transgenic	endothelial nitrate oxide synthase	cardiovascular, exercise physiology
Tie2-Catalase	transgenic	human catalase	cardiovascular, exercise physiology
PSMA1-GFP	transgenic	GFP-Proteasome Fusion Protein	proteasome research
Multi-Xeno	transgenic	α-1,3-galactosyltransferase knockout; human decay-accelerating factor (CD55); CD39; CD59 and thrombomodulin	xenotransplantation
NLS-CAG-eGFP	transgenic	nuclear localized signal-CAG- enhanced green fluorescent protein	cell tracking
CAG-Tomato	transgenic	CAG-Tomato	cell tracking
AFP-CD	transgenic	alpha fetoprotein promoter driving cytosine deaminase	liver research
ALB-TK	transgenic	albumin promoter driving thymidine kinase	liver research

Currently, there are many ways to produce genetically modified animals, however in the pig the most effective method is SCNT. [8] The first transgenic pig to model a human health condition[17] was produced in 1996. Since that time, several transgenics, knock-ins and knock-outs have been produced (for further details see these reviews[8,18]). Somatic cell nuclear transfer provides us with an advantage over previous methodologies in that it allows gene targeting versus random insertion. The exact genetic modification can be determined prior to creating the animal. Gene targeting is a valuable tool for in vivo studies of gene expression, signaling pathways, and developmental biology and more advanced cell based transgenic techniques such as a Cre/Lox-P system and Zinc Finger Nucleases to avoid embryonically lethal targeting events are now amenable to swine genetic engineering.[8] When designing the mutation to be made, the completed pig genome will allow us to have prior knowledge about alternative splice variants, isoforms, or locations in the genome where genetic modification can occur that have no effect on the animal (so called "safe harbors"). Comparative genomic analysis will help identify conserved cross-species genes and regulatory elements that can be utilized in the development of genetically modified pigs.

Swine Resources

There are many resources available for the pig as a biomedical model. With the release of the completed swine genome (Sus scrofa Build 10), there are several genomic resources available for the pig (ftp://ftp.ncbi.nih.gov/genbank/genomes/Eukaryotes/vertebrates_mammals/Sus_scrofa/Sscrofa10.2/ or www.ncbi.nlm.nih.gov/genome/guide/pig/). In 2003, the National Institutes of Health established the National Swine Resource and Research Center (NSRRC) (www.nsrrc.missouri.edu) at the University of Missouri. The goal of the NSRRC is provide the infrastructure to ensure that investigators have access to swine for their biomedical research through production of new genetically engineered pigs and reagents from animals already available. The NSRRC serves as a central resource for reagents, information, and training related to use of swine models in biomedical research. Investigators can donate swine models to the NSRRC for maintenance, production and distribution to the entire scientific community. Currently there are 29 different lines of pigs (wild-type and genetically engineered) available for request from the NSRRC as listed in Table 1 and the website. Furthermore, the NSRRC produces three to four new models for investigators each year.

Summary

Although the pig is the relative 'new kid' on the block, pig models of human disease will continue to have a significant impact on the scientific community. With a sequenced porcine genome[19] and advancements in genetic manipulations, the pig has become an optimal model for many human diseases. However, their greatest impact is likely yet to come with increasing recognition and use of these models by the biomedical research community.

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Disclosure

None reported.

MM

Not Lost in Translation: How Study of Diseases in Our Pets Can Benefit Them and Us

by Carolyn J. Henry, DVM & Jeffrey N. Bryan, DVM, PhD

Infectious diseases, cancer endocrinopathies, toxicities, and immune-mediated diseases are just some of the disorders that share causes and clinical signs across species.

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Abstract

Practice-changing medical discovery requires preclinical and clinical assessment be carried out using appropriate disease models. There is growing awareness of companion animals with naturally-occurring disease as such models. They offer significant advantages over more traditional *in vivo* models of induced disease. This review describes current efforts to promote translation of discoveries between human and veterinary medicine in order to more rapidly and efficiently make progress in improving the health of all human and animal patients.

Case Presentation

The patient sat in the exam room, uneasy in the hospital surroundings as I delivered the diagnosis: osteosarcoma. The family stared in disbelief as we reviewed the images together and their questions reflected the unfairness of the situation. Why him? Why so young? As I began to talk about treatment options, prognosis, and quality of life issues, the young patient was noticeably detached from the conversation—unable to comprehend the issues with which his family was already grappling. When presented with the option of clinical trials, some organized through the National Cancer Institute, there was a mixed reaction: concern about trying something unproven and hope that this new treatment would provide a better outcome; not just in this case but for those diagnosed in years to come. Eager to pursue the most cutting-edge therapy, the family agreed to speak with the Clinical Trials Coordinator that afternoon. They would leave the hospital weighing options to be discussed further when results of staging procedures were known. Complicating all of this was the fact that the patient was uninsured.

