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WHY THIS TOPIC?

Successful organizations understand that being able to communicate cross-culturally in the workplace leads to enhanced productivity, performance and employee engagement. Managing diversity drives profitability, leads to innovation and promotes an inspiring workplace culture. Within Canada’s population, 20% are foreign born with the top source immigrant countries being India, China, Pakistan and the Philippines.

INDIVIDUALISTS VERSUS COLLECTIVISTS

Individualistic values reflect individual tastes, goals, achievements and accomplishments. Collectivist values reflect common values among families, tribes, work divisions, communities. Every decision, conversation, and contribution is reflected in this value. The top collectivist countries in the world are Guatemala, Ecuador, Panama, Venezuela, Columbia and Indonesia. The top individualist countries in the world are the United States, Australia, United Kingdom, Netherlands, Hungary and Canada.

PERFORMANCE FEEDBACK

There is a close link between performance feedback and indirect versus direct communicators. In North American cultures, the ‘sandwich’ approach is utilized to offer performance feedback. Deliver positive news, followed by constructive criticism, end with positive feedback. Not all cultures will respond to the ‘sandwich’ technique. All cultures need praise. Ideally, offer specific praise – rather than positive or negative praise. Stating, “great job” will not provide enough feedback to model the behaviour repeatedly. Offering specific, positive feedback will reinforce the behaviour you are seeking ie. “your spreadsheet was well done because it was so detailed, delivered on time and easy to navigate”. Direct communicators do not always give positive feedback as it’s not part of their culture and doing good work is an expectation. This can be deflating for some and lead to employee disengagement. Indirect communicators need positive feedback but if they collectivists the praise would be better offered in person rather than in a group setting. If offering specific, constructive, negative feedback, indirect communicators will not respond well if the entire team is present. This should be done behind closed doors or with a human resource professional present so that the indirect communicator, who may also be collectivist, recognizes their job is secure as hierarchy can also play a part.

COMMUNICATION STYLES

Reflexive
Will repeat parts of the conversation utilizing the same tone and intonation; reflexive speakers show respect and understanding by repeating the conversation.

Interruptive
Interrupt the conversation without necessarily knowing it. Collectivists are often ‘interruptive’ in nature given they are more family and community-oriented. Unless someone asks for clarification, continue the conversation.
**Direct**
Use fewer words and less non-verbal communication. Unfortunately, the perception of direct communicators are that they are rude, abrasive and arrogant which may or may not be the case. Perception is not necessarily reality. Is this a communication style indicative of culture?

**Indirect**
A yes may mean yes, no or maybe. Indirect communicators are often collectivists where group harmony is much more important than disagreeing with someone which may result in a ‘loss of face’. With indirect speakers, ask clarifying questions and paraphrase.

**INTERACTION AT THE WORKPLACE**
There are three different ways to communication at the workplace; face to face, phone, email. There are differences between individualistic and collectivist cultures, particularly with interaction. With collectivist cultures, chit chat is about relationship building hence depending on where someone is from may center around family, community, school, politics, sports whereas in Canada, chit chat centers around weather and traffic. If English is a second language be aware of this during phone conversations. A helpful hint is to ask the employee/client to followup with an email to ensure something was not ‘lost in translation’. This assists if somebody has a strong accent and attributes to the concept of ‘saving face’. Lastly, greetings may differ in written correspondence. In Canada, titles such as “Mr. and Mrs.” are often used and even first names. This is not necessarily the case in most parts of the world where formality and hierarchy are important.

**NON-VERBAL COMMUNICATION**
The written word accounts for 7% of communication whereas non-verbal communication such as tone, intonation, gestures, paralanguage, posture, eye contact, smell, silence and personal space account for the remaining 93%. First impressions are made within the first seven seconds of meeting someone often before someone opens their mouths. Gestures can range from how handshakes differ around the world to something as simple as the ‘thumbs up’ sign being misconstrued for being offensive. In North America, direct eye contact is expected and respected, whereas, in many cultures, direct eye contact is seen as disrespectful.

Some cultures will avert a direct gaze by looking down or even at someone’s chin to avoid direct eye contact. Paralanguage refers to the tone and intonation of which we use. Some cultures expect their leaders to have very loud voices. The louder the voice, often the leader is more respected. However, in some cultures, such as in the Japanese culture, a loud voice signals someone is ‘out of control’. In North America, if someone is ‘silent’ it can be misconstrued as lack of interest or lack of contribution. In North America, we are rewarded with being able to ‘think quickly on our feet’. In many cultures, silence is considered to be a positive. It can mean that the person is reflecting upon what was actually said. When in doubt, mirror the image, the gesture, or even tone of voice. Companies such as Nike, Kellogg’s, Federal Express, Ikea and Ford have lost millions in revenues by not taking nonverbal communication into account.

*50 Shades of Beige*

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Honey bees are social insects. According to E. O. Wilson, the following three criteria are required for insects to be called truly social (eusocial): 1) caste division of labor, 2) cooperative care of brood/offspring, 3) overlapping generations (offspring stay with colony and contribute to general welfare). Under normal circumstances, honey bee colony consists of a queen, drones and worker bees. Both queen and workers are diploid females and have 32 chromosomes and drones are haploid males with 16 chromosomes.

1.1. Castes

1.1.1. Queen is the only mated egg-laying female with developed reproductive tract in a colony. She is slightly larger than a worker bee and her abdomen is elongated and only partially covered by the wings because it contains fully developed reproductive tract in addition to other abdominal organs. Three days after oviposition, a diploid larva hatches from a fertilized egg and is fed abundant amount of royal jelly during the entire larval period (5 days) after which queen cell is sealed with wax (8-9 days post-oviposition) and pupation occurs until eclosion (emergence) at 16 days post-oviposition. The newly emerged virgin queen will destroy other queen cells, if they exist, and during the first two weeks of life will fly out from hive to mate with 10-15 drones (polyandry) in the air. The more drones the queen mates with, the greater the genetic diversity of her offspring, and the greater the robustness of the entire colony - [important for the overall health of colony]. The queen stores the collected sperm from drones in the sperm-storing organ, called spermatheca, for the entire life (3-4 years). Once mated, the queen does not leave the hive again except during natural swarming. There are two major roles that the queen performs. The first role is laying eggs that develop into workers, drones and queens (if needed). During the peak season, queen can lay ~1500 eggs a day (close to her own weight). The second role is production of multiple pheromones that maintain functionality of the entire colony. Pheromones are produced in mandibular (and other) glands and distributed throughout the entire colony via direct contact and trophallaxis (food exchange) - [important behavior - > spread of diseases]. Pheromones have many important effects on the colony, including suppression of ovary development in worker bees, suppression of queen rearing (swarm and supersedeure cells) and enhancement of worker activity necessary for growth, productivity and overall health of the colony [important behavior - > spread of diseases] [1].

1.1.2. Drones are male bees whose main function is to mate with virgin queens after which the drones die. Drones are larger than worker bees but they are not as long as the queen. Three days after oviposition of a non-fertilized egg, a haploid larva hatches and is fed royal jelly during the first ~3 days and then pollen and nectar until the end of larval stage (day 9-10 post-oviposition). After pupation is complete, drones emerge at day 24 post-oviposition. During the first ~2 weeks of life, drones become sexually mature, and then fly out of the hive during the day to seek drone congregation areas where they join drones from other colonies which are waiting to mate with virgin queens. Drones that succeed to mate with a queen will die during copulation, while those that did not succeed to mate live for a few months during the summer and are expelled out of hive and die during the fall. During the summer, drones are accepted by all colonies, therefore they often drift from colony to colony - [important behavior - > spread of diseases].

1.1.3. Worker honey bees are ‘sterile’ females that have hypoplastic ovaries. Both queen and workers hatch from the identical fertilized eggs as diploid female larvae, and accordingly, they have the same genetic
composition. The quality and quantity of feed available to these diploid female larvae determines their phenotypic development. Namely, if larvae are fed with abundant quantities of royal jelly during the entire larval period, they will develop into queens within 16 days post-oviposition; conversely, if the same larvae are fed restricted amount of royal jelly for the first ~3 days and then with pollen and nectar, they will develop into worker bees within 21 days post-oviposition. The worker honey bees are responsible for all activities in colony except reproduction. The honey bee workers are divided into two major types based on seasonality and tasks: summer and winter workers.

**Summer workers** live ~6 weeks. Approximately the first half of life of an adult summer worker (3 wks) will be dedicated to housekeeping tasks in hive (cleaning brood cells, nursing brood, attending to and feeding queen, building wax comb and sealing brood and honey, processing nectar into honey, processing pollen into bee bread, removing dead brood and adults from the hive, ventilating and defending the colony) - [many of these behaviors are associated with spread of diseases]. The second half of an adult summer worker’s life (~ last 3wks) is dedicated to field work which consists of collection of nectar, pollen, water, and propolis. The most intense harvesting occurs within 2 miles but the forager bees can fly twice as far if nectar or pollen is not available closer - [important behavior -> spread of diseases]. Drifting of honey bee workers from hive to hive is not as common as in drones; however, it exists. During the honey and pollen flow any worker bee carrying the load will be allowed into the hive - [important behavior -> spread of diseases]. There are seasonal variations in the total number of worker bees per colony. To maximize honey production, the highest number of worker bees in a colony (50,000-70,000 bees) should coincide with the major blooming season of nectar producing plants (e.g. canola and alfalfa in Saskatchewan). In contrast, there are only 15,000 to 30,000 bees in a colony during the late winter.

**Winter honey bee workers** emerge at the end of summer and the beginning of fall (September to October in SK). During that time there is limited need for nursing (due to markedly reduced egg-lying activity) and foraging (reduced availability of flowering plants), so workers are not ‘worn out’ with these demanding tasks and their life span is on average 150 days [2]. The main role of winter worker bees is to ensure that queen survives during the winter until spring season starts and a new population of bees are generated. During the fall, surviving drones are expelled from the colony in preparation for winter. Honey bees do not hibernate. Instead, when the outside temperature falls, bees form a cluster surrounding the queen and generate heat by vibration of thoracic muscles and abdomens using energy from consumption of stored honey reserves. The density of the cluster is used as a mechanism for thermoregulation to achieve an optimal temperature of 29°C in the center around the queen and ~6-8°C at the periphery. Constant circulation of the bees within the cluster ensures exchange of the peripheral vs central positions of worker bees to freezing of bees at the periphery. The winter bees usually do not defecate within hive; instead, they accumulate waste within the rectum which can distend and occupy a substantial portion of the abdominal cavity. On sunny winter days when the temperature is above 0°C, bees will fly out of the hive for a short period to empty the waste from their rectum (cleansing flights).

### 1.1.4. Development stages of brood

Development stages of brood are the same for all three castes even though the total development time is different. The queen lays non-fertilized eggs in drone comb cells (which have a larger diameter than worker cells) and fertilized eggs in worker comb cells and queen cups. From fertilized eggs, diploid larvae emerge 3 days after oviposition and will develop into workers or queens depending on the larval food they are provided by nursing bees. Non-fertilized eggs will develop into haploid drones. After hatching, larvae are attended and nursed by many young nursing bees visiting each larva many times per day - [important behavior -> spread of diseases]. After completion of the larval stage (5-6 days), the comb cells containing larvae are sealed with wax (capped) and prepupal and pupal development occurs for 8-15 days depending on caste. Eclosion (emergence) of the imago (adult) stage occurs at day 16, 21 and 24 post-oviposition for queen, worker and drone, respectively. Larvae and pupae that die during the development process are removed and cells are cleaned by housekeeping worker bees - [important behavior -> spread of diseases]. In addition, worker bees have the ability to detected infected or infested pupae and remove them; this is called 'hygienic behavior' which interferes with disease progression (e.g. reproduction of Varroa) and...
improves colony resistance to disease. Hygienic behavior is a part of social immunity and contributes significantly to the overall health of a colony. This is an inherited trait and there are various queen-breeding programs that selectively enhance this behavior - [important management practice -> enhance disease resistance and colony health].

1.2. Reproduction

1.2.1. Natural propagating and reproduction of honey bee colony is accomplished by swarming during late spring and early summer. There are several predisposing factors that will result in swarming, including: 1) concentration of queen’s pheromones become too low to maintain colony cohesion – this could be due to rapid expansion of colony during the spring or decreased production of pheromones; 2) decreased/inadequate space for colony expansion and food storage – colony becomes too crowded; 3) decreased space for egg laying and brood rearing. Once the colony enters the “swarming mood”, queen cells with young queen larvae are produced. The old (mother) queen reduces egg-laying activity and her abdomen becomes smaller due to atrophy of ovaries in preparation for a swarming flight. Swarming will occur during favorable weather conditions (sunny and warm early afternoon), usually when the new queen cells are capped and the young queens are in the pupal stage. The old mother queen will leave hive with a substantial proportion of worker bees and form a transient swarm cluster, usually on a tree branch close to the original hive (less than 100 meters) until scout workers find a suitable location for their permanent new home, which could be a few kilometers away from the original hive - [important behavior -> spread of diseases]. Swarmed bees are docile and do not exhibit defensive behavior. The remaining portion of the original colony will wait for the new queen to emerge, mate and re-establish functional order of the colony.

1.2.2. Swarming is detrimental for a beekeeping operation due to loss of bees and subsequent decrease in honey production. Accordingly, good beekeeping management practices aim to decrease/eliminate swarming by removing predisposing factors for this natural reproductive and propagating behavior. There are numerous management beekeeping techniques used for multiplying colonies and some of them use queens produced by commercial queen breeders who ship queens worldwide. Large-scale commercial queen breeding and production by comparatively limited number of companies is considered to be a threat to genetic diversity - [important management practice -> impact on colony health].

2. Seasonal cycle in honey bee biology and beekeeping

2.1. Winter

European honey bees are adapted to a temperate climate and will survive winter providing that they are healthy, have abundant food stores and proper ventilation (to prevent condensation within the hive). At the end of winter and beginning of spring (March in SK), the bee population is at its lowest and the colony will start to rear brood to replace the old population of winter bees. [Disease management note: At this stage, there is no or very little brood hence the great majority of Varroa mites are in phoretic stage – this is the most effective treatment time with miticides].

2.2. Spring

During the winter, the colony consumes just enough food to generate sufficient heat in the cluster to protect the queen. However, when egg-laying and brood rearing resumes, requirements for energy and protein rapidly increase, and consequently, consumption of both stored honey and pollen is also substantially increased. Initiation of brood rearing (during early spring in SK) is the most critical time for overwinter survival of a colony, because if the colony does not have sufficient food stores it will most likely run out of supplies and die of starvation considering the lack of external sources of nectar and pollen in the environment at this time of year. Once spring blooming commences (crocuses and willows in SK ->
Apr-May), colonies start to expand rapidly. During this time, additional therapy against *Nosema* sp. and/or *Paenibacillus larvae* (American foulbrood) may be considered if necessary, to make sure that there is sufficient withdrawal time before major spring blooming (e.g. dandelions and caragana in SK -> May-Jun) if honey is to be harvested for human consumption. This is also a time period (May-Jun in SK) of intense beekeeping activity in the apiary, including, *inter alia*, spring inspections and clean-up, preparation for queen rearing, nucleus (replacement colony) establishments, and queen replacement. All of these activities are crucial for prevention of swarming, breeding of new queens and multiplication of colonies to be used for replacement or expansion of the operation. Under normal conditions, replacement of queens is done every second year, but this depends on management practices of each beekeeper. Nevertheless, the importance of high quality queens cannot be overemphasized, not only for optimal colony production, but also for the overall health of colony – [important management practice -> enhancement health of colony by high quality of queens]. An old or poor queen that does not produce sufficient quality and quantity of pheromones will compromise colony homeostasis and cohesion through alteration of several physiological and behavioral modifications in the worker bee activities such as reduced cleaning, guarding, foraging and brood care [1] that ultimately results in a weak colony and increased susceptibility to disease. Following natural instinct, the colony will try to replace the queen by supercedure but the progeny queen will still have the same poor genetics as its mother.

