As residents or new faculty members, we are faced with the expectation to carry out clinical research. As clinicians, we encounter frustrating clinical questions that we may want to resolve. However, many of us as clinicians have not received formal research training, and research may feel outside of our comfort zones. When trying to identify a good research question, trust your own clinical experience. Where are the controversies that are impairing your ability to manage your cases? What types of cases are commonly seen in your clinic? What is new and interesting about those diseases in the human field?

Clinical research questions may address new diagnostic tests or treatment options, adverse outcomes, prognostic indicators, or underlying etiology. Review your journal club papers and re-read papers that have interested you – is there a logical follow-up to a published study, or a study that could be repeated with a better design? Look through proceedings and research abstracts from your specialty meetings: where are the knowledge gaps? Talk with other colleagues or faculty mentors about their ideas for good clinical research questions. Your question should be relevant and interesting to you as a clinician.

The next step in approaching a clinical research question is to perform a thorough literature search to determine what is known and what needs to be answered. Start with the PubMed site (http://www.ncbi.nlm.nih.gov/sites/entrez), and use multiple search terms (scan relevant papers to see what words show up in the titles and abstracts). Supplement your PubMed search with additional publications from reference lists of papers you are reading. Read the entire papers, not just the abstracts. When reviewing research abstracts from conferences, checkPubMed to see whether older abstracts have reached the peer-reviewed and published stage (exercise caution if they have not). Summarize the results of your literature search in a bullet point outline. This summary should tell a logical story about why the problem is important, what is known, and what still needs to be discovered. This outline can later form the basis for the background section of a grant application, or the introduction portion of a manuscript. From your outline, ask yourself whether answering your original clinical question still makes sense, or whether it needs to be refined based on your literature review.

Once you have decided on a research question, generate a hypothesis about the likely outcome/answer to your question. Most granting agencies and many journals will ask you to overtly state your hypothesis(es). Your hypothesis should be concise; more detail can be added in your specific research objectives and study design. For example, your original clinical question might have been how to manage proteinuria that is refractory to ACE inhibitors in dogs. After your literature review, your research question might be to determine whether spironolactone is an effective adjunctive treatment to ACE inhibitors in dogs with persistent proteinuria (UPC > 3.0). Your hypothesis is that spironolactone will be more effective than placebo in reducing proteinuria in dogs that are refractory to ACE inhibitors.

The next step is to frame a specific research approach that will test your hypothesis in a clear and specific manner. This will flesh out your research question into full study objectives. Study objectives often start with active descriptors such as to “determine,” “evaluate,” “characterize,” or “compare.” One practical approach to refining your study objectives is the PICOT method, which identifies five components for your particular study, to include Population, Intervention, Comparator(s), Outcomes, and Time frame.

**POPULATION**

The study population should be as clearly defined as possible, and should encompass specific inclusion and exclusion criteria. If you are studying a pre-existing disease, your inclusion criteria should include a gold standard for diagnosis. Make sure that the gold standard is achievable in sufficient patients. If the gold standard is fairly invasive (for example, pancreatic biopsy for pancreatitis or intracranial biopsy for CNS tumors), consider whether there is a reasonably valid surrogate marker. You may be able justify your surrogate marker based on previous studies, or you may need to validate it first. This might shift the focus of your project to marker validation itself as your research objective. Think not only about diagnosis, but also about specific clinical presentations, age ranges, and specific breeds versus all breeds. For example, a study of serologic markers of chronic hepatitis in dogs might be more powerful if focused on a single breed, while breed might be less important in a study on medical management of pyothorax. Consider chronicity of disease – will you enroll only newly diagnosed patients, only patients with established stable disease, or both? Remember that the more heterogeneous your groups, the more patients you will need to demonstrate a difference.

Exclusion criteria should also be carefully defined at the onset. Consider severity of disease – will you exclude animals not expected to survive 24 hours? If so, is this valid for the question you are trying to answer? Will you allow prior treatments, and if so, what minimum washout periods are reasonable? Think about co-morbidities. Is it valid to include some FeLV- or FIV-positive cats in a study of antioxidant status in renal disease? The more homogeneous your groups, the fewer patients you will need to demonstrate a
difference. However, you need to make sure that your study groups are representative of the larger clinical population that you are trying to understand. In addition, restricting to a more homogenous group will require more time for recruitment, and might require multi-center involvement. Finally, there are practical and safety considerations in exclusion criteria. For example, small or anemic dogs may need to be excluded if a 30 ml blood sample is needed, and breeding animals may need to be excluded from a drug intervention study in a kennel of purebred dogs.

INTERVENTION
Most prospective studies have an intervention that is being evaluated, such as test drug versus placebo, a new procedure versus a standard of care, or a new diagnostic assay versus a commonly used test. Interventions should be well defined as to drug dosage, duration, and washout; specific procedure (and clinicians involved); and timing of testing relative to stage of disease. Decide which concurrent treatments will be allowed. Leaving much of case management to “clinician discretion” is not ideal and can cloud your results. You will also need to decide whether your study needs to be blinded (the client or investigator) or double-blinded (both the client and investigator). Anyone providing any subjective evaluation for the study should be blinded to the intervention group assignments; this includes owners, managing clinicians, vet techs, radiologists, cytologists, and pathologists.