Introduction

This is a difficult situation all too familiar in pediatric practice. However, in this case the patient was a 45-kg Rottweiler and the hospital is the Veterinary Medical Teaching Hospital at the University of Missouri. But before you dismiss the story as irrelevant to your medical practice, consider the

following: pet dogs are at approximately ten-fold risk of developing naturally-occurring osteosarcoma compared to people; genetic mutations involved in development of osteosarcoma are shared across species; therapy studies conducted using pet dogs with osteosarcoma have led to advances in limb-sparing procedures for people; and current clinical trials for canine osteosarcoma include investigation of inhalant chemotherapy, small molecule inhibitors, and novel immunotherapy approaches (http://www.vetcancertrials.org).[1-3] Many of these have direct translational application to human oncology.[4]

The Comparative Oncology Trials Consortium

The University of Missouri was one of the eight original institutions selected for inclusion in the National Cancer Institute's Comparative Oncology Trials Consortium (COTC) (https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home), the goal of which is to design and execute clinical trials in dogs with naturally-occurring cancer to assess novel therapies for translation to human patients.[5]

Since its inception in 2003, the Comparative Oncology Program, and specifically the COTC, has initiated 18 trials and has grown to 20 member institutions in North America. All member institutions must meet strict staffing criteria and have both CT and MRI imaging equipment, a dedicated clinical trials coordinator, tissue banking capability, radiation therapy on site, and demonstrated expertise in electronic data reporting.

The first trial conducted through COTC (COTC001) evaluated a targeted AAV-phage vector designed to deliver tumor necrosis factor (RGD-A-*TNF*) to $\alpha_v\beta_3$ integrins on tumor vasculature endothelium. This dose-escalation trial enrolled cohorts of three dogs (n = 24) to determine the optimal safe dose (5×10^{12} transducing units administered intravenously) of RGD-A-*TNF*.[6] A unique feature of this trial that would have been impossible in a human clinical trial was the ability to demonstrate selective targeting of tumor-associated vasculature and sparing of normal vasculature via serial biopsy of both tumor and normal tissue. Why would someone seek to enroll their pet in a clinical trial? The answer sometimes lies in the fact that health insurance and prescription plans are uncommon for veterinary patients and standard-of-care is less well-defined. Thus, treatment options for veterinary patients can vary widely and financial constraints are often the determining factor when medical decisions are made.

The upside to this economic dilemma is that clinical trials investigating new treatment options are often a win-win for pet owners and clinical researchers. The incentive to pet owners is three-fold: 1) trial funding means that pet dogs are treated at minimal expense to the owners, 2) trials offer cutting-edge therapy not available elsewhere, and 3) the opportunity to contribute to "the greater good" is attractive in terms of both altruism and the chance to turn individual misfortune into opportunity for future patients. The value of the latter cannot be overestimated. The sense of empowerment provided by turning a frightening diagnosis into an opportunity to advance the field of oncology in ways that could have tangible benefits for humans and animals affected by cancer is highly motivating for many people enrolling their pets in veterinary clinical trials.

In the case of the COTC001 trial, pre- and post-treatment biopsy of normal and tumor tissue confirmed targeting of the $\alpha_v\beta_3$ integrins on tumor vasculature. Repetitive dosing in a cohort of 14 dogs using the defined optimal dose was well tolerated and led to objective tumor regression in two dogs (14%) and stable disease in six dogs (43%). The study findings were used to inform decisions in design of ongoing Phase I clinical trials in humans. Other COTC studies that are currently underway are facilitating protocol optimization and elucidating mechanism of action for novel anti-cancer therapies that will undoubtedly enter human clinical trials if success is demonstrated in canine models of spontaneously-occurring cancer.

