Clinical trial design and execution in small animals
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Abstract
Exceptional clinical research in veterinary medicine requires careful consideration in study design as well as consideration regarding the risks, benefits, and ethics involved. Owners must provide consent after being thoroughly informed of all aspects of the study and all options for care of their pet. Each clinical study must be well thought out with a clear, concise hypothesis. Double-blinded randomized, controlled clinical trials are one of the best tools for answering important scientific questions about veterinary care. However, many options for study design are available and can be justified as scientifically sound. Researchers must write a plan for study activities in the form of a protocol. A complete clinical study protocol should contain information on the item to be tested (drug, device, procedure), information on the control, a clear hypothesis and objectives, sample size (and how it was calculated), enrollment criteria for study animals, randomization procedures, blinding information, detailed study procedures, study timelines, data collection forms, and a data analysis and statistics plan. Clinical research in veterinary medicine is a rewarding and exciting field. Full comprehension of the components discussed will support future trial design as well as assist in the evaluation of current veterinary clinical trial literature.

Keywords: Clinical trials, cats, dogs, ethics, study design

Introduction
The veterinary scientific literature has been deficient in high quality clinical research to answer many of the important questions in the field. Veterinarians receive little training in scientific methods in veterinary school and without additional training, most lack the expertise needed to conduct or evaluate scientific research. Although many veterinarians go on to obtain further training, and many are involved in published, well-designed “bench science”, it is uncommon to see well-designed, well-controlled, adequately powered clinical trials in veterinary medical publications. In order to publish more meaningful veterinary clinical research, an investigator must formulate a reasonable and testable hypothesis. They then must design and conduct a clinical trial protocol based upon measurable outcomes. Using the results of that study, an investigator must prove to the veterinary community (and possibly regulatory authorities) that the item tested (drug, food, device, technique, etc.) is efficacious for the intended use and is safe in the species for which it is intended.

Regulations and ethics in small animal clinical research

Ethics
Ethics in veterinary clinical studies are often vague and open to individual interpretation. Safety testing, patient risk/benefit analysis, using client owned animals for human research studies, and obtaining informed consent are all topics to contemplate.

Use of laboratory dogs and cats to either test the safety of a drug in normal animals or to test its effectiveness in a created laboratory model (e.g. arthritis models1-3) is one area of debate. Is it more ethical to use research dogs to prove safety and effectiveness, or is it more ethical to use fewer animals but be less certain of safety or efficacy prior to administering the drug to people’s pets (euthanizing and/or breeding more research dogs versus risking people’s beloved pets)? Safety issues in clinical trials in small animal theriogenology become a larger concern because many of the therapies to be tested do not treat a disease but are used in the process of breeding healthy animals, for example a drug to bring females in to heat.

Risk/benefit analysis is essential when designing and evaluating clinical studies. For veterinary trials, because most owners do not have health insurance for their pets, a huge benefit for clients and patients is the coverage of the cost of veterinary care. Even if the effect of the drug is uncertain, the overall effect of enrolling the pet in a clinical study may outweigh some risks because the animal receives better veterinary care. The options for many owners are to enroll their pet in a clinical trial or to euthanize because standard of care treatment for their pet’s condition is not affordable. An additional consideration for the owner is the chance of their pet being enrolled into the study’s control group (placebo or active comparator). Another consideration for the researcher is the ethics of removing animals from study treatment at the end of the trial. Treatment following study termination is especially important for new life-saving therapies or for therapies that dramatically increase quality of life. In these cases, an extended use or compassionate use study may be needed if the drug is not commercially available. Overall, some
studies may have clear medical benefit to the individual dog or cat, whereas, others may benefit the general population of pets and not the individual animal in a strictly medical sense.

In all cases, obtaining informed owner consent is essential to any trial in client owned animals. An informed consent document should contain all the information on study procedures, study drug(s), previous studies in this and other species, as well as any known side effects. The researcher should clearly identify the risks and benefits of study participation in simple language, approximately fifth grade level. Trained study staff should go through the consent in detail with the owner and the owner must be allowed to ask questions of the veterinarian investigator. The owner is required to sign the informed consent document prior to any study procedures being performed on their pet.

