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COMMENTARY

Evolving Value and Validity of Animal Models in Veterinary Therapeutic Research: Impact of Scientific Progress

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Abstract

In veterinary medicine, experimental *in vivo* animal models have long been integral to advancing our understanding of disease mechanisms and assessing the safety and efficacy of potential therapies. However, the value and validity of these models warrants reassessment in light of emerging scientific evidence, evolving standards in animal welfare, and the development of alternative methodologies. Such a reassessment is essential for maintaining ethical scientific practices and ensuring that research approaches remain both relevant and justifiable, especially as our awareness of animal pain, sentience, and consciousness deepens. While interspecies extrapolation of findings from these models poses challenges when applied to human medicine, what about cases where an animal species serves as both the experimental subject and the intended veterinary patient? Additionally, what alternative tools are potentially available to replace *in vivo* studies in these contexts? This commentary explores how veterinary research may improve efforts to meet the principles of the 3R's by integrating alternative *in vitro* and *in silico* models early in the investigative process and utilizing specialized tools within the target veterinary population during clinical trials.

Keywords:

3Rs, Veterinary Medicine, In Silico Modeling, Organoids, Alternative Methods

Introduction

Experimental *in vivo* animal models have been fundamental for advancing our understanding of disease mechanisms and for evaluating novel therapies across all scientific disciplines, including veterinary medicine. Nevertheless, the acceptability of these models must be subjected to continuous reassessment aligned with recent advances in modern neuroscience, especially in understanding animal pain, sentience, and consciousness.

Observational research in veterinary clinical settings has driven progress in areas like population pharmacokinetics (PK), pharmacodynamics (PD), physiologically based PK (PBPK) modeling, and biomarker validation. These advancements allow for more detailed, non-invasive investigations, enabling researchers to gather crucial data on disease progression and therapeutic efficacy while reducing reliance on invasive experimental procedures on healthy animals.

This commentary explores how efforts to support the principles of the 3R's (Russell and Burch, 1992) in veterinary research can be significantly enhanced by incorporating alternative in vitro and in silico models early in the investigative process and by employing observational investigative tools within the target veterinary population during clinical trials.

Animal Species as Experimental Models for Target Patients in Veterinary Medicine

Within human medicine, preclinical *in vivo* models nearly always involve artificially induced conditions such as those used to study bacterial or viral infections, inflammation, pain, cancer, congestive heart failure, chronic kidney failure, or metabolic diseases. They are ineluctably associated with differences as compared to what is naturally observed in the actual patient population, and uncertainty about their ability to accurately replicate the disease in the human patient (Miao et al., 2024),(Saura et al., 2022),(Mukherjee et al., 2022).

But what about studies where the experimental unit is also the targeted veterinary patient? This duality of status magnifies an inescapable obligation to be concerned with animal welfare and to implement 3Rs principles. Moreover, if a model does not ***significantly*** advance knowledge beyond that which existing methods provide, its use should be critically questioned.

Recent Innovations and Methodological Advancements in Knowledge Acquisition Surrounding the use of Animals Studies.

Within the U.S Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM), efforts are underway to identify alternatives to artificial infection and terminal clinical endpoint studies for the evaluation of non-systemically absorbed drugs i.e., where blood level

bioequivalence studies cannot be used to establish product bioequivalence (Helal et al., 2024),(Martinez et al., 2024),(Dong et al., 2018).

Upstream, *in vitro* systems such as 3D organoids generated from animal-derived adult stem cells (Quisenberry et al., 2024),(Mochel et al., 2017),(Gabriel et al., 2024),(Chandra et al., 2019) or induced pluripotent stem cells (iPSC) (Baird et al., 2015), deserves close attention. Human derived 3D organoids have been used to study a multitude of infectious diseases [bacterial, viral and parasitic] (Han et al., 2021),(Clevers, 2020),(Korwin-Mihavics et al., 2023), genetic diseases (Na et al., 2024), and cancer (Drost and Clevers, 2018)]. Alternatively, *in silico* approaches have been invaluable for human therapeutics. Such approaches include virtual screening (Chang et al., 2024),(Li et al., 2023), molecular docking (Agu et al., 2023), PBPK (Rasool et al., 2021), and artificial intelligence (FDA, 2025).