The key to successful translational studies is the appropriate choice of disease model and who better to choose that model than Mother Nature? While the phrase "cancer researcher" has traditionally conjured visions of laboratory personnel assessing tumor response in rodents with artificially-induced tumors, animals with naturally-occurring cancer offer distinct advantages. Rodent models of human cancers either develop within an incompetent immune system as xenografts or arise artificially homogeneic in knockout models. They lack heterogeneity, progress rapidly, and may not recapitulate the tumor microenvironment found in human cancers to the degree that spontaneously occurring cancer in companion animals does. The internal and external environments in which cancer develops in pets share similarities with those of people. The environments encompass everything from exposure to carcinogens to fluctuations in hormone levels. Given the abundance of

examples where cures in rodents have not translated to effective therapies in people, this side-step of medical discovery into the field of companion animal medicine offers a logical new path.

Beyond the applications of canine disease models to cancer drug development, the field of comparative medicine extends into many other disciplines including neurology, infectious disease, immunology, cardiology, orthopedics, and ophthalmology to name just a few. Researchers at the University of Missouri's College of Veterinary Medicine have gained international recognition as leaders in the study of animal models of retinal degeneration, asthma, degenerative neurological disease, and osteoarthritis. Mizzou researchers have modified existing technology to validate these animal models with comparative genomics and epigenomics, flow cytometry, ex vivo immunological evaluations, and bioengineering.

The concept of comparative medicine is gaining interest of late, both in the scientific literature and in the lay press. In articles from the *New York Times* to the *New England Journal of Medicine* this past year, authors have given examples of medical discoveries that have crossed species boundaries, benefitting patients of the two-legged and four-legged varieties.[7,8] In her newly published book, "Zoobiquity, What Animals Can Teach Us About Health and the Science of Healing," cardiologist Barbara Natterson-Horowitz coins the term "zoobiquity" to describe what she calls the "fusion of veterinary, human and evolutionary medicine."[9] She describes the tremendous potential for advancement of medical science if barriers between physicians, veterinarians and evolutionary biologists are broken down, stating "we are uniquely situated to explore the animal-human overlap where it matters most urgently - in the effort to heal our patients." Admittedly, when veterinarians use the phrase "patient" to refer to a pet presented for medical care, our physician counterparts do a double-take. But the reality is that precisely the same technology and treatment options found in local hospitals for human patients are available at academic and referral institutions for veterinary patients. In certain specialties such as veterinary oncology, drugs marketed for use in people are routinely prescribed for use in pets, as comparable veterinary-labeled drugs do not exist. Ignoring that the patient is a dog or a cat, the underlying etiology and pathology are similar, demanding the same diagnostics and drugs.

Viewing diseases with this lens, the similarities become obvious. Infectious diseases, endocrinopathies,

Efficient and rapid scientific discovery through comparative genomics are enhanced by clinicians and researchers sharing knowledge between human and veterinary medicine.

toxicities, and immune-mediated diseases share causes and clinical signs across species. Most canine patients come from large "families" of siblings/littermates, often with defined pedigree information available through kennel clubs such as the American Kennel Club. This facilitates the genetic mapping of disease susceptibility traits by breed, enhancing the power of comparative studies to elucidate underlying genetic causes. If one breed is uniquely susceptible to Disease X and another breed rarely affected, comparing the genomics of these two breeds to the rest of the population often defines the underlying etiology for Disease X, as well as giving clues as to how to manage or mitigate it.

Compatibilities in Genomic Studies

The field of genomics was bolstered in 1989 when the National Center for Human Genome Research was established in response to the charge to map the human genome as part of the International Human Genome Project. With sequencing of the human genome completed in 2003, biomedical research has experienced the opening of a floodgate of opportunities for discovery in areas as broad and varied as understanding genetic mechanisms of disease to developing a more sustainable food supply. Fast forward to 2005 when the completion of a genome sequence of the domestic

dog expanded the horizons of comparative medical genomics. Opportunities for efficient and rapid scientific discovery through comparative genomics are now limited only by the imagination of clinicians and researchers and the shared knowledge between human and veterinary medicine. An inherited cancerous disease in German Shepherd dogs, renal cystadenocarcinoma and dermatofibromatosis, is found to be caused by a mutation in the gene for folliculin, bearing a striking clinical similarity to the human disease, Birt-Hogg-Dube syndrome.[10] A form of progressive retinal atrophy (Leber's congenital amaurosis type 2) that leads to blindness shortly after birth shares a similar genetic mutation between dogs and people. Using gene therapy to restore the RPE65 protein that is lost through the mutation restored vision in affected dogs and led to subsequent successful therapy in people.[11,12] Veterinary neurologists, geneticists, and physicians at the University of Missouri have worked together to identify the genetic cause of degenerative myelopathy in dogs that is analogous to human amyotrophic lateral sclerosis (aka, Lou Gehrig's Disease).[13] NIH funding for a University of Missouri-led therapy trial in affected dogs was announced in September 2012. Clearly, the pace of scientific discovery and medical innovation has quickened and we are well positioned to lead the way in this critical endeavor.