Regulations

Regulations for development of veterinary pharmaceuticals are clear. The sponsor must submit a request for an investigational new drug (INAD) to the Food and Drug Administration Center for Veterinary Medicine (FDA/CVM) prior to testing in client owned animals. Several studies must be performed to provide evidence to the FDA/CVM that the drug is both safe and efficacious for the intended use and species. The sponsor then submits the results of those evidence based studies as part of a New Animal Drug Application (NADA) and the FDA/CVM reviews the submission. If the evidence is substantial, the FDA/CVM approves the drug.

When drugs are being tested in veterinary studies in an academic setting, the regulations are more obscure. Academic (or private practice) investigators often choose to evaluate drugs licensed for use in other species (human or other veterinary) in new ways. This type of research falls in a regulatory grey area and the FDA has no specific research guidelines. The research does however fall under animal welfare laws and must be reviewed by an Institutional Animal Care and Use Committee (IACUC), if being conducted at an institution with such a committee.

Study design

All well designed studies follow a complete written protocol. A well-written protocol is especially important if more than one person is responsible for study execution. The protocol defines which animals can be enrolled and what specific procedures will be performed at each visit. Most importantly, the protocol helps maintain study procedure consistency. The protocol must be finalized prior to the first animal being enrolled in the study and ideally, not changed from that point forward (there are, of course, cases where change in the form of a protocol amendment is necessary). The complete protocol should contain information on the item to be tested (drug, device, procedure), information on the control, a clear hypothesis and objectives, sample size (and how it was calculated), enrollment criteria for study animals, randomization procedures, blinding information, detailed study procedures, study timelines, data collection forms, and a data analysis and statistics plan.

Defining the question

Investigators must define a very specific question in order that a complete protocol may be written to explain exactly how the study will enroll patients and collect the data needed to answer that specific question. The question should be formatted in such a way that it has a yes or no answer and is very specific to the population being studied and the primary measurement used to answer the question. The following is an example of the evolution of a question:

- Does deslorelin work to bring bitches into heat?
- Does the deslorelin implant work to bring bitches into heat?
- Does the deslorelin implant work at 1.05 mg to bring bitches into heat?4
- Does the 1.05 mg deslorelin implant bring bitches in anestrus into heat followed by ovulation (as evidenced by progesterone above 5 ng/dL) 2-4 weeks following insertion into the mucosa of the vulva as compared to a placebo group?

The question may be made more complex to obtain additional data, but a scientist should never attempt to answer too many questions with one study. If so, the focus on the primary purpose may become lost and the study then becomes a data fishing expedition. For example:

- Does the 1.05 mg deslorelin implant bring bitches in anestrus into heat as evidenced by a progesterone concentration above 5 ng/dL 2-4 weeks following insertion into the mucosa of the vulva as compared to a placebo group and is the normal inter-estrus interval maintained following use?

Now that the question is defined, it should be used it to write a hypothesis. Two hypothesis examples are below:
1) The number of anestrous bitches ovulating 2-4 weeks following implantation of a 1.05 mg deslorelin implant or placebo implant will not be different. (This is a success/fail on a per dog basis type hypothesis.)

2) There will be no difference in maximum progesterone values in the 2-4 week period following the implantation of a 1.05 mg deslorelin implant or placebo implant in anestrous bitches. (This hypothesis will measure a continuous variable, progesterone concentration, and compare the population averages among each group.)

Controls
A method of assessing a “baseline” for any measured variable is essential to demonstrating effectiveness. For this purpose, a control group is often part of the research design. A placebo-group is most common but an “active control” group (receiving another medication commonly used to treat the same disease) could also be used. It is recommended that a placebo group be used whenever possible and especially when no other treatment exists for the condition. If a treatment is available, the ethics of having a placebo group versus using the standard of care treatment as a comparator should be considered. Although animals may not be influenced by a placebo effect, owners are and subtle differences in owner behavior (especially if the owner is recording observations) may bias the study. Rarely, in a sound scientific study, is a drug compared to historical data. Historical controls are the least scientifically sound but have their place when the disease progression is very well known, disease outcome is poor, a placebo might be inappropriate, endpoints are objective, and/or each animal can be compared to its own baseline.