### 2.3. Summer

During the summer (end of June to August in SK), the colony is at the peak of its strength and the majority of colony activities are centered on intense harvesting of food reserves (i.e. nectar and pollen) to be stored as honey and beebread for use during times of dearth (winter). However, “clever” beekeepers exploit this prolific behavior to generate profit from “stolen” honey using well established beekeeping practices.

### 2.4. Fall

At the end of August (in SK) all honey stored in honey supers (above brood chambers) is removed for extraction. Fall treatment and feeding is initiated to ensure that overwintering colonies are as healthy as possible and have sufficient food stores. The most important, and very often necessary treatment is against Varroa mite. For many beekeepers in North America, metaphylactic treatment against American foulbrood is equally as important and it is also done at this time (September in SK). The third potential fall treatment is against *Nosema apis* and *Nosema ceranae*; this last treatment is recommended/applied based on infection rates determined in forager bees, or based on history of Nosema disease in this particular operation. Since beekeepers harvest the majority of colony honey stores accumulated during summer, in September, honey bee colonies are provided with abundant feed in the form of sugar syrup to ensure that colonies have enough food stores during the winter. During mid-October in SK, miticide strips are removed from colonies and colonies are prepared for overwintering (according to the local winter climate).
3. Transmission of disease

3.1. Mode of transmission of disease within a colony

A honey bee hive contains thousands of bees with biological behavior that requires close interaction (e.g. trophallaxis), direct contact (e.g. pheromone spread) and housekeeping duties (e.g. removal of dead brood and adult bees). These behaviors facilitate horizontal transmission of pathogens between individual bees. In addition, there are certain pathogens (e.g. viruses) that can be transmitted vertically from queen to progeny.

3.2. Mode of transmission of disease between colonies

Once the disease is established within a colony it can spread from colony to colony by natural or anthropogenic means.

3.2.1. Natural transmission of diseases between different colonies may be facilitated by 1) drones and workers drifting to adjacent colonies [3], 2) foragers from different colonies foraging on the same crops [4], 3) queens mating with infected drones [5], 4) colony swarming, and most importantly 5) foragers robbing infected, weak or dead colonies.

Robbing behavior of honey bees is the most important natural mode of transmission of honey bee diseases between colonies. Robbing is a special behavior of forager bees that find an unprotected source of honey that is collected and brought back into their hive. This behavior intensifies at the end of summer when there is reduced availability of nectar from flowering plants and large number of foraging bees. Unprotected stores of honey could be available in dead colonies that died due to various diseases, hence the “robber” bees become contaminated and bring infectious pathogens back to their hives. Weak colonies are also often targeted by robber bees because their guard bee population is depleted and, consequently, easily overpowered. One of the major causes of colony weakness is disease; accordingly, pathogens from weak colonies are transmitted to healthy and strong colonies by their strong foraging population (robber bees). Thus, it is extremely important to remove dead-out colonies from the apiary and, if infectious disease is identified, destroy or disinfect equipment (frames, comb, etc.) to minimize disease spread. In addition, if infectious disease is not identified as the cause of weak colonies, weak colonies should be re-queened and/or merged to create strong colonies. It should be emphasized that robbing is not restricted to colonies in the same yard, but it can occur anywhere within the ~5 km (flight radius of forager bees) [important behavior -> spread of diseases].

3.2.2. Anthropogenic transmission of diseases (e.g. fomites, equipment, trade, etc.) is also extremely important. Using contaminated fomites and equipment, beekeepers can spread diseases from hive to hive or from yard to yard within the same operation if optimal biosafety practices are not implemented. Potentially devastating disease outbreaks can occur due to the sale and purchase of contaminated equipment or infected bees among beekeepers. National and international trade of potentially infected bees and products (e.g. packaged honey bees, queens, semen, honey, etc.) has been a major contributor to the global spread of honey bee pathogens during the last several decades [6] in spite of best intensions, strict regulations and high quality inspections. Migratory beekeeping practices also contribute significantly to transmission of diseases among colonies and dispersal of pathogens over wide geographical areas.
4. Treatment and prevention of diseases

In beekeeping industry, the integrated pest management (IPM) strategy is a commonly used term for prevention and control of diseases that includes: 1) genetic selection for resistance to disease (e.g. hygienic behavior); 2) management practices to reduce incidence and spread of diseases (e.g. frequent inspection, maintenance of strong/healthy colonies, prevention of robbing); 3) physical control (e.g. destruction of infected equipment/colonies, segregation of infected colonies, “shaking” method for control of brood disease, regular replacement of equipment/frames, interruption of parasite cycles, screened bottom boards for Varroa management etc.); 4) chemical control (e.g. chemical therapy of infected colonies and disinfection of contaminated equipment).

4.1. Chemical treatments

Chemical therapy with synthetic or natural chemicals is used in the Canadian beekeeping industry against mites (Varroa and tracheal mites), fungi (Nosema apis and N. ceranae) and bacteria (Paenibacillus larvae -> American foulbrood, and Melissococcus plutonius -> European foulbrood). Miticides and antimicrobials are used as both therapy and metaphylaxis depending on disease conditions, season of production and management practices. Unfortunately, resistance to antimicrobial and antiparasitic synthetic drugs has become a big concern for beekeeping industry.

4.2. Administration of therapy

Two major routes are used to administer treatment to honey bees: 1) administration in feed (antibacterial and antifungal medication) and 2) external contact administration (direct contact between external surfaces of bees and therapeutic chemicals impregnated in plastic strips, dissolved in solution or vaporized in hive).

5. Major Bacterial Diseases of Honey Bees in Canada

Short summary of those diseases for which chemical therapy is approved in Canada.

5.1. American foulbrood [7, 8]

American foulbrood is a devastating, contagious brood disease that develops rapidly, kills the colony and spreads to other colonies by robbing, drifting bees and anthropogenic modes.

Etiology: Paenibacillus larvae is a Gram-positive, spore forming, rod-shaped bacterium. Spores survive in contaminated equipment for decades. Approximately 2.5 billion spores are produced in each infected larva [8].

Pathogenesis: Larvae (up to 2-day old) ingest spores which germinate and proliferate in the intestine and subsequently spread throughout the body causing fatal septicemia.

Gross pathology: Brood frames have spotty brood pattern (shotgun brood); punctuated and sunken capping of brood cells; color of dead larvae changes from dull white to brown at which stage ‘ropiness’ test* is positive; and desiccated, dead larvae which form dark brown, brittle scales firmly adhered to the ‘ventral lateral’ wall of the brood cell (scale cannot be removed without destroying the cell wall). In advanced stages of disease there may be a strong decaying odor when the colony is opened, hence the name of the disease, ‘foulbrood’.

*’Ropiness’ test – The large number of vegetative P. larvae bacteria within macerated dead larvae will generate a typical glue-like consistency that can be detected by the ‘ropiness’ test. A dead larva is macerated with a matchstick within a cell and then slowly withdrawn. If the macerated tissue can be drawn out and stretched more than 2 cm, it is indicative of AFB infection.

Diagnosis: Gross pathology, especially a postive ‘ropiness’ test and the presence of scales, are highly
characteristic, or could be considered even pathognomonic, for AFB. Nevertheless, submission of samples of affected brood (including scales, if present) is recommended for confirmation of diagnosis by bacterial culture and/or PCR.

**Therapeutic treatment:** treatment with antibiotics of clinically affected colonies is not recommended, and in some jurisdictions, prohibited (contact provincial apiculture specialist for more information). The safest approach is to burn the entire colony and contaminated equipment. Alternatively, if infection rate is low, contaminated equipment could be irradiated, and in some jurisdictions, adult bees may be salvaged by the “shook-swarm method”*

* The “shook-swarm method” is used to salvage adult bees from colonies affected by brood disease (e.g. EFB and AFB). Adult bees with the queen are transferred/shaken into a screened box and kept in a cool place for a several ours to allow time for consumption and digestion of contaminated honey present in the gastrointestinal tract. These adult bees are subsequently transferred to a hive with new frames/foundation. This artificial method of separating of adult bees from infected brood reduces substantially the number of spores within a newly established colony, terminating, but not eradicating the disease [9]. Concurrent antibiotic therapy of the newly established colony will enhance efficacy of disease termination.

**Metaphylaxis:** In certain countries, antibiotics are prohibited in the beekeeping industry. In Canada and the USA, metaphylactic use of oxytetracycline (Oxytet-25, Oxsol 62.5, Foul Brood Mix) and tylosin tartrate (Tylan Soluble) against AFB is permitted and used regularly by many commercial and hobby beekeepers. The label instructions for Oxytet-25 are as follows:“Thoroughly mix 454 g of OXYTET-25 with 3.5 kg of powdered sugar. Apply 32 g of medicated mix per colony on the outer parts or ends of the frames 3 times at 4 to 5 day intervals in the fall and in the spring at least 4 weeks before the main honey flow.” Administration of oxytetracycline in syrup is also possible but it is not practiced as commonly. Tylosin is recommended only in beekeeping operations in which *Paenibacillus larvae* developed resistance to oxytetracycline. Potential residues in honey for tylosin are much higher when administered during the spring than for oxytetracycline.

**Integrated Pest Management (IPM):** Strategies for AFB management include re-queening with hygienic genetics; routine renewal of comb in the brood chamber (20-30% per year) to minimize contamination; frequent inspection to identify early stages of disease; prevention of spread of disease by robbing, contaminated equipment or feed; destruction of infected colonies and equipment; irradiation of equipment to destroy both vegetative stages and spores.

5.2. European foulbrood [8]

European foulbrood is an often self-limiting brood disease that is a consequence of reduced/suboptimal larval feeding due to an insufficient number of nursing bees to care for rapidly increasing numbers of larvae. A deficiency of brood care and feeding is most likely to develop during vigorous spring build-up of colonies in temperate climates (usually during the first major nectar/pollen harvest) [8, 10].

**Etiology:** *Melissococcus plutonius*, a Gram-positive coccus, is the main causative agent of EFB. However, it is often isolated with other bacteria (e.g. *Paenibacillus alvei, Brevibacillus laterosporus, Enterococcus faecalis* etc.) that may be secondary pathogens or saprophytes that may contribute to the typical sour odor of the infected colony as well as to “pseudoropiness” of affected brood (see above ‘ropiness’ test) [8, 10].

**Pathogenesis:** Larvae (less than 3-day-old) ingest food contaminated with *M. plutonius* bacteria which proliferate in the intestinal tract, competing with the larva for nutrients. During certain stages of colony expansion, the nursing bee population is insufficient to feed the expanding larval population, which, if infected with *M. plutonius*, will die due to starvation. Once the deficiency in nursing bees and larval nutrition is corrected, the symptoms of EFB will disappear [8, 10].

**Gross pathology:** Brood frames contain spotty brood pattern (shotgun brood); color of dead larvae
changes from dull white to brown at which stage tracheal network becomes visible; macerated dead larvae exhibiting ‘pseudoropiness’, but consistency of macerated larvae is granular and not as stretchable (less than 2 cm) as in AFB; and desiccated dead larvae which form dark brown, C-shaped, rubbery scales that are loosely attached to the bottom of brood cells. The presence of a ‘sour’ odor depends on the presence and composition of additional saprophytic bacteria [8, 10].

Diagnosis: Gross pathology could be used to distinguish EFB from AFB. Nevertheless, submission of samples of affected larvae is recommended for confirmation of diagnosis by bacterial culture and/or PCR. Submission of larvae affected at early stages (live larvae) will facilitate diagnosis because at early stages of infection, saprophytic bacteria are not as prevalent, and overgrowth by secondary bacteria in culture will be reduced [10].

Therapeutic treatment: Mild cases of EFB disappear once nectar follow becomes steady and/or nursing bee population is increased. Severe cases of disease can have a considerable impact on honey production due slow spring build-up of colonies and subsequent suboptimal population of foragers during the main honey flow. Heavily infected colonies (more than 50% brood affected) should be destroyed together with equipment. For low or moderate infections, therapy with oxytetracycline can be implemented (as described above for AFB) as long as an appropriate withdrawal period is observed. Nevertheless, the disease will usually recur the following year, therefore it is advised to use additional IPM strategies for prevention (e.g. shook-swarm method, re-queen, etc) [8, 10].

Beekeeping operations that use metaphylaxis against AFB are also protected against EFB in most instances.

Integrated Pest Management (IPM): Strategies for EFB control include re-queening with hygienic genetics, routine renewal of comb in brood chamber (20-30% per year) to minimize contamination, and the ‘shook-swarm method’ for colonies with low to moderate infections [8, 10].

Nota bene: These are the only two diseases of honey bees in Canada for which beekeepers will require a veterinary prescription to obtain antibiotics, because both tetracycline and tylosin are categorized by the Health Canada as medically important antimicrobials (MIA).

6. References:
5. Amiri, E., M.D. Meixner, and P. Kryger, Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. Scientific Reports, 2016. 6.
Veterinarians are animal advocates who have an obligation to respond appropriately and sensitively to the needs of animals and humans when health and welfare are at-risk. When presented with animal health challenges, veterinarians graduate with a solid foundation in how to work through a presenting complaint, gather information through a history and physical exam, create a problem list, a diagnostic and treatment plan, a plan for long term care, and patient follow up. Where animal welfare is concerned, however, many veterinarians identify feeling ill-prepared to identify and clearly articulate the nature of the concern and respond appropriately. Often it is clear that gaps in animal care result mainly from gaps in client education, or gaps in the personal wellness of the clients themselves. Cases in which there are human health and welfare challenges co-occurring with animal care issues can be particularly perplexing.

The concept of farm stress is understood as a response to financial pressure, poor psychological work environments, and vulnerability to changes in weather, interest rates, debt load, work demands and competing work/home responsibilities. Moreover, rural communities and families are culturally inclined to pull together and keep familial concerns ‘quiet’ managing crises on their own. In the short term, this collective approach is a strength allowing the opportunity to manage the practical side of farming challenges. However, this independent nature also has the potential to create vulnerabilities. As a result, the agricultural sector is at considerably higher risk for incidence of psychological injury, lack of treatment, and delaying treatment because of the perceived stigma around receiving support. When studied, farmers report increased levels of anxiety, depression, poor coping strategies and in some cases suicide. Farmers also identify seeking advice and support from those individuals known to them (eg. veterinarians) before reaching out to formal resources.