One Health/One Medicine

Few sites in the U.S. can boast the atmosphere of creative, innovative and collaborative research found at the University of Missouri. The phrase "One Health/One Medicine" refers to the sharing of resources, knowledge and effort toward the common goal of improving the health and well-being of all species. This One Health/One Medicine concept is a key component of the MU's strategic plan. The presence of a School of Medicine, School of Nursing, College of Veterinary Medicine, University Hospital and Clinics, Center for Clinical Research, Life Sciences Center, and tremendous strength in basic science research, biomedical innovation, and bioinformatics makes MU an ideal setting in which to translate medical discovery from idea to clinical implementation efficiently and at a pace to have a real impact on patients in our lifetime. This translational approach is being extended to other sites throughout the State. University of Missouri is partnering with other Missouri academic centers, the Kansas City Area

Life Sciences Institute, the Institute for Conservation Medicine, the St. Louis Zoo, and the "Animal Health Corridor" extending west along I-70 and connecting MU veterinary and human medical researchers and clinicians with animal health companies and academic institutions in Kansas City.

We are also reaching outside of the state to Kansas State University's College of Veterinary Medicine and the University of Kansas to speed clinical translation of novel diagnostic and therapeutic approaches. We are rapidly moving towards a day when the patient in the examining room with osteosarcoma will receive better news of individualized therapy developed in comparative trials. Through One Health/One Medicine efforts, whether the patient is human or canine will matter less than the underlying, shared disease mechanisms for therapy decision making.

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Disclosure

None reported. **MM**

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Animal Models in Today's Translational Medicine World

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Recent advances in molecular technology are leading to the development of superior animal models and providing unprecedented opportunities to test both gene and pharmacological therapies prior to clinical trials in humans.

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Abstract

Translational medicine drives progress of research along the continuum from basic biomedical research findings into clinical practice. Animal models play a central role in the above continuum. The recent explosion in molecular biology and generation of human physiological system in animals has led to an increasing use of in vivo animal models in today's translational medicine.

Translational Medicine: Introduction

Early Definition of Translational Medicine

Discovering new treatment and prevention of disease depends on a research continuum from basic biomedical research findings into clinical practice. Various methods and strategies have developed to bridge the gap between the discoveries generated in laboratory and implementing those findings in human clinical trials. The term "translational research" appeared in early 1990s but was used in the context of bench research involving molecular genetics and immunology spanning basic and clinical research.[1]

"Bench to Bedside" Definition of Translational Medicine

In basic and clinical research literature, multiple attempts have been made to define 'Translational Science' or 'Translational Medicine' or 'Translational Research.' The National Institutes of Health (NIH) offered the following definition:

"Translational research includes two areas of translation. One is the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second process of translation concerns research aimed at enhancing the adoption of best practices in the community. Cost-effectiveness of prevention and treatment strategies is also an important part of translational science."[2]

Translational research according to the above NIH definition is continuous process with the ultimate goal of improving patient health by moving, efficiently and expeditiously, laboratory research discoveries into clinical human trials and finally to the patient bedside. The first stage of translational research also known as T1 in literature is a bridge between laboratory research and human clinical trials in which findings generated in laboratory though the means of animal models, cell culture and molecular studies can be used and forwarded for application in human clinical trials. The next stage in the continuum is considered as T2 which moves results from clinical trials eventually to clinical practice with the ultimate goal of improving community health (see Figure 1).

Broad Multidirectional Definition of Translational Medicine

Recently, an argument was made that the concept of translational

research demonstrated in Figure 1 defines translational research too narrowly.[3] Rubio et al.[4] added a T3 arm with a new definition for translational research such that it is defined as multidirectional integration of basic, patient oriented, and population based studies leading to ultimate goal to improve public health (see Figure 2). T1 research involves bidirectional interaction between basic and patient oriented research for better scientific understanding with T2 coordinating between patient-oriented and population research for improvement of patient outcome, and finally T3 interacting between population and basic research for understanding human health and disease. Figure 2 demonstrates a dynamic interplay between basic, patient oriented, and population research with bidirectional arrows.[4] Basic research is often considered as the first step of the translational process and as per the American Cancer Society it provides the foundation of clinical research and involves laboratory studies, including animal models.[5]

Use of Animal Models in Translational Medicine

Introduction to Animal Models

The history of animal models goes back more than a millennium, when animals were used for experimental surgery. The first textbooks on anatomy were based on dissection on pigs and apes, not on human cadavers. Use of animal models is well known for some of the greatest discoveries in history. William Harvey's great work on circulation[6] and Louis Pasteur's work in microbiology[7] are few examples of use of animal model for great discoveries. Nowadays, the main use of animal models is for translational medicine and that role is considered the central point in the multidirectional paradigm of translational research.