Outcome measurements
The measurement used to prove or disprove the hypothesis becomes the primary variable. The study is statistically designed based on this measurement. In the hypothesis 2 example above, the primary variable would be progesterone levels. Secondary variables are measurements/data collected in the study that are also of interest. To continue with the hypothesis example above, the following items could be secondary variables: luteinizing hormone levels, days to onset of heat, drug levels in plasma, other hormone levels, etc. Investigators may consider several secondary outcome measurements (variables) if the research is at a very early stage and they are unsure which variables are the best measurements for answering the question. Other variables that could also affect the outcome of the study must be considered. These “tertiary variables” (age, parity, reproductive status, breed, etc.) are mostly considered demographic data but may need to be taken into account when analyzing the results.

Statistics
Once the outcome measurements are defined and the hypothesis is written, it is time to consult a statistician. A major clinical trial should always be designed with the assistance of a statistician with experience in veterinary clinical trial analysis. If a biostatistician is not available through your place of employment (statistics or epidemiology department), there are several well-respected statisticians who work as consultants in the animal health field. It is worth every penny of expense (and is usually not that expensive in the long run) to involve a statistician in the trial design phase before crucial mistakes are made which keep investigators from obtaining meaningful study results. A statistician can help consider items that are difficult to understand for the non-statistically minded, such as continuous vs. dichotomous variables, repeated measures, “intent to treat” designs, survival, non-inferiority, blocked randomization, covariates, and stratification.

Blinding
Blinding is a condition imposed on a study meant to hide the knowledge of treatment assignment from observers. Anyone who collects data used in the analysis should be blinded. Blinding limits observer bias. A study is single blinded when the owner is unaware of the treatment group assignment. Owner blinding is helpful to eliminate subtle differences in interaction with the pet. When both investigator (and other study personnel) and owner are blinded, the term is called double-blinding. Double-blinding can eliminate investigator observational bias as well as owner bias. Triple-blinding, or masking, also blinds the person(s) performing the analysis. The maximum blinding possible should be incorporated into the design of a protocol, including the use of placebos that are indistinguishable from the investigational treatment.

Study design
Many design possibilities exist for testing a hypothesis and this paper cannot describe them all in detail. The most common is the dual-arm, randomized controlled study. Other possible designs include multi-arm (compares multiple treatments), crossover (reduces the number of patients needed because each patient receives all
treatments usually with a “washout” in between), factorial (used to test treatments alone or in combination), dose escalation (for minimal effective dose or toxicity studies), etc. Each study design has its own set of pros and cons.

Animal numbers

Once the statistician is involved, he or she can help the investigator decide how many animals will need to complete the study in order to answer the question. Calculations to derive number of animals are based on the determinant of a clinically relevant success or the limit of detection for the primary variable. For example, a 30% increase in blood flow or six months additional survival might be considered determinants of success. It is helpful to look at previous similar studies to determine what numbers (differences and standard deviation in each group) to use to estimate the results of the study being planned. Most studies are designed with a minimum of 80% power (many at 90% or 95%) and an alpha of 0.05. This means that there is an 80% chance that you will detect a difference (if a difference exists), and a 5% chance that there is not a difference (if you believe a difference exists). For small pilot/exploratory studies (not intended for publication) meant to help design larger studies, the investigator may estimate animal numbers (or power if animal numbers are fixed) with the help of statistical software or online tools.

Once the number of animals needed to complete the study is known, the percentage of animals that will be enrolled but then not complete the study and how many will fail to comply with the required treatments or visits should be considered. The percentage of animals predicted to complete the study can be used to determine how many animals will need to be enrolled in total. Then, the investigator must decide how many animals will need to be screened for every one animal enrolled. Be realistic when determining the failure rate at initial evaluation. Most people overestimate the number they will be able to enroll in a study. Investigators should not use enrollment challenges to justify a decrease in the number of animals targeted to complete the study. Decreasing enrollment goals may have the consequence that the study does not have the power to accept or reject the original hypothesis. In small studies with few animals, the possibility of accepting the wrong answer to the question is very real. In the scientific community, it is becoming increasingly less acceptable to include only ten animals on study due to an inability to enroll patients or due to financial concerns. The objective analysis of the numbers of animals or the amount it will cost to do the study should drive the decision to do the study at all, not if it should be done well or poorly. The advantage of conducting research in the veterinary community is that it is an inclusive group and there are likely others in theriogenology (or other specialties) who would be happy to recruit and enroll patients using the identical protocol (identical protocol is crucial). If study related cost is a factor, consider reducing the number of secondary variables to be tested or visits required instead of risking a wrong or ambiguous result due to low enrollment. The older (and sometimes newer) veterinary literature is replete with underpowered studies that enrolled only a few animals. These studies contribute very little to the profession and in some cases have come to erroneous conclusions leading many in the profession astray until later demonstrated to be incorrect.