Although still limited, the accessibility of alternative approaches in veterinary medicine reflects advancements in data analysis tools within clinical setting such as population pharmacokinetics (POP-PK) (TOUTAIN, 2024),(Bon et al., 2018) and population pharmacokinetics/pharmacodynamics (POP-PK/PD) (Zamir et al., 2024),(Toutain et al., 2021). These tools, widely employed in human clinical medicine, provide an opportunity to translate traditionally investigational study questions into clinical applications without compromising the well-being of the target population. The importance of adopting these alternative approaches have been recognized by the European Medicine Agency (EMA) (EMA, 2024). Similarly, the FDA recently published an article advancing the use of alternative methods to reduce animal testing (FDA, 2025).

To emphasize the need to re-evaluate the risk-benefit balance of artificially induced animal disease models in veterinary medicine, we will use chronic kidney disease (CKD) as a case study to illustrate the importance of humane observational approaches in clinical settings for assessing drug safety and effectiveness.

The Remnant Kidney Model: A Case Study in Reassessing the Validity and Value of Animal Models

CKD is commonly observed in older human (CDC, 2024) and veterinary patients (Reynolds and Lefebvre, 2013). It is not a single disease but is rather a heterogeneous syndrome that results in the loss of functioning renal mass (Finch et al., 2016). It is associated with numerous complex physiological changes that affect the functions of many organ systems, with the extent of impact being related to the severity of renal impairment (Zoccali et al., 2017).

While glomerular damage is a primary cause of CKD in humans and dogs, it is rare in cats (Billington and Webb, 2024). Proteinuria, when present, is commonly a manifestation of abnormalities in the glomerular basement membrane (Suh and Miner, 2013) and is routinely used as a negative prognostic factor for dogs and cats (Vaden and Elliott, 2016). In humans, dogs, and cats, the final common CKD pathway often involves renal fibrosis and hypoxia. In cats, CKD may be acquired, familial or congenital (Breton, 2012) with kidney problem disposition in certain breeds (Breton, 2012).

One of the challenges in conducting clinical trials for evaluating the effectiveness of feline CKD drug candidates has been its relatively slow progression and the confounding effects of comorbidities and concurrent medications (Breton, 2012),(Schmiedt et al., 2023). These limitations have encouraged the development of animal models that serve as surrogates for actual patients. An example of this is the remnant kidney model, developed to overcome the difficulties encountered when studying CKD patient populations, be they humans (Chow et al., 2003) or veterinary (Schmiedt et al., 2023),(Brown, 2013) and to provide insights into the progression of renal failure.

Schmiedt et al. (2023) (Schmiedt et al., 2023) suggest that the remnant kidney surgery model can mimic both acute and chronic feline CKD outcomes and minimize sources of patient variability contributing to difficulties in clinical data interpretation. This laboratory model involves the surgical reduction of renal mass through partial arterial ligation of one kidney, followed by nephrectomy of the contralateral kidney. According to Brown (2013) (Brown, 2013) and Schmiedt et al. (2023) (Schmiedt et al., 2023), the remnant kidney model has been used in a variety of species (mice, rats, dogs, and pigs) to study the progression, clinical outcomes, and potential treatments for CKD. The core similarity between the clinical presentation of human and canine CKD and the remnant kidney model is nephron adaptation to “overwork”, leading to hyperfiltration and glomerular hypertrophy. However, this adaptation does not typically occur in the naturally occurring feline disease (Billington and Webb, 2024). Conversely, there are some comparable disease manifestations during the initial chronic phase of the feline remnant kidney model and the proportion of normotensive CKD cats remaining normotensive during disease progression (Schmiedt et al., 2023) (Bijsmans et al., 2015).