Examples of Animal Models in today's Translational Medicine

Translational research is primarily developed through the use of animal models. Examples in cancer research include xenografting, in which human cancer tissue is transplanted into nude mice (immunosuppressed to avoid rejection) allowing cancer development to be studied in vivo. Subsequently, the focus has shifted to development of "human physiological systems" within the mouse model. One of the steps in the development of humanized models is the production of mice with targeted mutations in genes to knock out further immune response. Human hematopoietic stem cells are then engrafted into these mice to colonize the bone marrow and differentiate into

Figure 1

The first stage of translational research (T1) is often carried out using animal models, cell cultures, or other experimental systems. Clinical research in the figure encompasses human subject research studies that involve direct interaction between investigators and human participants. The second translational process (T2) takes results from studies in humans and applies them in clinical practice with the ultimate goal of improving community health.
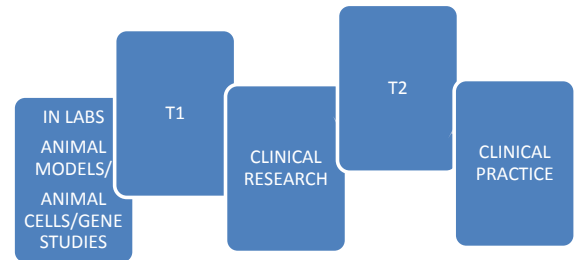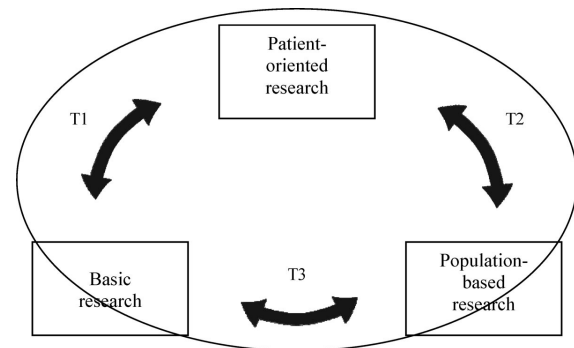

Figure 2

Model for translational research, as proposed by the Evaluation Committee of the Association for Clinical Research Training (Adopted from reference 4)

the multiple cell lineages that constitute a human immune system. These models are used in various research fields including immune, infectious and oncology research and are considered central to recent and future advances in translational research, including pharmaceutical development and personalized medicine. In a recent study by Thomas et al utilizing cultured hepatocellular cancer cells from a humanized mouse model, an experimental combination therapy was found to be effective in reducing tumor burden.[8] The above observation led to an investigator-initiated Phase 1B-2 dose escalation trial with combination drugs in patients with HCC.[8]

In addition, selective breeding, genetic modification, and advances in molecular imaging have provided a better understanding of disease processes and insights into possible interventions that were not possible previously. For a long period of time, neurodevelopmental disorders

such as autism and fragile X syndrome were considered medically untreatable. However, selective breeding and creating mouse models for behavioral phenotypes, such as the BTBR T+tf/J mouse model, have made it possible to better understand the behavioral phenotypes and design potential pharmacological interventions with the possibility that a single targeted pharmacological intervention may alleviate multiple diagnostic behavioral symptoms of autism.[9] The robustness of such data increasingly contributes to the translation of biomedical breakthroughs from preclinical studies to clinical applications. For instance, a protein known as Sema3A was found to facilitate bone regeneration in mice by simultaneously reducing bone destruction and increasing bone synthesis, and could lead to a new class of dual-action therapeutic agents for osteoporosis in humans.[10]

Use of mice is invaluable for translational research because of the ease of genetic manipulations to produce mice models for human disease. A trans-NIH initiative, the Knockout Mouse Project (KOMP), was conceived in 2003 with the aim of "knocking out" each of the genes in the mouse genome to create multiple new lines of knockout mice.[11] This project will make knockout mice available to researchers through live mouse models, embryonic stem cell clones, or frozen embryos and sperms with the goal of developing better models of human disease.