The complex nature of these cases can become a source of stress, empathy fatigue, and burnout in veterinarians and their teams. Veterinary professionals often feel as though they have to be ‘everything to everyone’ and many find themselves acting as the counsellor for clients as well as the advocate and doctor for patients. Not only is this exhausting mentally and emotionally, it is actually inappropriate and unethical. As a profession there is need to stay within scope of practice, appropriately utilizing existing networks of aligned paraprofessionals in the community. These allies in multifaceted family veterinary care are equipped and trained to support the health and wellbeing of clients while veterinary teams focus on the needs of patients.

Advanced veterinary communication skills are essential to the appropriate navigation of complex cases involving human and animal health and welfare challenges, and many veterinarians lack training in this area. Veterinary teams also need education around locating and connecting with complimentary resources, and appropriately communicating these human referrals to clients. Developing these skills ensure proper support for clients, allowing veterinary teams to focus on patient care within an appropriate scope. By examining relevant core and ancillary skills, we can better prepare to manage these situations while building rapport with clients, acting as advocates for patients, and maintaining an ethic of care and compassion in the veterinary-client-patient relationship.
Core Skills and Ancillary Supports:

Open Ended Questions:

Stems:
Who; what; where; when; why; how; please tell me; explain; describe

Examples:
What can you tell me about your winter feed plans?
What helped you decide to make that feeding choice for your horses?
Describe for me what you think is making everybody sick?
How do you keep your cows stay out of your grain storage?

Ancillary supports:

Prefacing: giving context before you ask an open ended question; maximizes the value of the answer
Example: It’s good for me to know what challenges you have around medication so that we can improve and optimize herd health. With that in mind, tell me about the way you keep track of your treatments?

Chunks and Checks: giving information in manageable chunks and then checking in with client to gauge understanding

Signposting: like a road-sign, allows clients to be aware of what is coming next and how things will unfold during the visit; provides context and reasonable expectations

Ask-tell-ask: A tool for explaining new concepts that involves first gathering client’s perspective as well as their level and nature of understanding of the situation, diagnosis, etc. you are about to discuss. Then you can explain the new information using terminology and details that are informed by their unique situation and perspective.

Non-verbal Communication:

Background: Roughly 80% of communication is non-verbal, so this is an essential element to pay attention to in developing elevated communication skills. This is particularly important when difficult decisions (euthanasia, costly procedures, welfare discussions) are involved. Nonverbal sensitivity improves client satisfaction with their veterinarian over and above other elements of practice, so this might be the most important element of communication for us to pay attention to.

Not only is it important to pay attention to non-verbal signals in ourselves, but also in our clients. This form of communication can tell us a lot about how a client is reacting to an interaction and may alert us to problems before anything verbal will. We can then alter our communication (both verbal and non-verbal) in response to these cues to improve the outcome.

Important Elements:

Kinesics – facial expressions, body tension, gestures, touch, body position and movements

Proxemics – how space is shaped between client, animal and vet
- Vertical height
- Interpersonal distance
- Angles of facing
- Physical barriers
Paralanguage – non-word phenomena (pause, pitch, rate, intonation, volume, emphasis) – the background of language

Autonomic shifts – don’t have control over these; things like facial flushing or blanching, tearing, sweating, breathing rate, etc – happen when people are having strong feelings

Examples:

Shaping space – sets the stage in communicating to the client our views on the relationship. Pay attention to the way the space is arranged – should be conducive to collaborative non-verbal interactions (sitting beside or at an angle to the client; removing physical barriers; allowing both client and veterinarian to sit/face each other)

Developing non-verbal rapport – matching (moving as client moves) and leading (use of interpersonal synchrony that has been set up by matching) – these concepts can help alter the emotions or tension in the room in positive or negative ways so if we know about them, we can use them to our benefit.

Reflective Listening:

Background: Reflective listening can take the form of summarizing, paraphrasing, and hypothesizing to review shared info. The client hears their story as understood by you. This allows clients to add further information where necessary, clarify things, and correct misconceptions. Importantly, it also allows client to feel their perspective is recognized and valued, emphasizing that they are being heard.

Understanding perspective is fundamental to the ‘art’ of veterinary medicine. If we don’t invest in this part, we miss out on opportunities for relationship building and important client education that otherwise may not happen. Without seeking to understand our client’s perspectives on things, we can completely misinterpret everything they tell us or lead the consultation in a completely inappropriate direction. Reflective listening is an important partner to empathy, which is one of the skills we can rely on most heavily when addressing difficult conversations with clients whose own health and wellbeing might be challenged.

Stems:

- I hear you saying that...
- So if I get all this correctly...
- So the way things went was...

Empathy:

Background: Empathy is a way of suggesting an appreciation for what an experience may be like for another person. In using empathy, we express active concern for and curiosity about the emotions, values and experiences of others. Empathy is different from sympathy; where empathy drives connection, sympathy drives disconnection. Empathy involves identifying what another person might be feeling, and connecting with something in ourselves that knows the same feeling.

Methods of using empathy:

- Non-judgmental response – ‘this is a tough decision call and I’m not sure that there is a right or wrong answer’
- Normalization – ‘it makes sense that you’d struggle with this decision’
- Appropriate disclosure – ‘I’ve been through something similar and found these decisions really hard to make” **be careful about your relationship with the client here, as this dictates whether these statements seem appropriate or inappropriate; also be careful to only disclose details about experiences with which you already feel resolved – do not use the client’s experience as a therapy session for you**
- LISTENING – silence is a wonderful tool for empathy – giving clients space to think and figure out how they feel – learn to be comfortable with silence and use it to your advantage! Silence can also be a great tool to encourage a response.
All of these communication skills, when used together, allow us to connect with our clients even in challenging situations so that we can maintain de-escalated interactions and a patient and client-centered care approach.

**Human Factors and Connecting with Allied Supports:**
There are multiple human mental and physical health issues that, when co-occurring in cases of disrupted animal health and welfare, can create challenges for the veterinary team. Being able to identify some of the common challenges that can be present for our clients is useful not only in developing empathy for them, but also in having productive discussions with allied professionals on the human health side.

**Common Human Health Issues:**
- Mental/Physical health challenges
- Substance misuse/abuse
- Poverty
- Social exclusion

When any of the above challenges are presumed or identified in our interactions with our clients, it can be helpful to know ‘who to call’ when the client needs more support. For further training in this area, veterinarians should consider Mental Health First Aid©, a course designed to provide individuals with the ability to support a person living with a mental health crisis or problem. Below you will find links to support services province-wide for direction on client referral.

### Opening Statements for Vets Offering Supports

<table>
<thead>
<tr>
<th><strong>Connect</strong></th>
<th>“This situation has been just (awful, horrible, shocking, terrifying)”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empathize</strong></td>
<td>“It makes sense you’re feeling the way you are, given everything that has happened”</td>
</tr>
<tr>
<td><strong>Elicit</strong></td>
<td>“What changes have you noticed about yourself since this has happened?”</td>
</tr>
<tr>
<td><strong>Reflect</strong></td>
<td>“Sounds like you’re not sure how to manage all of this”</td>
</tr>
<tr>
<td><strong>Normalize</strong></td>
<td>“Seems to me that just about anybody who’s had to go through this might need a hand”</td>
</tr>
<tr>
<td><strong>Offer</strong></td>
<td>“How about I leave you a list of people you could contact if you needed someone to talk to, just in case you ever wanted it?”</td>
</tr>
</tbody>
</table>

**General Inquiries**
- Healthline (province-wide)-811 -or-
  - [https://www.saskhealthauthority.ca/Services-Locations/Pages/Home.aspx](https://www.saskhealthauthority.ca/Services-Locations/Pages/Home.aspx)

**Crisis Lines**
- Emergency Services-911
- Healthline- 811
- Saskatoon Crisis Intervention Service-306-933-6200
- Southwest Crisis Service (Swift Current) -306-778-3386
- West Central Crisis & Family Support (Kindersley)-306-463-6655
- North east Crisis Intervention Centre (Melfort) -306-752-9455
- Hudson Bay & District Crisis Centre-306-865-3064
- Prince Albert Mobile Crisis Unit-306-763-8181
- Regina Mobile Crisis Unit-306-764-1011

**Support for Veterinarians**
- Professional Psychologists & Counsellors (PPC) -306-664-0000
  (Province-wide support provided by the SVMA-More detailed information available under Resources & Information “Member Wellbeing” at www.svma.sk.ca)
- For more information or to enhance your skills consider Mental Health First Aid©
References:


Rhabdomyolysis in horses can be sporadic or recurring, with the latter being associated with a number of underlying disease mechanisms.

**Known diseases presenting as recurring rhabdomyolysis**

**Recurrent exertional rhabdomyolysis (RER):** This form of rhabdomyolysis most commonly occurs in Thoroughbreds (about 5% of Thoroughbreds affected in the USA) and is likely inherited as an autosomal dominant trait. In its expression, the condition is thought to be multifactorial, influenced by the fitness level of the horse, as well as diet, age, gender, temperament, exercise schedule and the presence of any lameness.

**Type 1 Polysaccharide Storage Myopathy (PSSM):** Horses suffering from PSSM have recurring episodes of rhabdomyolysis, usually within the first 30 minutes of exercise. Type 1 PSSM is caused by a genetic mutation in the glycogen synthase 1 gene, inherited as an autosomal dominant trait.

**Type 2 Polysaccharide Storage Myopathy (PSSM):** All other forms of PSSM, i.e. negative for PSSM type 1 on genetic testing, are referred to as PSSM type 2 and are characterized by abnormal staining for muscle glycogen in histological assessment of muscle biopsies.

**Equine Myofibrillar Myopathy:** This newly recognized disease was initially identified in Arabians and has since then also been described in Warmbloods. It receives its name from the physical changes identified in muscle cells involving disruption of the orderly alignment of contractile proteins called myofibrils. A common clinical sign, especially in Arabians competing in Endurance races, is intermittent tying up. The disease may also present as poor performance.

**Glycogen Branching Enzyme Deficiency (GBED):** This disease of Quarter Horses and related breeds is caused by a defect in the glycogen branching enzyme gene. Clinically affected animals are homozygous with heterozygous animals being the carriers. The disorder is fatal and treatment should not be attempted.

**Seasonal Pasture Myopathy (atypical myopathy):** This muscle disease of horses is fatal in over 90% of cases. Clinical signs include stiffness, difficulty walking or standing, voiding dark urine and eventually rapid breathing and recumbency followed by death.

**Diagnostic testing**

**Recurrent exertional rhabdomyolysis (RER):** Diagnosis is based on history, documentation of elevated muscle enzymes and histological examination of muscle biopsies. Muscle biopsy shows rhabdomyolysis, regeneration, an increased number of central nuclei, and absence of abnormal polysaccharide.

**Type 1 Polysaccharide Storage Myopathy (PSSM):** A genetic test for PSSM type 1 is available, performed at the University of Minnesota Veterinary Diagnostic Laboratory.
Type 2 Polysaccharide Storage Myopathy (PSSM): PSSM type 2 is diagnosed based on history of recurrent rhabdomyolysis, often associated with exercise, clinical signs of tying up, elevated muscle enzymes and abnormal staining for muscle glycogen in histological assessment of muscle biopsies.

Equine Myofibrillar Myopathy: Diagnosis is based on muscle biopsy. Histological features of muscle biopsies include internalized myonuclei, mild to moderate myofiber atrophy, aggregates of the cytoskeletal protein desmin and myofibrillar disarray. Genetic testing is currently not available.

Glycogen Branching Enzyme Deficiency (GBED): A genetic test for this condition is available and should be utilized to identify affected animals and carriers. The test is performed on mane or tail hair (with roots intact) at the University of California Davis and Vetgen, Inc. Muscle biopsy of affected animals will show complete lack of normal glycogen staining.

Seasonal Pasture Myopathy (atypical myopathy): This disease is most commonly seen in the fall and associated with a toxin in the seeds of the box elder tree (Acer negundo).

Nutritional management

Recurrent exertional rhabdomyolysis (RER): Treatment aims at reducing the frequency of clinical episodes as much as possible. Modifications to exercise routine and diet are essential to successful management of affected horses. If possible, trainers should modify the horses training schedule to minimize stress. Diet should provide adequate but not excessive amount of calories for the level of training. Calories should be provided as fat rather than carbohydrates.

Type 1 and 2 Polysaccharide Storage Myopathy (PSSM): Horses with PSSM can perform well in many cases, if managed appropriately. Routine daily exercise helps with the metabolism of glucose. Horses should be exercised regularly at a level appropriate for their level of fitness. Increases in exercise intensity should be made gradually. Depending on their caloric needs, horses should be fed a diet of only grass and alfalfa hay with provision of a vitamin and mineral supplement. If concentrate needs to be provided to meet caloric needs, dietary management includes provision of diets low in carbohydrates (less than 5% digestible energy as starch) and high in fat (greater than 12% digestible energy). Possible fat sources include corn or vegetable oils, linseed oil, or rice bran.

Equine Myofibrillar Myopathy: Management recommendations include enhancement of muscle strength through a consistent graded exercise program focused on strengthening the back and core muscles. Recommended dietary management includes provision of a balanced diet with an amino acid supplement given around the time of exercise.

References

1. https://cvm.msu.edu/research/faculty-research/valberg-laboratory/for-veterinarians
   last accessed on May 3, 2018
2. Reed, Bayly, Sellon, Equine Internal Medicine, 3rd ed.
How do you decide when drugs are needed - when drugs will change the course of disease? In the early phases of veterinary careers, the decision is usually expert-based: you were taught in a class or on a clinical rotation how to intervene. Expert-based medicine can lead to success in practice, but because our brains work in interesting but predictably inaccurate ways, we must be cautious about using expert opinion and observations of our own clinical cases to guide our decisions. Expert opinion and clinical impression are subject to significant bias. Bias is:

A systematic distortion, due to a design problem, an interfering factor, or a judgement, that can affect the conception, design, or conduct of a study, or the collection, analysis, interpretation, presentation, or discussion of outcome data, causing erroneous overestimation or underestimation of the probable size of an effect or association (J. Aronson, https://catalogofbias.org/2018/06/15/a-word-about-evidence-6-bias-a-proposed-definition/)

An exhaustive catalog of biases makes interesting reading (https://catalogofbias.org/), and it also provides some important characteristics of bias. Bias is often not intentional nor is it necessarily avoidable. The potential for bias must just be recognized, and the types of studies or evidence with higher potential for bias should be weighted less heavily in drug (or other health) decisions.

Because of bias and because our brains can work in inaccurate ways, we will be better served by being systematic in how we make therapeutic decisions because not all evidence is equal. This is typically portrayed as an evidence “pyramid,” but there are numerous ways to conceive of levels of evidence. The unifying factor is a hierarchy of evidence, with less biased evidence being weighted more heavily. Additional weighting factors are then subjectively added to account for factors such as external validity, i.e., how well does the evidence reflect your clinical setting.