As with any animal model, the question is whether these models, created within the target species, serve as the best substitute for actual patients when evaluating the safety and effectiveness of drug products? Considering the remnant kidney model, investigators observed differences between the extent of renal adaptation occurring in the young, model animals versus that in feline CKD patients. For example, in terms of treatment responses, although benazepril

was shown to decrease systolic blood pressure in the feline remnant kidney model, systolic blood pressure decreased over a six-month period in both the benazepril-treated group and the placebo group (Brown et al., 2001). This type of normalization of systolic blood pressure does not typically occur with the naturally occurring disease (Lawson and Jepson, 2021).

Furthermore, the typical feline CKD patient often presents with numerous concomitant pathologies not accounted for in the young surgical model subjects (Hori et al., 2018). Cats with naturally occurring CKD are often administered multiple medications such as calcium channel blockers (Magalhães et al., 2023) or treatments for hyperthyroidism (Reynolds and Lefebvre, 2013). Ignoring these concomitant pathologies, the significant impact of age (given that the target population is largely geriatric) and the presence of concomitant medications can compromise our ability to effectively evaluate the safety and effectiveness of potential treatments under actual conditions of use.

Importantly, the model's invasive nature and associated adverse outcomes present challenges in aligning with the goals of the 3R's. *In light of these uncertainties, might humane alternative approaches be just as, if not more informative, especially when combined with data from actual CKD patients?*

The Importance of Alternative Preclinical Models in Advancing the 3Rs Principles in Human and Veterinary Medicine

Efforts to reduce the use of *in vivo* animal model studies raise the question of how best to fill the resulting knowledge gaps.

The use of 3D organoids has been transformative, particularly in cancer research. These patient-derived organoids, grown from stem cells obtained from various tumor types, allow for a personalized approach for studying tumor biology and potential treatments (Fang et al., 2023). By eliminating the need for animal models with (artificially) induced cancers that can differ significantly from natural occurring human cancers (Gordon et al., 2009), this approach enables insights directly applicable to human health. Patient-derived organoids not only help scientists better understand tumor heterogeneity but also allow for high-throughput drug screening for specific cancer subtypes.

Three-dimensional kidney organoids derived from human-induced pluripotent stem cells, when combined with artificial intelligence and morphometric assays, have recently been used to study both therapeutic and nephrotoxic drug effects (Shi et al., 2024; Gupta et al., 2021). These organoids offer a biologically relevant context that more closely mirrors the human kidney environment, enabling the investigation of drug–tissue interactions. Despite remaining

limitations such as scalability and batch-to-batch variability (Na et al., 2024), these models show strong potential as efficient and predictive tools (Oishi et al., 2024). The development of co-culture systems—integrating kidney organoids with other cell types, such as immune or endothelial cells—has further expanded their utility for studying complex intercellular dynamics within the kidney (Nishinakamura, 2019). These systems support investigations into immune responses, vascular contributions to renal function, and potential responses to drug treatment or injury (Shankar et al., 2024). Although additional validation is needed, *in vitro* systems such as 3D organoids and organ-on-a-chip technologies (Vunjak-Novakovic et al., 2021; Ingber, 2022) offer the opportunity to use patient-derived tissues to explore therapeutic targets and assess drug toxicity in a minimally invasive manner. Notably, the first description of adult stem cell-derived kidney organoids from canines was recently published as a preprint by Zdyrski et al. (2024). To the best of our knowledge, no reports of adult stem cell-derived kidney organoids from felines have been published to date.