Although mice may be considered as a standardized translational device, various other animal models including primates are being developed and available for translational research and drug discovery.[12] Similar to the Knockout Mouse Project (KOMP), the National Primate Research Centers (NPRCs) established Working Groups (WGs) for developing resources and mechanisms to facilitate collaborations among non-human primate (NHP) researchers as well as to develop Genome Banking. The Genetics and Genomics Working Groups are developing resources to advance the exchange, analysis and comparison of non-human primate genetic and genomic data across the National Primate Research Centers.[13]

Limitations of Animal Models in Translational Medicine

Similar to human clinical studies, use of animal models for translational research with the goal of translation of bench research to clinic has few limitations. Involving young and healthy animals for research always carries a risk of selection bias.[14, 15] Natural dissimilarities between physiological and pathological system of various animal models and humans is one of the challenges of translation of bench research to clinical practice. Various remedies and approaches are underway to circumvent these differences. This includes work at the genetic, molecular, cellular, and

clinical scale to understand the link between these elements within animals and humans. The ultimate goal of translating data between species via interdisciplinary approach will require techniques and expertise from mouse genetics, stem cell science, clinical research, comparative genomics, pathology, and medicine.

Conclusion

In the present era, animal modeling is considered the backbone of understanding various disease pathophysiologies and provides enormous opportunities for novel, effective therapy for a wide spectrum of presently untreatable disease and injuries. Recent advances in molecular technology are leading to the development of superior animal models and providing unprecedented opportunities to test both gene and pharmacological therapies prior to clinical trials in humans.

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The Laboratory Animal Veterinarian: More than just a Mouse Doctor

by Cynthia G. Alvarado, DVM & Lonny M. Dixon, DVM

Laboratory animal veterinarians have become irreplaceable contributors to the advancement of medical and scientific knowledge through their involvement in collaborative and independent research that ultimately benefits both humans and animals.

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Abstract

Use of animals in research is strictly regulated by federal laws that define how the animals can be humanely housed, studied, and sold. Veterinary care for these animals is also required. Laboratory animal veterinarians serve as a unique bridge between the humane use of laboratory animals and the advancement of scientific and medical knowledge.

Introduction

It is not common knowledge that laboratory animal veterinarians and their support staff work every single day monitoring the health and welfare of the animals used in biomedical research. The role of laboratory animal veterinarians as multi-disciplinary contributors to biomedical research has grown significantly over the past fifty years. This article will give our colleagues and the general public a glimpse into the realm of laboratory animal medicine and the role that veterinarians play to ensure the welfare of animals used in research while also contributing to discoveries that benefit both humans and animals.

The History of Laboratory Animal Medicine

The history and development of the veterinary specialty of laboratory animal medicine began in 1915, when a veterinarian, Simon D. Brimhall, VMD, was employed by the Mayo Clinic.1 As the first veterinarian appointed to a research animal management position at an American medical research institution, Dr. Brimhall's role at that time mirrored some of the same responsibilities of present day laboratory animal veterinarians: providing veterinary care to research animals, overseeing animal husbandry, managing animal facilities and breeding colonies, studying animal diseases, and performing collaborative and independent research. However, until the 1940s Dr. Brimhall continued to be one of only a handful of veterinarians involved in laboratory animal medicine.

In 1944, Congress passed the Public Health Service Act, which resulted in the post-war expansion of biomedical research by increasing funding to the National Institutes of Health (NIH). As the NIH grew to become the largest federal funder of biomedical research, the demand for veterinarians in research

also increased.[2] Although veterinarians of that time were well-versed in the care of common domestic and agricultural animals, their knowledge was minimal regarding proper husbandry, veterinary care, and diseases common in research animals, especially rodents. In an effort to formalize education, training, and research in laboratory animal medicine, a group of approximately 20 dedicated veterinarians sought approval from the American Veterinary Medical Association (AVMA) for a new specialty board. In 1957, the American Board of Laboratory Animal Medicine became the third veterinary specialty to be recognized by the AVMA. The Board, now known as the American College of Laboratory Animal Medicine (ACLAM), establishes standards for training and board certification in laboratory animal medicine, organizes continuing education opportunities, and promotes research in laboratory animal science and medicine.[2]