Weighting of evidence

The most common studies used to support drug decision-making, in the order of lowest to highest likelihood of bias are:

- Systematic review of randomized controlled trials RCTs (with or without meta-analysis)
- Critically appraised topic that includes RCTs
  - These are like mini systematic reviews
- Large RCTs (>150 animals per group)
- Small RCTs (<150 animals per group)
- Cohort studies
  - Cohort studies may not be identified as such, but they follow a group of animals over time, and comparisons are made between the groups with different treatments
- Case series
- Narrative reviews
Opinion – written or oral; or one’s own clinical experience
Pharmacokinetic studies (more accurately, bias cannot be assessed)
In vitro studies (more accurately, bias cannot be assessed)

Within these study types, an estimate of how much difference the drug will make, or the “treatment effect,” can usually be estimated in systematic reviews (depending on the depth of data extraction), RCTs and cohort studies. Case series, narrative reviews, opinions, pharmacokinetic studies, and in vitro studies cannot be used to estimate treatment effect. In case series, the reason is the lack of a comparison group, and in the other two, treatment effects can’t be reported. While a treatment effect is tempting to extract from opinions (yours or an expert’s), opinion can be colored by a lack of a comparison group, confounding by indication bias, and loss to follow up, so it should be used cautiously to evaluate and compare treatment effects.

Treatment effect

The characteristic of evidence that determines its usefulness, in addition to its quality, is whether it provides an estimate of a treatment effect and what the size of the treatment effect is. As mentioned above, treatment effect is a quantification of the difference the drug made in the outcome. Here’s an example:

After arrival at a feedlot, when calves showed signs of respiratory disease, they were randomly assigned to receive Drug A or a placebo. The outcomes measured included mortality and need for retreatment. At the end of the study, 200 calves had been treated, 100 with Drug A and 100 with the placebo. In the Drug A-treated calves, 50% of calves had to be retreated, and 5 calves died. In the placebo-treated calves, 75% of calves had to be retreated, and 20 calves died. Two different treatment effects can be calculated:

The treatment effect of eliminating need to retreat was: 75% - 50% = 25%
The treatment effect of reducing mortality was: 20 – 5 = 15 calves

What do those mean? Drug A reduced the need to retreat by 25%, and reduced mortality by 15 calves. These seem like a straightforward calculations, but usually the inclination is to say that the treatment effect of reducing the need to retreat is the TOTAL amount of reduction, or 75%, when in fact, the drug only made a difference in 25% of animals. For mortality, Drug A reduced mortality by 15 calves, not 20, as one might be tempted to say.

An excellent tutorial on treatment effect (which can sometimes be quantified as NNT) is available: https://www.students4bestevidence.net/number-needed-treat/

Steps to evidence-based practice

How can you incorporate weighting of evidence and estimate of treatment effects into decision-making? A commonly used step-wise approach that provides a systematic way to gather those data to make a diagnostic, prognostic, or therapeutic decision is:

Step 1: Ask a clinical question using the PICO format
The PICO format includes specific descriptions of Patient, Intervention, Comparison, Outcome. For example, in recently arrived feedlot calves diagnosed with respiratory disease (P), will Drug A (I) decrease mortality and decrease need for re-treatment (O) compared to not treating (C).

Step 2: Search for evidence to answer the question
Information resources continue to expand, but peer-reviewed literature should be relied on for drug information. Free access to databases of literature is available, but should be considered carefully as to their true reach – for example, Google Scholar may provide results that differ from day to day, and access to full text of articles may also change from day to day. A relationship with a medical librarian or medical library will greatly enhance your ability to access evidence.

Step 3: Critically appraise the evidence

Critical appraisal is the essential difference between reading a journal article and evidence-based practice. Criteria for quality differ among different study types, but a thorough reading results in better appraisals than simply reading the abstract.

Step 4: Answer the clinical question with the appropriately weighted evidence.

The answer may directly lead to a clinical recommendation (a strong or a weak recommendation), but it may need to be added to other evidence, such as evidence on the probability of an adverse drug event, or evidence about the cost effectiveness of the intervention.

(Step 5: Evaluate the process)

An excellent tutorial which includes links to worksheets for appraising literature, is available at EBVM Learning (http://www.ebvmlearning.org/).

In addition, there are increasingly sources of evidence synthesis that do the steps for you. The Center for Evidence-based Veterinary Medicine at the University of Nottingham curates BestBET for Vets (http://bestbetsforvets.org/), a database of current best evidence to answer specific clinical question using the steps outlined above. Major domestic species are represented, and more are being added all the time. Another source of evidence synthesis is published in the open access journal Veterinary Evidence (https://www.veterinaryevidence.org/index.php/ve/index) as so-called Knowledge Summaries in each issue of the journal. Using these resources as well as becoming proficient in performing the steps can lead to improved outcomes for animals and is an ethical and professional approach to making medical decisions.

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Animal Health
Several factors highlight the need to use a One Health approach when using antimicrobial drugs in animals:

1. all antibiotic use leads to selection for resistance at some level
2. genes encoding for antibiotic resistance in animal pathogens have been identified in live animals and animal waste
3. genes encoding for antibiotic resistance in human pathogens have been demonstrated in live animals, animal waste, and animal food products

To preserve the effectiveness of antibiotics and to ensure their availability for use in animal health, veterinarians must implement antimicrobial stewardship in their practice of medicine. One definition for the veterinary profession comes from consensus among entities represented by the American Veterinary Medical Association that antimicrobial stewardship:

“...refers to the actions veterinarians take individually and as a profession to preserve the effectiveness and availability of antimicrobial drugs through conscientious oversight and responsible medical decision-making while safeguarding animal, public, and environmental health.”

This definition aligns with the American Association of Bovine Practitioners’ guidance:

“...the commitment to reducing the need for antimicrobial drugs by preventing infectious disease in cattle, and when antimicrobial drugs are needed, a commitment that antimicrobials are used appropriately to optimize health and minimize selection for antimicrobial resistance.”

According to the AVMA definition, the core principles of antimicrobial stewardship include: Commit to stewardship; Advocate for a system of care to prevent common diseases; Select and use antimicrobial drugs judiciously; Evaluate antimicrobial drug use practices; and Educate and build expertise.

Applying these principles to practice then is the hard work of antimicrobial stewardship, some examples of which are presented below.

1. Commit to stewardship

Could you appoint a primary staff person to implement stewardship practices? Can you include antimicrobial stewardship-related duties in position descriptions and performance evaluations for staff? Could you develop agreed-upon protocols for antimicrobial prescribing based on consensus in the practice and based on available guidelines?

2. Advocate for a system of care to prevent common diseases
Can you identify barriers preventing adoption of disease prevention strategies? Do you make infection prevention and control supplies readily available?

3. Select and use antimicrobial drug judiciously

Do you make reputable current antimicrobial resources easily accessible? Could you critically assess the need, selection, and duration of prophylactic antimicrobial drugs? Do you record indication for antimicrobial drug prescriptions in medical records and client communications?

4. Evaluate antimicrobial drug use practices

Could you evaluate prescribing practices compared to published guidelines to assess compliance? Do you have evaluate how often (percentage of cases) antimicrobial drugs are prescribed? Could you engage with veterinary diagnostic laboratories to provide facility or regional antibiograms?

5. Educate and build expertise

Could you provide appropriate documentation to your clients on stewardship? Do you provide and engage in CE for veterinary and technical staff to stay current with guidelines and stewardship practices?

These and other activities of antimicrobial stewardship are a necessary part of the global effort to combat antimicrobial resistance and preserve the effectiveness of antibiotics for future generations.

References – additional references available on request

Antimicrobial Stewardship Definition and Core Principles, https://www.avma.org/KB/Policies/Pages/Antimicrobial-Stewardship-Definition-and-Core-Principles.aspx, accessed June 29, 2018


Key elements for implementing antimicrobial stewardship plans in bovine veterinary practices working with beef and dairy operations, http://aabp.org/resources/AABP_Guidelines/AntimicrobialStewardship-7.27.17.pdf, accessed June 29, 2018


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Animal Health
Drug decision-making should be approached systematically and with iterative data gathering to support decisions. The process may have become rote for the experienced practitioner, so a regular thoughtful review of how decisions are made is prudent. Making decisions about which drug to use (and how) can be generalized to the following cycle:

1. Recognize a physiological alteration in a patient
2. Define a therapeutic goal to address the alteration
3. Consider available options and alternatives (what drugs might address the therapeutic goal, and what drugs are likely to change the outcome?)
   a. For each drug option:
      i. Likelihood of desired effect
      ii. Estimate of magnitude of desired/therapeutic effect
      iii. Likelihood of adverse effect
      iv. Estimate of magnitude of adverse effect
   b. Additional considerations that may rule in or out drug options
      i. Legal or regulatory issues that impact use
      ii. Client desires and expectations about outcomes
      iii. Ability to administer drug
      iv. Cost
4. Make choice, dispense/provide prescription and client/caregiver education
5. Follow up in short- and long-term to evaluate outcomes

The process is the same, regardless of the alteration in the patient. However, the evidence to support the decision may be more or less available. Several examples are provided below that also incorporate the framework for using evidence discussed in a previous presentation (“Critical appraisal of evidence about drugs”).

In the context of providing evidence, a few studies provide estimates of the likelihood of desired effect, such as number needed to treat, or NNT, which is a relatively easy way to characterize therapeutic effect: NNT is the reciprocal of the difference in the probability of an event in the treated vs the control group. For example, if 80% of treated animals respond to a particular drug and 20% of placebo-treated animals respond, the difference in the likelihood (probability) of response is estimated to be 60%, so the NNT would be 1.7, which would be rounded to 2. An estimate of 2 animals would need to be treated to see that treatment response in 1. Aside from NNT, there are other approaches to estimating treatment effect. All must be compared cautiously between studies, however, since exposures and outcomes may not be the same. And NNT is only useful when the outcome is binary or when it can be reduced to binary.

One of the more challenging elements of the decision-making cycle about which to locate evidence is the estimate of the magnitude of treatment effect. Studies often report that the difference between treated and untreated animals is significantly different, but what is different is often underemphasized. For example, it may be reported that statistically significantly more animals with foot rot may have reduced lesion score when treated with an antibiotic compared to those who were
not treated, but was the lesion score reduced by 1 on a scale of 1-5 or 4? The magnitude of response should be included in the mental calculation leading to a drug choice along with the likelihood of that response.

Respiratory disease in cattle

Given the importance of BRD in cattle production, there are a number of antimicrobial drugs approved for control or treatment. Multiple comparative and placebo-controlled studies have been published investigating the effectiveness and the magnitude of the therapeutic effect (although this is uncommonly a stated objective of a study). Based on the therapeutic goal of control/metaphylaxis or treatment, systematic reviews with and without meta-analyses are available to provide evidence for decisions about antibiotics.

Two systematic reviews have been published in recent years, each of which took a different approach to asking the question about estimating treatment effect. The first (DeDonder, 2015) calculated NNTs for negative-controlled trials. The outcome definition impacted the median NNT: median NNT was 2 for treatment success, 6 for preventing mortality, and 6 for preventing an acute case of BRD when an antimicrobial is used metaphylactically (for control of BRD in high risk animals). In the other systematic review (O’Connor, 2016), a method was used to compare drugs that have not been compared in head-to-head trials.

The decision to add anti-inflammatories or other ancillary treatment is less well studied, although a systematic review has been published (Francoz, 2012). Data so far generated suggest that ancillary therapy does not impact clinically relevant outcomes such as weight gain or pulmonary lesions.

Respiratory disease in small ruminants

This disease is presumed to have similar prevalence in sheep and goats as in cattle, but there are few clinical studies, particularly comparative studies and large studies. Large studies (and multiple studies) are needed in order to provide more precise estimates of a treatment effect of each antimicrobial drug. In vitro data are the current best evidence for comparing across drugs, and that is fraught with potential bias. In addition, the interpretive criteria used to make susceptibility determinations are not validated in sheep and goats. There are a handful of placebo controlled trials as well as drug approval data that may be helpful but few direct comparisons of drug in studies with external validity applicable to North America.

Contagious abortion in small ruminants

Due to the sporadic, seasonal, and regional nature of abortion caused by bacterial pathogens such as Campylobacter in sheep and goats, there are limited data to support antimicrobial choices. In the US, chlortetracycline has been approved to reduce the incidence of abortion, but the studies supporting the label are decades old and were not as robust as might be required today for drug approval. Published data may not be convincing as to the treatment effect of CTC. In addition, resistant isolates have been reported. Since few isolates are subjected to susceptibility testing unless failures are perceived, and since the disease is sporadic in nature, the true prevalence of resistance is unknown. Pharmacokinetic studies suggest that concentrations achievable with in-feed dosing of CTC may not actually be effective. Clinical impression of effectiveness seems to drive much of the current usage.

Foot rot in cattle

One pseudo-systematic review (Apley, 2015) evaluated placebo-controlled studies based on data from drug approval studies published as Freedom of Information summaries as well as one
comparative trial. The NNTs ranged from 1.3 to 3 for reduction in lameness and/or lesion score. The comparative study, oxytetracycline vs ceftiofur, did not find a significant difference between the two drugs. These data together support the weak conclusion that 1-3 animals would need to be treated to see a reduction in lameness in 1 animal, and that one antimicrobial does not appear to be superior.

**Perioperative prophylactic antibiotics**

The therapeutic goal is to decrease the likelihood of post-operative infections. Very few controlled studies have been performed in veterinary medicine to provide evidence that prophylactic antimicrobials are necessary, or when they are necessary, or which ones are most effective, or how many animals need to be treated to prevent one infection. Data from humans is typically extrapolated, and these data may be even less applicable to the large animal setting, particularly if procedures are performed on farm.

Although controlled studies are lacking for cattle and common surgical procedures, indiscriminate use of antimicrobials to “prevent infections” should be avoided. Attention to surgical site preparation, surgical time, and other infection prevention strategies are more likely to yield results.

**Analgesia for castration and dehorning**

Assessing analgesia after castration and dehorning is challenging, and no single outcome has been validated. Peak cortisol, pressure algometry, behavioral measures, and other parameters have been used to compare efficacy of various analgesics, and it is quite challenging to find studies that compare different drugs or combinations. Evidence suggests that NSAIDs can reduce pain post-procedure, but the magnitude of the reduction is hard to estimate.

**Selected references** – Additional available on request


This session was sponsored by [MERCK Animal Health](http://www.merck.com/animal-health).
All veterinarians know that sometimes things go wrong. Camelids are predisposed to the same complications as other animals. We will highlight our experience with a few of these complications.

Communication is important

Communication is perhaps the most important single aspect of veterinary medicine. When things go wrong, the importance of communication among the veterinary team and with the client goes up. Often because of embarrassment and pride it may be tempting to run and hide and not let other see mistakes and failures, however early communication often prevents a more difficult conversation later.

Incisional complications

With any surgical incision complications such as dehiscence, hemorrhage, infection, and gangrene are possible. To minimize these complications, proper surgical technique is important. Hemostasis, surgical asepsis and surgical technique are critical. Compared to cattle, camels have a thinner body wall therefore one may speculate that infections at the level of the skin are more likely to penetrate into the abdomen causing peritonitis or the body wall is more likely to dehisce leading to herniation or evisceration.