Define the population

The study population should be defined in advance with clear, concise eligibility criteria. Specific inclusion and exclusion criteria should be created, keeping in mind that if these criteria are too strict, the study will not apply to the general population and patients may be difficult to recruit. However, if the criteria are too loose, the study may fail due to extraneous factors. Consider the following:

- Age, sex, breed effects on the study
- Will subjects be allowed to have other diseases?
- What is standard of care and how does the studied treatment fit in?
- Will study subjects be allowed other medications?
  - If not, is this applicable to the general population?
  - Do other drugs interfere with the study drug? Protein binding?
  - How might other medications affect the disease process studied?

Randomization

Randomization is one of the most effective tools for the elimination of bias from many sources. Randomization helps balance study groups and is the basis of tests for significance. Animals on the study should be assigned to groups in a random preconceived plan. This plan can be created by the study statistician or by the investigator based on the complexity of the study design. A helpful website for creating a randomization plan is: www.randomization.com. Often, animals are assigned to the plan in order of enrollment in the clinical study in a way that keeps the investigator and other study staff with direct client/patient contact blinded.
Study procedures

Create a study timeline with the measurements filled in (Table 1). The first day of drug administration is traditionally Study Day 0. There should be enough leeway in the follow-up visits so that cases are not lost due to the inability of the owner to return with the pet within the given timeframe. This is especially important for long studies.

Table 1. Sample study procedures and timeline

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Day -7</th>
<th>Day 0 (+/− 2)</th>
<th>Day 14 (+/− 2)</th>
<th>Day 28 (+/− 4)</th>
<th>Day 60 (+/− 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CBC, Chemistry, UA</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Begin or End Treatment</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td>E</td>
</tr>
<tr>
<td>Hormone Panel</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Abdominal Ultrasound</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Feasibility

Once the timeline is complete, the feasibility of the study for the investigators, study staff, and the owner is evaluated. Potential issues that may arise include fitting the study timeline into a typical workweek or workday, measurements that need to be done on weekends or after normal work hours, or time allowed to receive and evaluate the results of screening tests. In addition, the ability to recruit and enroll enough animals that fit the population criteria should be considered. If enrollment could be a problem, a multi-site study could be designed. A multi-site study requires agreement between investigators at the additional sites and an even more specific protocol (very detailed inclusion criteria, study procedures, etc.). Multi-site studies are more difficult logistically but can greatly increase enrollment on a clinical trial. All the investigators should be fully committed to running the trial for this approach to be successful. Thoughtful site selection can make or break a clinical study. The final assessment of feasibility is an evaluation of the financial aspects of the trial, especially if it is sponsored by industry or if it was designed elsewhere, to be certain the study can be conducted correctly for the money provided.

Budgeting and financing

A budget can be created from the table of procedures and events in the protocol. Recruitment and retention will be enhanced if the study pays for all veterinary care. In fact, for studies that rely on survival analysis, paid veterinary care is essential to eliminating bias or reaching an endpoint (e.g., canine parvovirus studies). Consider adding money to compensate the investigator for time lost from clinical practice and/or money for a technician to do the majority of tasks in which direct veterinarian involvement is not necessary (discussed further below). Many studies also include client incentives for participation depending on the inconveniences compared to the benefits of the trial. Many owners will enroll their pet in a trial in exchange for free services or drugs, and others for the benefit of science, but often, an additional incentive must be offered, especially if the trial is long and requires many return visits.