The History of Animal Welfare Regulations

Although biomedical research surged in the 1940s, federal laws regulating animal use were not passed until many years later. In 1965, *Sports Illustrated* featured the story of Pepper, a pet Dalmatian that was stolen from her family in Pennsylvania and was sold by her dognappers to a New York hospital where she died during an experimental surgery. Soon after, *Life* published an article exposing the neglectful treatment of animals by a Maryland dog dealer. These incidents prompted the public to lobby for legislation for the regulation of animal care, housing, sale, and use in research.[4]

In response, Congress passed the Laboratory Animal Welfare Act of 1966, which was renamed the Animal Welfare Act (AWA) in 1975. In its initial form, the AWA focused on the protection of pet dogs and cats. However, after numerous amendments, the AWA has evolved to become one of two key laws governing research animal care and use. The AWA requires licensing of all facilities using animals for the purposes of research, testing, or teaching in higher education and is enforced regardless of the source of funding. The AWA provides specifications for virtually all aspects of animal care including feeding and watering, sanitation, identification, ventilation, space/housing requirements, handling, transportation, recordkeeping, and adequate veterinary care. A special agency of the United States Department of Agriculture serves as the enforcement agency of the AWA and conducts unannounced yearly inspections at licensed facilities to monitor compliance.[4]

Another key law is the Health Research Extension Act of 1985. This act provides the legislative mandate for the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), which is enforced at all institutions that receive funding from any of the eight Public Health Service agencies, which include the NIH, Centers for Disease Control

and Prevention and the Food and Drug Administration. In accordance with PHS Policy, institutions must also comply with the guidelines set forth by the Guide for the Care and Use of Laboratory Animals (the Guide). The 220-page, 8th edition of the Guide was published in 2011 and provides specific guidelines for the care and use of all vertebrates. Though the AWA and PHS Policy share many similarities, the AWA does not cover agricultural mammals used in agricultural research, birds, or mice and rats bred for research, while the PHS Policy includes specifications for the care and use of all live vertebrates.[4]

Ensuring Animal Health and Welfare

To ensure that animals receive adequate veterinary care, both the AWA and PHS Policy require that all research facilities employ an attending veterinarian (AV) with experience in laboratory animal medicine.[5] Adequate veterinary care is defined as "what is currently the accepted professional practice or treatment for that particular circumstance or condition."[4] In addition to ensuring the well-being of research animals, the duty of the AV is to ensure that the animal care and use program at the institution has the appropriate equipment, facilities, and trained personnel necessary to provide adequate veterinary care. The AV also has the responsibility to provide guidance to researchers and their staff regarding any aspect of animal use including humane handling, anesthesia, analgesia, and euthanasia.[5] The regulations also require that all research animals are observed daily for general health and that emergency veterinary care be available at all times.[4]

In addition to the AV, most large institutions find it necessary to employ additional clinical laboratory animal veterinarians to work under the AV to provide for the daily care of their research animals. These veterinarians serve as the designee of the AV, who has the authority within the institution to suspend or terminate animal use at any time if it is not operating within the standards of the AWA or PHS Policy.[4]

Both the AWA and PHS Policy also require that the AV be a voting member of the Institutional Animal Care and Use Committee (IACUC). Required at all institutions using animals covered by the AWA or PHS Policy, the responsibility of the IACUC is to ensure compliance with federal regulations by overseeing the care and use of animals at the research institution.[4] To meet this responsibility, the IACUC reviews and approves animal use protocols, which outline in detail how a researcher intends to use animals in a study. As experts in animal care and medicine, laboratory animal veterinarians are frequently consulted by IACUCs to ensure that animal use protocols are in compliance with animal welfare regulations and current standards of animal care.

Training and Certification in Laboratory Animal Medicine

The first residency training program providing veterinary care to research animals for veterinarians was funded by the NIH at Wake Forest University's Bowman Gray School of Medicine in 1959.[3] The first formal training program in the uniformed services began in 1960 at the United States Air Force School of Aerospace Medicine.[3] Currently, there are over 45 ACLAM recognized training programs, which range from two to four years in length. Some programs offer residency training in conjunction with additional coursework or extensive research training, resulting in a Master of Science or PhD degree. ACLAM-recognized residency training programs are designed to prepare veterinarians for ACLAM-board certification by providing an environment for didactic and clinical training in laboratory animal biology, pathology, medicine and surgery as well as animal husbandry, resource management and responsible animal use. These residency programs allow trainees to become familiar with the numerous regulations and policies relating to the welfare of animals used in research. Trainees are also encouraged to be involved in collaborative or independent research.