It is important to make an assessment of how extensive the incisional compromise. It is more concerning if holding layers are involved vs. merely skin. For abdominal incisions, making an assessment of the health of the peritoneum is also important. An ultrasound exam can be useful to view the peritoneal surface, assess any free abdominal fluid, abdominal fibrin, or visceral adhesions. Keep in mind if gas is present within the body wall or abdomen, ultrasonic visualization will be impaired. Abdominocentesis can also be a useful diagnostic tool to assess peritoneal involvement. If abdominocentesis is performed it should not be done through a compromised body wall because that could potentially inoculate the abdomen with organisms from the superficial tissues.

If only the most superficial layers are involved, debriding necrotic tissue and allowing the incision to heal by second intention is optimal. If deeper tissues (holding layer) are involved, the same principles apply, however abdominal support in the form of an abdominal bandage may be warranted. It is important to change the abdominal bandage often as the anaerobic environment created by the bandage promotes growth of anaerobic organisms. Exudate produced by the wound will accumulate in the bandage warranting frequent bandage changes. Topical antiseptics can be useful to mitigate the growth of superficial organisms potentially preventing the spread to adjacent, healthy tissues.

Surgical resection of a compromised area en bloc and closure may be indicated. This is the most radical form of debridement where all of the necrotic tissue is debrided back to healthy tissue and closure is performed. The advantage of radical debridement is that we expedite the debridement phase, and we ensure removal of all diseased and damaged tissues. The disadvantage is that some healthy tissue will also be removed. Tetanus prophylaxis is indicated in the case of wounds that have necrotic tissue, as the anaerobic environment favors the growth of clostridial organisms.
Orthopedic Complications

The most common orthopedic injuries seen at the author’s practice are fractures and luxations. Orthopedic injuries can be frustrating and scary for owners and veterinarians. However one should bear in mind that camelids make good orthopedic patients. They usually tolerate external coaptation, and they have a smaller adult body weight compared to cattle and horses. However complications can arise. Some of the more common orthopedic complications include inability to reduce the fracture or luxation, arthritis, fixation failure, and infection at the fracture site.

Transfixation pin casting (TPC) is a common method of fracture fixation in camelids. TPC uses principles of external skeletal fixation with added rigidity provided by a cast. Transcortical pins are placed through the bone proximal to the fracture followed by casting which attaches to the transfixation pins. The cast replaces the sidebars of a conventional external fixator. Although the transfixation pins are necessary to better neutralize compressive, distraction, and rotational forces, they create weak spots and stress risers in the bones. Pin site fractures can develop.

To reduce the likelihood of developing a pin track fracture, the cast should not end immediately above the most proximal transfixation pins. Doing so will result in the concentration of forces at the location of the pins, a weak spot in the bone. To increase security, the cast should be extended above the joint proximal to the fracture.

Options for animals who develop pin tract fractures include use of another transfixation pin cast, true external skeletal fixation, and internal fixation to provide stability to the fracture. Although radiographs may not be necessary to make a diagnosis of a fracture, they can be very useful to assess the integrity of the bone and check for small fissure lines that may weaken the bone. If fissures are present, care should be taken to not place transcortical pins through fissures if at all possible. Doing so may result in fracturing of the bone.

Other problems including cast sores, tendon contracture or hyperextension, and decreased joint mobility can result if patients are left in a cast too long. Full limb casts accentuate these problems. The longer the patient wears the cast, the greater the likelihood of experiencing these complications.

Nonunion and delayed union are complications that can result if fractures do not heal properly. These can result from inadequate stability provided by the fracture fixation, infection at the fracture site, and decreased inherent bone healing ability. Inadequate stability can be caused by poor surgical technique or surgical error during fracture fixation. An undersized apparatus for the patient size can also lead to fracture instability. Infection at the fracture site can be due to an open fracture or contamination that is introduced during the surgical procedure. Older patients, patients with systemic disease, and those whose traumatic wounds compromised blood flow to the area of the fracture have decreased inherent ability to form new bone.

Animals with nonunion usually require surgery. Animals with systemic disease should be treated appropriately. Ensuring adequate levels of calcium, phosphorus, and vitamin D are necessary for proper bone healing. Rickets decreases the body’s ability to form bone in growing animals. Dark colored animals in winter are predisposed to rickets. Adequate colostrum in neonates is important to keep patients from becoming septic or to prevent local infections from developing at the fracture site. Local therapy such as injecting synthetic growth factors, bone grafting, or gene therapy may be other options to increase bone healing. Debriding the ends of the non-healing bone to expose “fresh” bone is also important to stimulate fracture healing.

Unless there is an obvious physiologic issue that is preventing new bone from forming (i.e. rickets or infection at the fracture site), providing adequate stability at the fracture site is likely the most important factor that the veterinarian can change to effect fracture healing. Internal fixation is more biomechanically stable and provides a more rigid, robust fixation compared to external fixation or
external coaptation. A disadvantage to internal fixation is increased disruption of the normal “biology” of fracture healing and an increased risk of introducing contaminants into the fracture site.

**Obstetrical Complications**

Management of dystocias in camelids often result in other obstetrical complications, most commonly vaginal and uterine lacerations. Due to the small size and friability of the caudal reproductive tract, tearing of the uterus or vaginal walls can occur with extensive fetal manipulation. Small hands are advantageous. Lubrication can decrease the amount of force necessary for fetal extraction and can decrease the incidence of trauma. A working knowledge of anatomy and attention to obstetrical technique are important.

Awareness that a tear occurred is important so that timely intervention can be provided. Vaginal tears can be challenging to close surgically. With the exception of lacerations at the most caudal aspect of the vagina, the tears are difficult to visualize, and any suturing needs to be done blindly. The vagina is friable following parturition, and its suturing-holding ability is decreased. Partial-thickness lacerations or full thickness lacerations that do not penetrate into the abdominal cavity, usually do not require surgical closure. Even patients with small communications into the abdominal cavity may do well with conservative management alone.

Antibiotics with adequate spectrum and good penetration into the abdomen to protect against peritonitis should be selected. Vaginal tissue has a well endowed blood supply, and hemorrhage may be a concern with even superficial lacerations. Pressure applied temporarily to the vaginal wall with a tampon is usually sufficient to limit bleeding. A camelid tampon can be made with 2 to 3 inch stockinet packed with cotton cast padding. The tampon can be sprayed with 10 cc of 1:1000 epinephrine. The epinephrine will cause vasoconstriction and limit hemorrhage.

Uterine lacerations are more problematic and should be addressed as soon as possible. Gaining access to the uterus is difficult to achieve through a vaginal approach. Uterine lacerations may be accessed via an abdominal approach. Minimally invasive techniques using laparoscopy may be possible depending on the facilities available and surgical expertise. Prolapsing the uterus under general anesthesia may provide the surgeon adequate exposure to visualize and suture the laceration.

Things will go wrong eventually. Don’t panic! Overall goals may need to be adjusted to work towards a realistic outcome. It is important to realize that most problems are fixable. If needed seek help and guidance. Most importantly, try to figure out what went wrong to prevent the same mistake in the future, and to make it a learning experience.
Pigs are gaining popularity as common pets in the US. As such, veterinary procedures and routine care such as general anesthesia, spays, and castrations are being more commonly performed in pigs by practitioners. Anesthetic procedures are often needed to enable veterinary surgeons to safely and humanely perform even simple and routine procedures because of the refractory nature of these patients to restraint. However, these animals are not merely a different breed of dogs and there are special anatomic and physiologic differences that can make these procedures challenging.

Anesthesia in Pigs

Restraint in pigs can be challenging and often some form of chemical restraint is necessary for even non-invasive procedures. However, there are unique anesthetic concerns of which the veterinarian needs to be aware. Endotracheal intubation and IV catheterization is more difficult in pigs when compared to dogs. Obesity which is common in pet pigs increases the difficulty in performing these as well as many surgical procedures and can also affect the accuracy dosing anesthetic drugs.

Injectable anesthesia may be necessary if equipment for inhalant anesthesia is not available. A common injectable formulation that works well in pigs is a cocktail using Telazol® (tiletamine HCl/zolazepam HCl), ketamine, and xylazine (TKX). A 500 mg bottle of Telazol® powder is reconstituted with 2.5 cc of ketamine (100 mg/ml) and 2.5 cc of large animal xylazine (100 mg/ml). The resulting solution will contain 100 mg/ml Telazol®, 50 mg/ml ketamine and 50 mg/ml xylazine. The drug combination is administered intramuscularly at a dose of 0.5-1 ml / 50 lbs of body weight. Patients often become recumbent within 5 minutes of injection. A surgical plane of anesthesia will usually last around 20-30 minutes. The main disadvantage of this cocktail is that recovery is generally longer than that with inhalant anesthesia. Patients can take in excess of 90 minutes to recover, making it less desirable for quick and minimally invasive procedures.

Obesity can also affect the metabolism of certain anesthetic drugs. This is especially true with injectable anesthetics. Ideally drugs are dosed according to lean body weight, but this can be a challenging estimation in obese pigs. Inhalant anesthesia is less sensitive to inaccurate weight estimation because it is “dosed to effect.”

Inhalant anesthesia works well in pigs. Many veterinarians have a concern with using inhalant anesthesia in pigs due to the risk of malignant hyperthermia. However, this condition is very uncommon in pet pigs, as it usually occurs in heavily muscled production pigs with a specific gene. Selective breeding has virtually eliminated malignant hyperthermia even in commercial swine operations today. The introduction of newer inhalants that have replaced halothane have also decreased the incidence of this condition.
Endotracheal intubation can be challenging because pig's mouths do not open wide limiting access to the pharyngeal region for visualization of the larynx for intubation. Obesity, a common problem in pet pigs, causes redundant pharyngeal tissue to encroach into the airway which can further inhibit visualization. Pigs also have a pharyngeal diverticulum that can entrap the tube and prevent it from passing into the trachea. The diverticulum is caudal to the opening of the arytenoid so even though the tube is visualized passing through the arytenoid opening, it can still become entrapped in the laryngeal diverticulum.

Fortunately, pigs make good candidates for mask anesthesia. Pigs don't salivate as much, and they rarely vomit or regurgitate while under anesthesia compared to ruminants. They will typically struggle initially when the mask is introduced but usually will calm quickly after they are breathing the gas for a few minutes. Isoflurane or sevofluane works well when delivered via mask induction. Short, routine, and non-invasive procedures such as examinations, foot trims, castrations, etc. are indications for a “light” plane of general anesthesia delivered via face mask. After discontinuation of the gas, the patient typically recovers within 5-10 minutes.

IV access is another challenge in porcine veterinary medicine. Although we typically prefer jugular catheterization for most of our farm animal species, the jugular vein in pigs is well protected by thick cervical musculature. This places the jugular vein deep within the cervical region, and it is not conducive for catheterization. Because of their “short & stubby” legs, cephalic or saphenous vein catheterization is usually not feasible as it is in most canine or feline patients. Fat deposits in the tissues around these vessels make identification of the vessels difficult in obese patients. Ear veins are most commonly used for IV access. There are challenges with ear vein catheterization; they can be difficult to place if the animal is not already under anesthesia due to inability to adequately restrain the head. The ear vein doesn’t permit a large gauge catheter, and therefore is less reliable for administration of large volumes or viscous fluids.

Ovariectomy / Ovariotomy

Spaying pigs is one of the most common surgical procedures that is performed on female pigs. Preventing unwanted pregnancies is the major indication for spaying female pigs. Decreasing aggression or reducing undesired behaviors associated with estrus cycles such as aggression and inappropriate urination are other indications. Pigs that are left intact are at risk of developing pyometra as well as various types of ovarian and uterine neoplasia.

In theory, the surgical anatomy is similar to that in their canine counterparts, however, distinct features make ovariohysterectomy in pigs more challenging. As in the dog, the ovary and uterus are supported by the broad ligament. The uterus is more tortuous than in other domestic animals and can be mistaken for loops of intestine. Pigs have a robust blood supply to the uterus. There is an ovarian artery that provides the major blood supply to the ovary and then anastomoses with the uterine vasculature. The uterus acquires its major blood supply from the uterine artery but also from the caudal uterine branch of the vaginal artery. The ovarian artery arises directly from the caudal aorta while the uterine and the vaginal artery arise from the internal iliac a. Although the porcine ovarian and uterine structures have a very robust blood supply, the thick broad ligament can obscure the vessels which can make identification of these structures
challenging. Obese animals pose even a greater challenge as fat within the broad ligament can further obscure these vessels.

Adequate ligations of these vessels are critical or excessive hemorrhage will result. Prior to ligation and removal of the ovaries and uterus, the vasculature should be studied, to make the most efficient use of ligatures and to identify proper location for transection. Do not blindly fenestrate with blunt digital dissection of the broad ligament as this tends to result in excessive hemorrhage. Once a truly avascular area within the broad ligament has been identified, a small fenestration can be made in this area to facilitate suture passage. When looking for vessels within the broad ligament, be aware that pulling or squeezing on the ligament can cause the vessels to blanch making them easy to miss.

Ligations with suture material that has good knot hold characteristics is very important to prevent the suture from becoming insecure. Vessel sealing devices such as the Ligasure can decrease hemorrhage and decrease operative times. These devices are especially helpful when spaying an overweight animal where vessels are difficult to identify. However, these devices are expensive and may only be options at tertiary veterinary facilities.

The fatty broad ligament is friable and excessive tension on the uterus and broad ligament commonly results in tearing and brisk hemorrhage. This is more common after fenestrations have been made or part of the ligament has already been ligated. A prior cut in the ligament creates a weak spot and tearing originating at the cut and extending though vasculature can occur.

Obesity, a common problem in pet pigs, adds challenges throughout the surgical procedure. First, obesity can make anesthesia more challenging as it can interfere with catheterization and intubation (see previous section on anesthesia in pet pigs). Although opening and closing of the abdomen is similar in theory to other species, an extensive fat layer will make the linea deep and difficult to identify. Following opening of the linea, additional fat is encountered between the body wall fascia and the peritoneal surface. The uterus is difficult to exteriorize in these patients, and blood vessels within the broad ligament can be challenging to ligate. Excessive fat accumulation within the broad ligament also increases the friability leading to tearing and hemorrhage which is exacerbated because of the excess tension required to exteriorize the uterus from the deep incision.

Ovariectomy is a viable alternative to ovariohysterectomy to eliminate the possibility of unwanted pregnancy and estrous cycles. Ovariectomy is a technically simpler procedure that has been shown to be successful in pet pigs. This is obviously not an option for patients that have preexisting uterine pathology, but ovariectomy at a young age is thought to decrease the incidence of uterine pathology later in life due to decreases in hormonal fluctuations.

Closure of the abdomen following a spay is routinely performed similar to other species. The linea or fascia of the external rectus muscle is the holding layer and should be closed separately with suture that has adequate strength for the patient. Interrupted or continuous suture lines are acceptable. Subcutaneous closure is generally necessary to reduce dead space and obese animals may require 2 or more subcutaneous closures to close the thick subcutaneous (fat) tissue.