While three-dimensional kidney organoids offer promising opportunities to reduce reliance on invasive animal models, important limitations must be acknowledged. The extent to which these organoids faithfully replicate the complex pathophysiology of CKD—especially in older patients with multiple comorbidities—remains unknown. In veterinary medicine, ongoing studies—such as the initial characterization of adult stem cell-derived kidney organoids from companion animals (Zdyrski et al., 2024)—represent important foundational steps toward the development of more predictive in vitro models. However, further validation is necessary to determine whether these systems can reliably model disease progression and treatment responses observed in naturally occurring canine and feline CKD.

The Critical Role of Innovative Approaches in Clinical Research

Recent strides have been made on alternative approaches that are consistent with the 3R principles. *In silico* PBPK methods can predict drug distribution to the targeted drug sites and assess the influence of disease or population polymorphisms on systemic drug exposure (Adiwidjaja et al., 2024) (Cross et al., 2025). Alternatively, POP-PK and POP-PK/PD models provide a top-down approach where clinical data from patient populations are used to understand dose-exposure-response variability under real-world conditions. In their pioneering work on the POP-PK of digoxin in canine cardiac patients, Whittem et al. (2020) (Whittem et al., 2000) identified both slow and rapid digoxin absorbers. This delay in absorption was attributed to the stress of hospitalization (Whittem et al., 2000), a factor that would have been impossible to identify in laboratory Beagle dogs accustomed to their environment.

The issue of lameness in horses can also be studied in a humane manner. Today, clinical evaluation of lameness assisted by devices designed to measure severity, such as wearable inertial sensor systems, enables objective gait analysis (Timmerman et al., 2022),(Crecan and Peștean, 2023). These devices are now routinely used in some clinical settings and could be further explored to determine their suitability for meeting regulatory requirements for drug development. Such approaches could serve as attractive alternatives to experimentally induced inflammation (Van De Water et al., 2021), or chondral lesions induced through arthroscopy (Yamada et al., 2022).

Also worthy of reconsideration is the clinical evaluation of antiparasitic drugs and the need to include not only field effectiveness data (obtained from veterinary patients using non-invasive methods of assessment) but also data based on parasite counts (adults, larvae) in dose determination and confirmation studies. Currently, these studies involve necropsy of dogs and cats, and subsequent worm counts in experimentally infected animals (control and treated groups) (CVM GFI#276; VICH GL19). While field effectiveness studies remain essential, there is a need to identify alternative methods for determining residual worm burdens in artificially induced infections without requiring necropsy. Such methods would align with the principles of the 3Rs and support the return of both control and treated animals to the colony at the conclusion of the study. One possibility includes the use of *in vitro* systems (e.g., organoids) to define drug exposure-response relationships (Duque-Correa et al., 2020). For systemic parasitic infections such as heartworm disease, bioavailability studies could be used to establish dose–plasma level–exposure relationships in healthy or diseased animals. When combined with organoid data, this information could serve as a basis for dose determination and confirmation. For non-systemically absorbed drugs targeting infections such as gastrointestinal parasites, organoid data—used alongside *in vivo* dose determination studies conducted in accordance with the 3Rs—could help identify parasite sensitivity to variations in drug exposure. These data could also inform assessments of sensitivity to potential changes in product dissolution *in vivo*. Regardless of the approach selected for dose determination and confirmation, the ultimate goal should be to rely primarily on the demonstrated effectiveness of the parasiticide in real patient populations, allowing for assessment within the more complex context of patient-specific ecosystems.

These examples demonstrate the importance of using clinical trials to provide a broader perspective on factors that can influence treatment responses (Sheiner, 1997). Referring back to CKD, by using the International Society of Renal Interest (IRIS) scoring system (Cowgill, 2023), the degree of kidney dysfunction in dogs and cats with naturally occurring CKD can be

objectively quantified and provide longitudinal monitoring of both the positive and negative effects of drug candidates.

Ultimately, these ethical and scientific approaches, could be equally, if not more, informative than traditional *in vivo* models while utilizing alternatives centered on non-invasive tools and model systems that align with the evolving therapeutic landscape.

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Author contributions

MM, JPM, and PLT have contributed equally to writing this manuscript.

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