Currently, licensed veterinarians interested in board-certification in laboratory animal medicine must have completed either an ACLAM-recognized residency training program or have six years of relevant, full-time experience in laboratory animal medicine. In order to ensure that candidates have a working knowledge of the scientific method, they must serve as first author on a hypothesis-based research paper published in a peer-reviewed journal. In addition, admission into the College requires passing the ACLAM board certification examination.[3]

The ACLAM board examination tests knowledge regarding the biology, husbandry, and clinical medicine of species commonly used in research. Currently, 60-70% of animal-related questions are based on species used most commonly in research, which include mice, rats, rabbits, non-human primates, dogs, and pigs. Other species, such as cats, frogs, ferrets, guinea pigs, zebrafish, and invertebrates make up the remainder of animal-related questions. Candidates must also have a solid knowledge base regarding the research uses for these animals and possess proficiency in research facility design and management, animal welfare regulations, and research methods and equipment. A contemporary knowledge of advancements in the biomedical research field and laboratory animal medicine is also required to pass the exam, which may include questions based on recent articles from selected peer-reviewed journals. Once candidates have

The Public Health Service Policy on Humane Care and Use of Laboratory Animals requires that all research animals are observed daily for general health and that emergency veterinary care be available at all times.[4]

passed the board examination they are awarded the title of "Diplomate of the American College of Laboratory Animal Medicine" (DACLAM).[3]

Careers in Laboratory Animal Medicine

After completion of residency training, many veterinarians trained in laboratory animal medicine may chose to remain in a clinically-oriented career, working as an institutional AV or clinical veterinarian in academia or industry. These veterinarians enjoy practicing veterinary medicine in a field with a wide range of animal species, including transgenic animals or those with other genetic mutations. For example, on a typical day a laboratory animal veterinarian's patients may include a genetically engineered mouse, a 500-lb transgenic pig, or a herd of sheep used for antibody production.

In addition to providing veterinary care to research animals, another duty of a clinical laboratory animal

veterinarian includes researcher support. Veterinarians are frequently involved with training and assisting researchers and their staff in specialized techniques and procedures and providing assistance with experimental design and protocol writing. Clinical veterinarians may have additional responsibilities, such as serving on the IACUC, teaching in higher education or training residents in an ACLAM-training program.

Other veterinarians trained in laboratory animal medicine may opt to pursue a career in research. Due to their training, these veterinarians are attractive as research collaborators due to their extensive knowledge of veterinary medicine and animal models of disease.

Laboratory animal veterinarians ensure the welfare of animals used in research while contributing to discoveries that benefit both humans and animals.

Laboratory Animal Medicine at the University of Missouri

Established in 1968, the Comparative Medicine Program (CMP) is the NIH-sponsored and ACLAM-recognized laboratory animal medicine residency training program at the University of Missouri (MU). In addition to providing training in laboratory animal medicine, the MU CMP also focuses on exploring the comparison of pathology and diseases in research animals to those of other species, including humans. Since the program began, more than 100 veterinarians have completed the program, of which 74 have become ACLAM diplomates. Of the 800 active ACLAM diplomates, the MU CMP has trained 62, which is approximately 8% of all active diplomates. The MU CMP allows veterinarians to combine their laboratory animal medicine training with a research program. Upon completion of the residency training program, relevant coursework, and research, a MS or PhD degree is conferred. As of 2012, there are 10 trainees in the MU CMP.

Unique components of the MU CMP include access to the NIH-funded Rat Resource and Research Center and the National Swine Resource and Research Center, the only centers of their kind in the country. The Mutant Mouse Regional Resource Center at MU is one of four in the US. These centers serve as repositories for cryopreservation, production, and characterization of genetically-engineered rodent strains and swine to ensure the continued availability of valuable genetically engineered animals to the biomedical research community. MU is also home to an animal biosafety level-3 research facility where select agents, such as *Bacillus anthracis* (anthrax) and *Yersinia pestis* (plague), are studied. In addition, MU is one of only six public universities in the country that have schools of medicine, veterinary medicine, engineering, law, and agriculture on one campus. This provides MU CMP trainees with extensive opportunities for interdisciplinary research collaboration.

Conclusion

Although rodents continue to be the predominant animals used in research, laboratory animal veterinarians are considered to be more than just "mouse doctors." They are recognized as valuable members of the research team as sources of extensive knowledge regarding laboratory animal medicine and the humane use of animals as research models.. Laboratory animal veterinarians have also become irreplaceable contributors to the advancement of medical and scientific knowledge through their involvement in collaborative and independent research that ultimately benefits both humans and animals.

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Disclosure

None reported.

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