Post operatively, the patient should have activity restricted for at least 2 weeks to reduce the risk of incisional complications such as herniation. Pig abdomens are close to the ground which increases the chances of incisional complications. Monitoring of the incision post-operatively is challenging since the incisions are not easy to visualize. They commonly get contaminated or experience physical trauma as their abdominal skin drags along the ground. They should be kept in a relatively clean and dry environment to reduce these risks. If the surgical procedure was prolonged or complicated, prophylactic antibiotics can be useful to decrease the risk of surgical site infections. Achieving therapeutic blood concentrations of antibiotics at the time of the surgical procedure is the most judicious and effective way to use prophylactic antibiotics.
Castration

Castrations are relatively simple procedures to perform in pigs. It is technically similar to that in dogs or other domestic animals. Scrotal or pre-scrotal approaches are possible. Scrotal approaches are common in neonatal production pigs, but I prefer the pre-scrotal approach for older and pet pigs, and that is the approach that will be discussed here.

The patient is placed in dorsal recumbency, and although general anesthesia is not a requirement, it can greatly reduce stress during the procedure. Because the procedure is quick, patients usually do not require intubation, and mask anesthesia works well for most castrations. Local anesthesia can be used to augment a light plane of general anesthesia.

Following a surgical prep, the testicle is pushed cranially and a skin incision is made over the testicle. With cranial pressure maintained on the testicle, continue the incision deep through the subcutaneous tissues until the testicle (covered in vaginal tunic) is exposed. If the testicle does not “pop” through the skin incision, extend the incision just long enough to allow the testicle to be exteriorized. The spermatic cord is isolated by breaking down the cremaster muscle by pulling distally on the testicle and pushing proximal on the cremaster with a gauze sponge. Following isolation of the spermatic cord, the cord can be ligated or emasculated routinely. The opposite testicle can be removed through the same skin incision or a separate incision on the opposite side can be created. Be careful not to cause iatrogenic damage to the penis on midline when trying to remove the opposite testicle through the original incision. Identification of the testicles is more challenging, and iatrogenic penile damage is more likely in obese animals.

Pet pigs are predisposed to inguinal herniation following castration. Closing the external inguinal ring following castration can reduce the risk of a patient developing an inguinal hernia following castration. Monofilament, prolonged absorbable suture such as polydiaxone (PDS) suture is a good choice for ring closure.

If the surgical procedure has been performed using aseptic technique, the skin can be closed. If aseptic technique has not been practiced, at least part of the incision should be left open for drainage. Post operatively exercise should be restricted and the patient kept in an area where the incision can be monitored and kept clean and dry. Typically, antibiotic usage is not necessary following castration.

Wound Management

The etiology of wounds in pet pigs is varied but commonly due to dog or wild animal attack. The principles of wound management is similar to that of other species. Wounds should be evaluated and hemorrhage should be controlled as part of the initial management. Necrotic and severely damaged tissue should be debrided, contamination reduced, infection controlled, and dead space eliminated and/or drained.

Fresh wounds that have ample healthy tissue, may be closed primarily. If a lot of dead space exists, drainage should be provided if the wound is to be closed entirely. The wound should be left open to heal by second intention if there is a lot of contaminated tissue, if the tissue on the wound’s margin is not healthy, or if there is inadequate tissue to primarily close the wound. Delayed primary closure is another option for contaminated and unhealthy wounds. With delayed primary closure, the wound is left open for initial management, and then closed after health of the tissues has been restored.

Antibiotics may or may not be indicated depending on the extent of the wound and the systemic health of the patient. Local or topical antibiotics tend to be more effective at treating wounds that do not have a systemic component.
Many surgical procedures in cattle are performed without the benefit of General Anesthesia. Procedures such as routine flank surgery such as DA correction, C-section, and exploratory, dehorning, castrations, and invasive hoof procedures are typically performed on animals awake. Nerve blocks are very useful to anesthetize the area to facilitate the procedure and increase animal comfort. Nerve blocks are used commonly in conjunction with general anesthesia or sedation to decrease the amount of anesthetic drug required. Although some procedures are quick enough to be performed without the benefits of a local block, local or regional anesthesia should be considered whenever the pain of the surgical procedure or the post operative pain of the procedure places unnecessary stress on the animal.

**Regional vs. Local Anesthesia**

Local anesthesia refers to loss of sensation to a specific topographic area of the body. This is in contrast to general anesthesia. Regional anesthesia refers to local anesthesia that is achieved by anesthetizing a specific nerve or a group of sensory nerves. Regional anesthesia is frequently performed at a location remote from the surgical site as opposed to other forms of local anesthesia which are performed at the intended surgical site.

Advantages of regional anesthesia include blocking away from the incision, versatility in exact surgical site, and the use of less local anesthetic per area anesthetized. Lidocaine (the most commonly used local anesthetic in food animals) can have inhibitory effects of the healing of wounds. Therefore it is desirable to decrease the amount of lidocaine at the surgical site. Local anesthetic when infused into the tissues creates edema within the tissues which decreases visualization of important structures and makes the surgical site messy. A regional block offers more versatility for intraoperative changes such as extension of the incision or slight modifications in the location of the incision. If larger sensory nerves are blocked, relatively large areas of anesthesia can be achieved with relatively small amounts of anesthetic.

Disadvantages to regional anesthesia include the need for a working knowledge of the anatomy of the nerves and landmarks can be difficult to identify in certain animals. This makes regional anesthesia technically more challenging to perform. In certain circumstances, it may be disadvantageous to block surrounding areas. For example overzealous epidural anesthesia may block nerves that innervate the hind limbs and can cause a patient to become recumbent or ataxic.

**Paravertebral Nerve Blocks**

The paravertebral nerve blocks are regional blocks used to anesthetize the flank in the area of the paralumbar fossa. The nerves that innervate this area are the last thoracic, and the first two lumbar nerves (T13, L1, and L2). Anesthetizing L3 may also be beneficial if incisions are to be made very caudally in the paralumbar fossa. Paravertebral nerve blocks can be divided into proximal and distal which refer to the proximity that the nerves are blocked to the spinal cord. The proximal paravertebral nerve block, anesthetizes the nerves right after they emerge from the vertebral column. The distal paravertebral nerve block anesthetizes the nerves at the lateral edge of the transverse processes of the lumbar vertebrae.
Regardless of the block used, the same area of the flank should be anesthetized. Always remember to check your block before the cow is cut. The only reliable method of checking the block is by assessing the cow’s response to pain sensation. If she responds to a painful stimulus, then she should be re-blocked. She may not need all nerves blocked again. The most common nerve to miss during a block is T13. It is also critical to make certain that enough time has been allowed for the block to take effect. The closer to the nerve the anesthetic was injected, the faster the block will work. Other signs that are suggestive that a block was successful is the presence of scoliosis due to the relaxation of muscles on the side which was blocked. A successful block will also cause vasodilation and increase blood flow which will make the cow feel warmer on the blocked side.

**Proximal Paravertebral Nerve Block**

The proximal paravertebral nerve block is generally regarded as being more technically challenging. The technician must have a better working knowledge of the anatomy. Landmarks can be difficult to find, especially in fat cattle. A distinct advantage of this block is that it uses less local anesthetic since the accuracy of drug placement is increased.

Landmarks to find the nerves are the cranial edge of the lateral aspect of the transverse processes of L1, L2, and L3. Due to the shape and angle of the transverse processes, if a straight line is drawn from the cranial edge of the lateral transverse processes to midline, that will be close to the base of the immediately cranial transverse process. In that way, the transverse processes of L1, L2, and L3 are used to find the base of the transverse processes of T13, L1, and L2.

A canula is used to puncture the skin and protect the long needle which is used to find the base of the transverse processes. A 14 gauge, 1 inch needle is used as a canula and an 18 gauge, 6 inch spinal needle is used to penetrate through the lumbar musculature and find the transverse processes. The 18 gauge spinal needle will telescope inside the 14 gauge needle.

Following correct positioning, the canula should be inserted through the skin. The canula should be inserted about an inch from midline. The spinal needle should be inserted through the canula perpendicular to a plane through middle of the cow. This needle can be angled cranially and caudally to find the transverse process. Remember we are looking for the transverse process in front of the one previously used as a landmark on the lateral flank edge, so the needle may need to be aimed a bit cranially. The needle should be inserted. If no bone is encountered, then the needle removed partially, re-directed and again inserted. After the transverse process is encountered, the needle should be “walked off” the caudal edge of the transverse process to find the nerve. The nerve runs directly caudal to the transverse process after which it is named.

Since most cattle have a lot of musculature and soft tissues on top of the transverse processes, the spinal needle must be almost completely pulled out before redirection occurs. It is helpful to mark the spinal needle at the level that the transverse process is encountered, so that this depth can be approximated when the needle is no longer over bone. The needle should be inserted an additional 1 to 1.5 cm.
Prior to injection of local anesthetic, aspiration should be performed to assure that a vessel has not been penetrated. Five to six cc’s of local anesthetic should be injected per site. I prefer to block each nerve twice to increase my chances of achieving a successful block. A trail of 1-2 cc of local anesthetic should be left when removing the spinal needle. When the correct depth is reached, a small “pop” should be felt when needle penetrates the ligament that runs across the transverse processes. The cow will likely react when this occurs which is a good sign that the nerve is nearby. If ever a strong reaction from the cow is elicited, local anesthetic should be deposited in that area, regardless of the needle positioning.

Distal Paravertebral Nerve Block

Advantages of the distal paravertebral nerve block include being less technically challenging than the proximal paravertebral and more commonly used needle sizes. Since the nerves are blocked distally from where they emerge from the vertebral bodies, the exact location is not as precisely located and therefore a larger dose of anesthetic is required as compared to the proximal paravertebral nerve block. Fat cows can also be difficult to block due hidden landmarks.

The landmarks are the lateral aspect of the 1st, 2nd, and 4th lumbar vertebrae (L1, L2, and L4). The equipment used is an 18 gauge, 1.5 inch needle and a 20 cc syringe.

The lateral edge of each landmark is palpated. The needle should be inserted parallel to the surface of the transverse process and felt as it scrapes directly above and below each transverse process. 15cc of local anesthetic should be injected above and 20 cc of anesthetic should be injected below each process. I typically attempt to block each nerve twice to increase my chances of achieving a successful block.

Local Anesthesia for Flank Surgery

Line blocks and inverted L blocks are commonly employed in the field to achieve local anesthesia for surgery. These blocks are very technically easy to perform. Disadvantages include local anesthetic at the surgical site which can make surgical visualization less and can also decrease the healing of surgical wounds. Increased doses of anesthetic is required and if intraoperative changes with the surgical location or incision size needs to be made, less versatility is afforded by these blocks.

A line block is performed by injecting local anesthetic at the intended surgical site. In addition to the skin, all muscle layers and peritoneum should also be infiltrated with anesthetic. An inverted-L block is performed by injecting local anesthetic along the ventral aspects of the lateral edges of the lumbar transverse processes and then down along the caudal border of the last rib. The goal is that all nerves innervating the paralumbar fossa are intercepted by infiltrating local anesthetic along these lines.
Epidural Anesthesia

Decreasing pain and straining during obstetrical procedures is a very common indication for performing epidural anesthesia. Epidural anesthesia can also be useful for achieving regional anesthesia of the perineal region so that surgical procedures in that area may be performed. In cattle the sacrococcygeal joint is most commonly used while in small ruminants and calves the lumbosacral joint is typically employed.

When using the sacrococcygeal joint, the tail is moved upward and downward while the sacrococcygeal joint is palpated. The junction between the last sacral and the first caudal vertebral is identified. An 18 gauge, 1.5 inch needle is inserted through the skin. A drop of local anesthetic is placed on the hub of the needle. Once the needle is inserted through the skin, the soft tissue structures occlude the opening of the needle and the air pocket trapped within the needle keeps the drop on the top of the hub. The needle is slowly advanced straight down between the vertebral bodies. Once the tip of the needle reaches the epidural space, negative pressure will be encountered and the drop on the hub will be pulled into the space. A syringe can be carefully applied and local anesthetic injected. If correctly placed, the injection should be without resistance.

If the lumbosacral space is used, the procedure is similar. At this location it is possible to advance the needle too far and penetrate into the subarachnoid space. If this happens, local anesthetic can be injected but the dose should be adjusted and is approximately ½ of the epidural dose.

Typically 2% lidocaine is used to achieve epidural anesthesia for most procedures. Lidocaine may be combined with xylazine to increase the duration of effect. Morphine can also be given epidurally to decrease chronic pain. Morphine does not affect motor function, which may be a side effect of an overzealous lidocaine epidural. However, morphine does not provide surgical anesthesia.

Cornual Nerve Block

Blocking the cornual nerve will anesthetize the area around the horn base for dehorning cattle. The nerve that is blocked is actually the cornual branch of the lacrimal nerve, a portion of the ophthalmic division of the 5th cranial nerve. The site used for blocking is 2 – 2.5 cm from the horn base along the facial crest. The depth of the nerve can vary from about 0.7 to 1 cm deep. Frequently the nerve may be palpated between the frontalis muscle and the temporal muscle. 5-10 ml of 2% lidocaine should be injected at this site. With well developed horns, there may be innervation from the caudal aspect of the horn and a block at the back of the horn base may be necessary. I usually perform a ring block around the horn base to make certain that the horn is desensitized.

Adequate restraint is necessary to prevent the animal from throwing its head. The animal should be caught in a head gate with chute. The head should be tied tight to the side with a halter or held to the side with nose tongs. Head gates that have a nose bar or apparatus to secure the head are useful. Prior to blocking, hair should be clipped and asepsis should be practiced.

Blocks for ocular procedures

An auriculopalpebral nerve block can be used to prevent eyelid closure during examination of the eyeball or to facilitate the administration of intraocular medication. Blocking the Auriculopalpebral
nerve paralyzes the orbicularis oculi muscle. This does not desensitize the eyeball so is not sufficient for painful surgical procedures to the eye.

The auriculopalpebral nerve runs along the lateral aspect of the zygomatic arch. 10-15 ml of 2% lidocaine should be infiltrated along the nerve. The needle can be inserted near the junction of the zygomatic arch and facial crest and directed caudally or inserted at the base of the ear and directed cranially along the facial crest.

To achieve desensitization of the eyeball, a Peterson block or a retrobulbar block should be performed. These blocks anesthetize the nerves that emerge from the optic foramen. Indications include enucleation, encineration, tumor removal or other painful ocular procedures.

For performing a retrobulbar block, a 6 inch, 18 gauge needle should be used. A slight curve should be applied to the needle. The needle should be inserted through the eyelid and the technician should feel the needle scrape along the orbit. When the needle is behind the globe, approximately 20 ml of 2% lidocaine should be injected. Because the retrobulbar muscles are anesthetized and 20 ml of fluid has been injected posterior to the globe, proptosis of the eye should occur.