COMPARATORS (CONTROLS)
Clinically relevant controls are key to the structure of your research question. The control group(s) should reflect the accepted standard of care or a placebo. For most studies, concurrent controls are necessary, since they help to minimize bias associated with the passage of time. Such biases could include the development of better diagnostic tests or interventions during your study period compared to ten years ago, the adoption of a new medical records system with better search capabilities for certain adverse outcomes, or inherent differences in the consistency of evaluations between two time periods. Historical controls may be acceptable in some instances based on ethical grounds, such as when studying new interventions for well-established, uniformly fatal outcomes.

OUTCOMES
All studies should have at least one defined primary outcome. This is often the most important question that needs to be answered. Primary outcomes are strongest when they are objective, such as hematocrit, blood pressure, days in hospital, or survival. If surrogate markers are used, they should be validated against a clinically relevant outcome, such as force plate analysis for lameness in polyarthropathy, or cardiac troponin I concentrations for myocardial disease. Primary outcomes should be easily measured, clinically available, and validated for your species. When subjective assessments are needed, seek out a validated scoring system, such as the Glasgow Composite Measure Pain Score, or the Survival Prediction Index (SPI2) for illness severity. Alternatively, you might develop your own scoring system. Even if subjective scoring systems are chosen, it is best to complement these outcomes with objective or surrogate markers to strengthen your study. For invasive procedures compared to non-invasive procedures, consider masking the surgical/intervention site to the evaluators, or, if blinding is not possible, use only objective outcome measures.

To gain the maximal amount of information from the study, secondary outcomes can also be included. These may be of lesser importance, or exploratory in nature for things that are more difficult to prove, but provide depth to the study. For example, the primary outcome of a study on IV IgG in dogs with immune thrombocytopenia was days to a platelet count > 40,000 with IV IgG versus placebo. The secondary outcomes were the incidence of acute or delayed reactions to IV IgG, cost of hospitalization between groups, and 6-month morality rates.

Once you establish your primary and secondary outcomes, you need to determine the number of subjects you will need to enroll. This is a sample size calculation based on the desired power of the study to detect a clinically relevant difference between groups. This step can be approached in one of two ways: 1) start with a known sample size (e.g. number of retrospective cases available in each group, or number of cases likely to be recruited or that the budget will allow), and determine what power you will have to detect the difference you are interested in; or 2) start with a minimum power threshold (typically 80% or higher), and determine how many patients you will need in each group to reach that. The pieces of information that you will need for a sample size calculation are: the expected mean outcome, with SD, in your control group (either from a pilot study, or published veterinary or human studies); and the difference that you want to detect in your experimental/treated group (e.g. 20 mmHg of blood pressure, 50 umol/L of fructosamine, or 1 less day of hospitalization). Power is typically set at 0.05. Power is either calculated from your available sample size or set at a minimum of 0.8 (i.e. 80% chance to detect a true difference if it is present). Then use an on-line sample size calculator (see below for a good website) to calculate your sample size/power. Follow up with a statistician (before you start your study) if your study design is complex. Sample size/power calculations are essential for any research question that involves the statistical comparison of outcomes in two or more groups, and most granting agencies require a clear description defending the proposed sample size. This is true even for retrospective studies!

TIME FRAME
Finally, the time frame for study observation and follow-up should also be established. What time points for evaluation are clinically relevant? How much follow-up would you, as a clinician, find adequate? Is the proposed duration of follow-up feasible, or are many
cases likely to drop out over time? If your recruitment time frame is short (less than one year), does it take into consideration possible seasonal variables such as tick exposure (which could bias a fever-of-unknown-origin study) or seasonal vaccines (which could affect a study of immune function)?

Once you have refined your clinical research question, formulated your hypothesis, and framed your study in PICOT format, your study plan will fall into place quickly. Make sure that your study design still addresses your hypothesis, and has not “drifted” to address a slightly different question as you have formulated your PICOT. This can easily happen as you make practical decisions and concessions in your study design, and you may need to reframe or narrow your hypothesis at this stage. Remember that after your PICOT efforts, your research question should still be relevant to clinical practice and interesting enough to warrant your time and effort. It is even more critical that your research question be feasible, given your clinical expertise, caseload, support staff, available funds, and career time frame. The best advice for new researchers is to choose a disease that you see commonly, and start with a simple focused research question!

**Common roadblocks to new researchers in formulating successful research questions**

- Question is unfocused
- Question is not clinically interesting
  - “Because no one has looked at it” is not sufficient!
- Disease is uncommon
- Outcome to be studied is rare (e.g. uncommon side effect or complication)
- Data collection is invasive, labor intensive, or off-putting to owners
- Validated assays are not in place
- New pieces of equipment are needed
- Case identification is outside of your sphere of control
- Collaborators are not motivated to complete the study
- Prolonged follow-up is required

**Helpful websites**

  - Provides a worksheet to help define a research question and determine search terms to provide background research.
- [http://www.stat.uiowa.edu/~rlenth/Power/](http://www.stat.uiowa.edu/~rlenth/Power/)
  - A useful on-line sample size calculator from Dr. Russell Lenth at the University of Iowa. Sigma = SD; n = sample size; difference of means = clinical relevant difference that needs to be detected (in same units as SD)

**References**