Study execution

Recruitment

Study subjects may be obtained from current clinical patients in the practice database, from referring veterinarians, or by reaching out to owners directly. Consider if there will be any benefit to referring veterinarians for study patient referrals. If there will be no benefit available it may be best to advertise directly to owners. Unless you work in a private practice that is actively competing with other veterinarians for study patients, it is best to assure referring veterinarians that you will send their patients back to them once the study is completed. If you are in private practice, clinical trials can be a great way to attract new clients and increase your revenue, especially if you are involved in industry sponsored studies.

Data collection

Keep data collection to the minimum necessary to fully evaluate the endpoint variables. Create data collection forms that include space for every data point that MUST be obtained. Specific forms for each visit ensure all pertinent data are collected. The medical record is very often missing information that is essential for the study
(heart rate for example) and cannot be relied upon to obtain data for a study. In form development, it is best to use check boxes or bubbles when possible and try not to include free form text areas, especially on forms completed by owners. Limiting free text areas keeps study personnel and participants from adding information that is not needed to answer study question(s) and increasing the workload for data entry. Checklists for each visit to help the investigator and other study staff guarantee all protocol specific procedures have been completed during each study visit.

Study staff

It is worth repeating that, unless the investigator has a large amount of free time, a clinical trial coordinator or study technician should be hired. This person is usually a very detail oriented and organized veterinary nurse or assistant who will execute the details of the study including scheduling appointments, organizing and completing paperwork, calling owners, communicating with the sponsor, and generally keeping the study running smoothly. The investigator then performs only items that are specifically required to be done by the investigator (examination, diagnosis, prescriptions, etc.), decreasing his/her workload. In general, it is more cost effective for the veterinarian to see additional patients than complete study paperwork.

Protocol adherence

*Stick to the protocol!* Unless a true flaw is definitively identified, the protocol should be strictly followed. A reasonable change might be allowing smaller dogs to be enrolled because the investigator discovers that the tablets can be accurately cut in half (only whole tablets were anticipated). A poor reason for deviating from the protocol would be allowing one dog to be on antibiotics for a minor topical infection when your protocol specifically excludes antibiotics. If antibiotics are truly not important to the study, the protocol should be amended to allow antibiotics. A protocol amendment is a change that you make to the protocol before you initiate that change. A protocol deviation is the departure from the protocol, written after it occurred. Deviations should be avoided, if possible, to maintain the prospective nature of the study and data integrity.

Data analysis

After the data are collected and before they are given to the statistician for analysis, they should be checked for quality and accuracy. Ideally, 100% of data points are verified however, a 100% verification is often not realistic and the percentage of data checked is dependent on time and money. A plan for how to handle missing data points, deviations, skipped doses, early withdrawals, etc. should be made prior to beginning the process of including or excluding data or cases from the final analysis.

Working with industry sponsors

In general, there are two types of studies that are done in partnership with industry sponsors, investigator initiated trials and traditional safety and efficacy trials used for product registration or line extension. The level of sponsor involvement varies widely and can range from total control of the study to merely providing supplies or drug. It is absolutely essential that the role of the investigator is spelled out specifically in a contract prior to the initiation of the study. The ability of the investigator to publish the results of the study is a key issue that must be addressed prior to study initiation.

While some pharmaceutical companies enlist the most inexpensive clinics to perform clinical studies for them, most are looking for the most effective sites for clinical trials. Effective sites are those clinics that can provide excellent patient care, complete data, and are great to work with, as well as performing the studies at a reasonable (not necessary the least expensive) rate. There are very specific guidelines, such as Good Clinical Practice (GCP) and International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), which have been set out by government regulators that must be followed for these types of studies. Most companies provide training to support learning these regulations and how to conduct trials in support of regulatory approvals.

Conclusion

Jerry Avorn said that “The randomized controlled clinical trial is nothing less than the single most important development in the revolution of modern therapeutics, the most powerful intellectual medicine we have—the one that makes all others possible. Like Newton’s laws of motion, the concept is both breathtakingly simple and enormously strong.” Sound design in veterinary clinical studies is crucially important for evaluation of new therapies and techniques. Consideration of each of the discussed components can help in evaluation of published veterinary research as well as planning future research.
References