For performing a Peterson eye block, 4½ inch 18 gauge needle should be used. The needle is directed perpendicular to the skull in the notch formed by the zygomatic arch and the posterior rim of the orbit and the coronoid of the mandible. The needle is advanced until the bony plate around the foramen orbitorotundum is reached. The needle should be withdrawn slightly. When the desired depth is reached, mild spasms of the eye may be noted. 15-20 ml of 2% lidocaine should be injected just anterior to the foramen rotundum. At least 5 minutes should be allowed for the block to take effect. As with the retrobulbar block, paralysis of the retrobulbar muscles will allow proptosis of the eyeball.

References

Concerns about animal welfare are often centered on negative affective states, such as pain. These concerns are increasingly reflected in regulatory changes concerning animal agriculture, evidenced by bans of specific painful husbandry procedures such as tail docking. Consumer assurance programs, the largest driver of animal-welfare change in the US, are also increasingly specifying how and when pain relief must be provided to farm animals. For cattle, the common painful procedures addressed by such programs include castration, dehorning or disbudding and branding. There is considerable scientific evidence that all of these procedures cause immediate pain, but less is known about pain experienced through the healing process.

Hot iron branding is the most common form of herd identification in the US beef industry (45% of cattle and calves, USDA, 2008). Pain responses during branding include tail flicking, kicking, and falling down, escape attempts, and vocalization. In addition to the immediate response, healing of hot-iron brands can take longer than 10 weeks in other species and burns can remain painful until the healing process is complete. Initially, work from the University of Saskatchewan demonstrated that brand wounds are inflamed at least 7 days after branding and that there were no effects of the procedure on weight gain and handling ease in the weeks that followed the procedure.

More recently, we have found that hot-iron brands take at least 8 weeks to heal. When a known and increasing force is used to quantify sensitivity of these wounds (stimulus-evoked pain responses), branded cattle are more sensitive than unbranded controls for at least 10 weeks. In addition, brand wounds are more sensitive at the center of the wound than 5 or 10-cm above it, supporting the idea that the degree of tissue damage increases the response to palpation. The sensitivity of the tissue corresponds to the degree of healing: cattle with hot-iron brand wounds further along the healing process are less responsive than at earlier stages.

As with other painful procedures, methods of alleviating pain associated with the procedure or those that hasten healing would improve animal welfare. As an initial step towards understanding of how interventions affect healing of hot-iron brands, we have explored two options: administration of a non-steroidal anti-inflammatory (NSAID) or use of a cooling gel at the time of branding.

**NSAID administration**

Unlike dehorning or castration, little is known about how to alleviate branding pain. In terms of pain in the hours afterwards, the effects of a single injection of NSAID is beneficial for other these procedures in cattle. Less is known about how reducing inflammation after these procedures affect healing in cattle. NSAIDs have either no effect or slow healing in soft tissue wounds in humans and rodents. The effects of a single injection of an NSAID has been suggested as a practical method of mitigating pain in the hours after branding. However, we found this approach has limited to no biological benefit in terms of wound sensitivity, surface temperature, healing rate or lying behavior. These results are, perhaps, unsurprising, given that the effectiveness of the drug we tested, flunixin meglumine, is short.
Cooling gel

Effective cooling of a burn using water or a gel (active ingredient, tea tree oil) has been demonstrated to improve the rate of wound healing and decrease tissue damage in pigs (Jandera et al., 2000). In cattle, application of a room temperature gel either once immediately after or twice (immediately after and 1 day later) after hot-iron branding immediately cools the tissue, but this change does not result in improvement of long-term outcomes such as sensitivity or healing rate.

Other factors

Other factors that may influence healing of hot-iron brands remain largely unexplored. Aspects of branding method, such as iron temperature and contact time, do not correlate with healing within the range tested, but further work in this area may be warranted to optimize the process. The age of the animals is another consideration. Branding younger calves may hasten healing for several reasons. Firstly, the amount of tissue damaged could be smaller in younger calves than in older, bigger animals. Secondly, calves grow more rapidly in the weeks after birth than at the industry-typical age for processing on cow-calf operations (10 to 11 weeks, USDA, 2008) and this faster growth may aide healing. Finally, anecdotal reports suggest that the shape (e.g. curves vs. straight lines) and concentration of surface area affected may affect healing. For example, the center of the brand may remain more sensitive than the outer edges because the burn is more severe in this area.

The immediate pain associated with hot-iron branding has been well documented and new evidence suggests that these wounds remain painful throughout the healing process (8+ weeks). At least 2 possible practical solutions, a single injection of NSAID or a cooling gel applied at the time of branding, do not hasten healing. Alternatives are needed.

References

Animal welfare assessments are being widely used in other sectors of animal agriculture. We recently developed an assessment for cow-calf operations and used it to examine the health and behavior of beef cows being worked in a chute on 30 California ranches.

We found relatively little variation among most important health outcomes. Injury, lameness and low body condition were all rare. Nasal and ocular discharge and diarrhea were more common, as were hairless patches.

In contrast, we found that ranchers varied considerably in how they handled their animals and how these animals responded. For example, some ranchers never used an electric prod, while others used it on 75% of their animals. Using this variation, we identified that cows touched with an electric prod were more likely to balk, vocalize, stumble and fall in the chute, and stumble and run as they exited.
In addition to generating knowledge about how management practices affect cattle behavior, we also provided each participating ranch with a benchmarking report, showing them how they compared to the other 29 ranches in the study. The stars indicate how we showed the ranches where their observations fit into the patterns across the others.

- = 1 ranch
- = 30 ranches

We developed a website to allow training for this assessment online. This website explains how to measure each parameter and then allows users to test their consistency of scoring with approximately 30 pictures or videos: [https://www.ucdcowcalfassessment.com/](https://www.ucdcowcalfassessment.com/)

This session was sponsored by

![Easy Boss E](https://example.com/easy-boss-e.png)
The largest driver of changes in animal welfare in the US are corporate and industry-led assessment and audit programs. These programs aim to ensure a minimum level of care for food animals. Understanding the components is an important aspect to understanding these programs. There are three main components: 1) standards of care, 2) verification and 3) enforcement.

Standards of care cover what is checked on the farm and in this system, often rely on information about the animals themselves. These types of measures, such as body condition or lameness, are called outcome-based because they are a way to gauge animal welfare of the system by looking at the animals themselves. Protocol-based measures include paperwork or standard operating procedures about how the operation is run. For example, documentation of employee training for animal care is a common protocol-based measure included in audits or assessments. Finally, facility-based measures include presence of specific resources, such as shade or water, or minimums for aspects of housing, such as space/animal.

Verification involves measuring how the operation stacks up against the standard of care. This can be done by the owner or employees of the operation (1st party), others that have connections to the farm or its products (2nd party), such as the herd veterinarian or by an independent auditor (3rd party). For some programs, the farm is audited by a 3rd party once a year, but to prepare for these visits, 1st and 2nd party involvement is also needed. Indeed, a majority of change occurs through “managing what you measure” at the 1st and 2nd party level.

Finally, many market-driven welfare assurance programs involve some type of enforcement. This can be as simple as the ability to access the market and sell the products in the first place. In other situations, operations meeting a specific set of standards of care may label their products and this may provide a market advantage or a premium.

Often a precursor to a full-blown market-driven audit is an assessment. These often are structured in a manner similar to an audit in terms of the standards of care. However, they are often broader and lack much of the guidance about verification found in formal audits. These assessments also usually have educational, rather than enforcement goals.

Summary of the components of animal welfare assurance programs, specifically market-driven audits and programs that are more of an assessment than an audit.

<table>
<thead>
<tr>
<th>Component</th>
<th>Market-driven audits</th>
<th>Assessment, rather than audit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards of care</td>
<td>includes outcome, protocol and facility based measures</td>
<td>includes outcome, protocol and facility based measures</td>
</tr>
<tr>
<td>Verification</td>
<td>on all farms; 1st/2nd and 3rd party</td>
<td>on all farms; 1st or 2nd</td>
</tr>
<tr>
<td>Enforcement</td>
<td>ability to sell products (market access); label participating products</td>
<td>no substantial enforcement; goals are educational</td>
</tr>
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</table>
This non-regulatory approach to animal welfare has a number of benefits in terms of widespread and rapid improvements and the ability to update programs on a regular basis. The challenges include 1) the scale of assessment in terms of both number of operations to visit and with what frequency, 2) determining key welfare criteria to include standards of care, in terms of validity and feasibility 3) ensuring consistency among auditors during verification, and 4) the emergence of competing assurance programs within a sector.

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[Image of Easy Boss E logo: Oral Distraction Device]
Veterinarians are animal advocates who have an obligation to respond appropriately and sensitively to the needs of animals and humans when health and welfare are at-risk. When presented with animal health challenges, veterinarians graduate with a solid foundation in how to work through a presenting complaint, gather information through a history and physical exam, create a problem list, a diagnostic and treatment plan, a plan for long term care, and patient follow up. Where animal welfare is concerned, however, many veterinarians identify feeling ill-prepared to identify and clearly articulate the nature of the concern and respond appropriately. Often it is clear that gaps in companion animal care result mainly from gaps in client education, or gaps in the personal wellness of the clients themselves. Cases in which there are human health and welfare challenges co-occurring with animal care issues can be particularly perplexing. These cases are complex and dealing with them can become a source of stress, empathy fatigue, and burnout in ourselves and our teams.

As veterinary professionals, we often feel as though we have to be ‘everything to everyone’ and many of us find ourselves acting as the counsellor for clients as well as the advocate and doctor for patients. Not only is this exhausting mentally and emotionally, it is actually inappropriate and unethical. As a profession there is need to stay within scope of practice, appropriately utilizing existing networks of aligned paraprofessionals in the community. These allies in multifaceted family veterinary care are equipped and trained to support the health and wellbeing of clients while veterinary teams focus on the needs of patients. Advanced veterinary communication skills are essential to the appropriate navigation of complex cases involving human and animal health and welfare challenges, and many veterinarians lack training in this area. Veterinary teams also need education around locating and connecting with complimentary resources, and appropriately communicating these human referrals to clients. Developing these skills ensure proper support for clients, allowing veterinary teams to focus on patient care within an appropriate scope.

We can begin to distill the art of client communication by examining relevant core and ancillary skills. These allow us to effectively gather information, build rapport with clients and act as advocates for patients while maintaining an ethic of care and compassion within the veterinary-client-patient relationship.

**Core Skills and Ancillary Supports:**

**Open Ended Questions:**

**Stems:**

Who; what; where; when; why; how; please tell me; explain; describe

**Examples:**

- Please tell me about the housing situation for your cat?
- What helped you decide to make that feeding choice for your dog?
- How do you ensure your pets do not have access to household cleaning products, toxic plants, prescription or other medications?
Ancillary supports:

**Prefacing:** giving context before you ask an open ended question; maximizes the value of the answer

Example: It’s important for me to understand any challenges around housing that might need to be improved to optimize health and safety for you and your cat. With that in mind, please tell me about the housing situation for your cat?

**Chunks and Checks:** giving information in manageable chunks and then checking in with client to gauge understanding

**Signposting:** like a road-sign, allows clients to be aware of what is coming next and how things will unfold during the visit; provides context and reasonable expectations

**Ask-tell-ask:** A tool for explaining new concepts that involves first gathering client’s perspective as well as their level and nature of understanding of the situation, diagnosis, etc. you are about to discuss. Then you can explain the new information using terminology and details that are informed by their unique situation and perspective.

**Non-verbal Communication:**

**Background:** Roughly 80% of communication is non-verbal, so this is an essential element to pay attention to in developing elevated communication skills. This is particularly important when difficult decisions (euthanasia, costly procedures, welfare discussions) are involved. Nonverbal sensitivity improves client satisfaction with their veterinarian over and above other elements of practice, so this might be the most important element of communication for us to pay attention to.

Not only is it important to pay attention to non-verbal signals in ourselves, but also in our clients. This form of communication can tell us a lot about how a client is reacting to an interaction and may alert us to problems before anything verbal will. We can then alter our communication (both verbal and non-verbal) in response to these cues to improve the outcome.

**Important Elements:**

**Kinesics** – facial expressions, body tension, gestures, touch, body position and movements

**Proxemics** – how space is shaped between client, animal and vet

- Vertical height
- Interpersonal distance
- Angles of facing
- Physical barriers

**Paralanguage** – non-word phenomena (pause, pitch, rate, intonation, volume, emphasis) – the ‘music’ of language

**Autonomic shifts** – don’t have control over these; things like facial flushing or blanching, tearing, sweating, breathing rate, etc – happen when people are having strong feelings

**Examples:**

**Shaping space** – sets the stage in communicating to the client our views on the relationship. Pay attention to the way an exam room is arranged – should be conducive to collaborative non-verbal
interactions (sitting beside or at an angle to the client; removing physical barriers; allowing both client and veterinarian to sit)

**Developing non-verbal rapport** – matching (moving as client moves) and leading (use of interpersonal synchrony that has been set up by matching) – these concepts can help alter the emotions or tension in the room in positive or negative ways so if we know about them, we can use them to our benefit

**Reflective Listening:**

**Background:** Reflective listening can take the form of summarizing, paraphrasing, and hypothesizing to review shared info. The client hears their story as understood by you. This allows clients to add further information where necessary, clarify things, and correct misconceptions. Importantly, it also allows client to feel their perspective is recognized and valued, emphasizing that they are being heard.

Understanding perspective is fundamental to the ‘art’ of veterinary medicine. If we don’t invest in this part, we miss out on opportunities for relationship building and important client education that otherwise may not happen. Without seeking to understand our client’s perspectives on things, we can completely misinterpret everything they tell us or lead the consultation in a completely inappropriate direction. Reflective listening is an important partner to empathy, which is one of the skills we can rely on most heavily when addressing difficult conversations with clients whose own health and wellbeing might be challenged.

**Stems:**

- I hear you saying that...
- So if I understand you correctly...
- So the sequence of events was...

**Empathy:**

**Background:** Empathy is a way of suggesting an appreciation for what an experience may be like for another person. In using empathy, we express active concern for and curiosity about the emotions, values and experiences of others. Empathy is different from sympathy; where empathy drives connection, sympathy drives disconnection. Empathy involves identifying what another person might be feeling, and connecting with something in ourselves that knows the same feeling.

**Methods of using empathy:**

- Non-judgmental response – ‘this is a tough decision and there is no right or wrong answer’
- Normalization – ‘it is completely normal to struggle with this decision’
- Appropriate disclosure – ‘I’ve been through something similar with a pet of my own and found these decisions very difficult to make’ **be careful about your relationship with the client here, as this dictates whether these statements seem appropriate or inappropriate; also be careful to only disclose details about experiences with which you already feel resolved – do not use the client’s experience as a therapy session for you**
- LISTENING – silence is a wonderful tool for empathy – giving clients space to think and feel – learn to be comfortable with silence and use it to your advantage!

All of these communication skills, when used together, can allow us to connect with our clients even in challenging situations so that we can maintain de-escalated interactions and a patient and client-centered care approach.
**Human Factors and Connecting with Allied Supports:**

There are multiple human mental and physical health issues that, when co-occurring in cases of disrupted animal health and welfare, can create challenges for the veterinary team. Being able to identify some of the common challenges that can be present for our clients is useful not only in developing empathy for them, but also in having productive discussions with allied professionals on the human health side.

**Common Human Health Issues:**

- Mental/Physical health challenges
- Substance misuse/abuse
- Poverty
- Social exclusion

When any of the above challenges are presumed or identified in our interactions with our clients, it can be helpful to know ‘who to call’ when the client needs more support. For further training in this area, veterinarians should consider Mental Health First Aid®, a course designed to provide individuals with the ability to support a person living with a mental health crisis or problem. Below you will find links to support services province-wide for direction on client referral.

**General Inquiries**

- Healthline (province-wide)-811
  or
- [https://www.saskhealthauthority.ca/Services-Locations/Pages/Home.aspx](https://www.saskhealthauthority.ca/Services-Locations/Pages/Home.aspx)

**Crisis Lines**

- Emergency Services-911
- Healthline- 811
- Saskatoon Crisis Intervention Service-306-933-6200
- Southwest Crisis Service (Swift Current) -306-778-3386
- West Central Crisis & Family Support (Kindersley)-306-463-6655
- North east Crisis Intervention Centre (Melfort) -306-752-9455
- Hudson Bay & District Crisis Centre-306-865-3064
- Prince Albert Mobile Crisis Unit-306-763-8181
- Regina Mobile Crisis Unit-306-764-1011

**Support for Veterinarians**

- Professional Psychologists & Counsellors (PPC) -306-664-0000
  (Province-wide support provided by the SVMA-More detailed information available under Resources & Information “Member Wellbeing” at [www.svma.sk.ca](http://www.svma.sk.ca))

- For more information or to enhance your skills consider Mental Health First Aid®
References:


Recognizing animal abuse is a difficult thing for veterinarians to do. Lack of training combined with our compassionate natures leaves us frequently blind to potential abuse victims in our practices. This lecture will address factors to increase suspicion of abuse as a diagnosis, as well as how to approach making the diagnosis. We will discuss terminology commonly used in abuse and neglect cases.

Animal abuse is not a medical determination, it is a legal one. Non-accidental injury (NAI) is a term used to describe any injury that does not have a potential accidental or ‘natural’ cause. NAI is a medical determination made in cases of abuse. Generally, criteria that should increase a veterinarians index of suspicion that they are dealing with a case of NAI include;

1. The nature of the injury – the type of injury itself, repetitive injuries, or multiple occurrences in the same house.
2. Features in the history – a history inconsistent with the injury, violence in the home, a lack of history, behavior of the animal or the owner.
3. The implication of a specific person
4. Socioeconomic class
5. Unexplained deaths
6. Clinic hopping
7. Large number of animals on file that have never been seen.

Developing a robust differential diagnosis list is the basis for any good diagnosis in clinical practice. Including NAI as a potential differential diagnosis in any case with vague or non-descript clinical signs will help increase the chance that veterinarians will recognize cases of abuse when they present. If an implication of NAI is made, then it’s important all other differential diagnosis be excluded.

Non-accidental injury is a term used in both animal abuse and child abuse cases and helps draw parallels between the two crimes in both the type of perpetrator, and victim. Both scenarios see a perpetrator in a position of power or authority and an essentially voiceless victim. As veterinarians we are the natural advocates for animals and are uniquely positioned to witness the effects of animal abuse and to be their voice. Mandated reporting creates a legal obligation to reaffirm the moral obligation we already feel as veterinarians to speak up when an animal is abused or neglected.

Animal maltreatment broadly covers both neglect and abuse of animals. The two terms differ based on intent and this typically correlates directly with any potential charges. Neglect is the result of acts of omission and refers to failure to provide necessaries of life and encompasses unintentional maltreatment. This typically includes failure to provide food, water, shelter, veterinary care, grooming. Abuse refers to acts of commission and is the intentional maltreatment of an animal. Abuse encompasses physical, sexual and emotional abuse. Neglect on a large enough scale can be considered intentional and then classed as abuse.

Provincial legislation in the animal protection act covers liability based offences and acts of omission. The penalties and jeopardy associated with charges under this act reflect the relatively lesser severity of the offense and the potential for ignorance on the part of an owner. Federal legislation in the criminal code covers acts of commission and the penalties and jeopardy reflect the relative severity of the offenses. For a criminal charge to be upheld the prosecution must prove intent on the part of the perpetrator. Both provincial and federal legislation regard animals as property and as such the
collection of evidence of potential crimes must be carried out in a legal manner. As long as an animal is owned, collection of evidence from that animal can only be carried out by consent or by warrant. Once an animal is seized, the seizing agency is in care and control of the animal and can direct collection of evidence that may otherwise not have been legal to gather. Local bylaws may affect at what point an animal is technically property of a law enforcement agency, city or province.

Animal maltreatment is commonly seen in conjunction with maltreatment of people and other antisocial behaviors. Understanding the link between animal abuse and other crimes is essential to understanding the larger role in the safety of society that veterinarians can play. In the complex wheel of power that describes how abusers control their victims there are numerous ways that animals can be used to exert power or dominance and therefore exert control. Many of these forms can be seen in clinical veterinary practice settings and may be the only point of contact for human victims of violence.

Creating a plan for addressing cases of animal abuse starts with a discussion in practice and the designation of reporter and contact person for law enforcement. Using a pre-formatted template can help ensure all team members know who to contact and when. Having a discussion on how to handle such cases will help relieve some of the stress of reporting.

Veterinarians don’t report cases of animal abuse for a variety of reasons. Mandated reporting helps allay some of these fears and shows that society supports vets in reporting. Fears of vet avoidance, economic impact on the practice, and legal ramifications are demonstrably under realized. It’s important to remember in reporting that you need only have a suspicion. That your role is to report and to collect evidence to support your suspicion as legally allowed. As a veterinarian you are not a law enforcement officer, you are not the judge and you are not the jury. If there are mitigating circumstances to an offense or a decision to not pursue charges or levy a minor penalty, that is up to law enforcement and the judiciary.
Collecting the necessary information and documenting what you find ultimately makes for solid evidence in the prosecution of the offender in court. Veterinarians are essential in helping build that solid case for court in incidences of animal abuse. This session will discuss the live forensic exam and collection of evidence including photography, documentation of findings, and necessary diagnostics specific to legal cases.

Documentation of potential legal cases must be much more thorough than a standard case in practice. If you don’t document it, in the eyes of the court, you didn’t look at it. Also, for court purposes if you didn’t photograph it, then your description can be called into question.

A live forensic exam begins with ensuring you have all the history you need to perform a good evaluation. Consider what you expect to find, what the law enforcement expectations are and what samples/evidence you may need. Ensure you are legally placed to collect the evidence you feel is pertinent. Examination of live victims may begin with evaluation of the scene. In these cases consider what impact the environment has on the health and welfare of the animals. What conditions/disease states might you expect to see based on specific environmental conditions ie; crowding, lack of shelter, dirty conditions, high ammonia.

**Photography:**

Photography is the first step to properly documenting your observations. Use a digital camera and ensure the time and date stamp is turned on. Ensure good lighting, the quality of your photos will color law enforcements confidence in your skills overall.

The first photo in every series should be a slide with case documentation, including the case number, the investigating agency, the date, and any other pertinent information. All photos taken must be provided in disclosure. For best photos try for a grey background and good lighting, include a scale (ABFO), and try not to include people. A photo series should be something that someone who has never been to the scene, or seen the animal, can skim through without explanation and understand.

Initial overall photos should include 6 full body views of the animal – left, right, dorsal, ventral, front and back. Then focus in on any abnormalities seen, starting with distance shots progressing to close-up then close-up including scale.

A thorough external exam should be carried out noting any normal, as well as abnormal findings. Using an alternate light source or a flashlight held at an angle will assist in finding trace evidence like fluid, hairs or foreign particulates. Any matting, wounds or lesions should be documented by description and using a diagram. Leave no stone unturned! Evaluate the inside of the mouth, the ears, the eyes, and the paws, places you may glean over in a routine exam. Take care especially when there are clear signs of trauma or evidence to not get tunnel vision. Always body condition and muscle mass score an animal.
Collecting evidence:

Any evidence found on an animal should be photographed in situ prior to sampling or removing. If samples of fluids, hair, DNA are suspected then reference samples from the animal and anyone who has been in contact with the animal must be collected. Wearing gloves and masks will reduce the chance of contamination by staff. All evidence collected must be sealed and stored in a secure manner prior to transfer to a law enforcement agency.

Fluids: suspect fluids on an animal should be examined and photographed under UV lighting. They should be swabbed using sterile cotton swabs. Dried fluids can be collected by moistening a sterile swab with saline and rolling it over the dried substance to rehydrate it, then collecting with a second sterile swab. All swabs should be allowed air dry and packaged in paper.

Hair: Suspect hairs should be packaged in paper

Nails: Animals nails in suspected trauma cases should be examined closely. If any damage, all nails should be trimmed and preserved in a paper evidence bag.

Projectiles: Projectiles should only be handled by gloved hands or with plastic utensils so as not to create tool marks.

Blood: Collect only when legally placed. Sampling is considered a medical procedure and cannot be carried out without consent. Urine collection by cystocentesis is the same principle.

Urine and Feces: if these are naturally voided they can be collected.

Blood/Urine/Feces should be collected in all cases of suspected abuse or neglect prior to initiation of any therapy, even before food and water is provided. Collecting a minimum database will help rule out any underlying disease states and detect other contributing causes. They may not always be required but you can’t undo your treatment and may be happy for more evidence later.

Toxicology: toxicological sampling depends on what substance is being tested for. Check with your lab for specific samples required, preservation and packaging. Most commonly used in house are Innovacon urine drug tests.

Entomological evidence: preserve largest larvae in EtOH. If you have an entomologist, collect live samples for rearing.

DNA sampling: may be required in some cases. Buccal swabs are excellent, as are frozen muscle biopsies, hairs with follicles, or frozen feces. Never expose samples to formalin if you may want DNA later.

Providing a Report:

The more complete and thorough your report, the less likely you will be required to go to court. If all your findings and interpretations are well laid out there will be no reason to call you for questions. A sample template for reporting will be discussed and is briefly included here.

Contact: who the report is written for

Case Summary: A brief synopsis of the case, what information you were provided with including previous medical history. One or two paragraphs summarizing your findings and interpretations.

Subject of Exam:
Medical History:

Examination Findings:

Specimens Received

Assessment in this case involved physical exam performed on xxxx at xxxx. As well as review of blood work collected xxxxxx and review of radiographs taken xxxx

Radiographic Interpretation:

Physical Exam

Eg. The dog is a roughly 2year old (as per Officer Bailey) intact male Australian Cattle dog type.

HR: 140bpm, RR: 20bpm, CRT 2s, mm pink tachy, skin tent is severely prolonged.

Detail ALL physical exam findings without interpretations.

Assessment: eg. Severe emaciation, very poor coat, marked dehydration, moist dermatitis, ocular infection.

Ancillary Procedures and Results

Eg. Histopathology, cytology, DNA, drug testing.

Diagnosis:

Eg. Starvation. Emaciation with no medical cause.

Pg Brk -Interpretations

This is where you write you interpretation of your findings along with your opinions.

Pertinent information to include is;
Was the animal suffering? In distress? In pain?
If so, for how long?
What would owners without medical training have seen?
What could have been done? How much would it cost?
Will there be lasting consequences for the animal?
This session will cover the approach to the forensic necropsy. We all have the tools to perform a good necropsy for diagnosis of cause of death. This session will cover a more in-depth approach and discuss drawing conclusions and making interpretations from findings. We will conclude by discussing writing statements for law enforcement, what they want out of your statement, and what will be expected of you in court if you are asked to testify.

The forensic necropsy ideally begins with evaluation of the scene or ensuring a good description of the scene. Obtaining any history available, whether that is medical history or witness accounts. This will help guide your examination and help you determine what samples you may need/want, what processes you need to rule out/in. Take time prior to starting the necropsy to properly prepare for what you expect to find and want to get out of the process. Confirm with the requesting agency what their expectations are.

The necropsy initially begins with observation;

1. Document how the body was received and ensure chain of custody
2. Full body radiographs – radiographing the full body will ensure signs of chronic or historical abuse are not missed. Ideally three view skull rads (lateral, VD, Cr-Cd), 5 view chest rads (L/R lateral, L/R lateral oblique, VO or DV), 3 view abdomen and pelvis (Lateral and VD/DV), views needed to capture extremities entirely.
3. Photography as for live exam – 6 view followed by focused views
4. External exam - focus on coat, nails, signs of veterinary intervention, openings (natural and unnatural). Flex and extend all joints, palpate the entire body. Focus in on any abnormalities found.
5. Reflect the skin, inspect for any injuries, muscle and fat present.
6. Reflect the right fore and hind limbs
7. Fully open the abdomen and the thorax
8. Inspection of internal organs – bread loaf
9. Open the cranium – inspection of the contents

Next you want to describe all lesions

1. What organ is affected/location?
2. What color is it?
3. What texture is it?
4. Size?
5. Shape?
6. Well demarcated/poorly demarcated?
7. Odor?

Lastly, interpret your findings. This is using your knowledge to explain what happened. This portion of your report should include the same important information included in the interpretations in the live exam.
The most frequent use of the necropsy in practice is to diagnose cause of death. The goal of a forensic necropsy is to determine the COD, document the extent of the disease or injury, determine the degree and duration of pain and suffering, determine the manner and mechanism of death and sometimes estimate the post-mortem interval.

Post mortem changes can help in the estimation of post mortem interval. Livor mortis, rigor mortis and algor mortis are most commonly used despite a lack of validation for a variety of breeds and species. Gastric emptying time can be useful with a short post mortem interval. Decomposition is heavily influenced by the environment the carcass is exposed to and so may not be a reliable indicator of PMI. In cases of decomposed bodies entomological evidence can provide a colonization interval that may approximate the post mortem interval.

**Providing Expert Witness Testimony:**

As an expert you can provide not only testimony as to what you saw, heard, smelled, but also your interpretations and opinions of that evidence. Lay people cannot provide opinion evidence in court. You can testify to your opinion of the cause of injuries, the cause of death, the extent of suffering, the duration of distress.

Providing testimony begins with a ‘voir dire’ where your credentials will be read by the party calling you as a witness. You will have previously been asked to provide a current CV and this will be submitted to the court as an exhibit. The defendant or their council, and the judge will then have the right to question you on your credentials. You will be proffered as an expert in veterinary medicine, as long as you hold a current license in the province you are testifying in you shouldn’t encounter any objections.

Testifying in court can seem daunting but consider that veterinarians are second only to firefighters in their credibility as a witness on the stand. Legal cases in Canada are not like law and order, it is very rare for defense to be represented by legal counsel on provincial charges. If there is defense counsel, they are highly unlikely to question you in an adversarial manner as they appreciate that they do not have the knowledge to do so. Their questions are typically just to provide clarity, not to refute your evidence or opinion.
Flea and tick infestation is a major health problem in dogs and cats, and control presents an economic burden to their owners. Recent advances in product technology have greatly expanded the available options for veterinarians and pet owners. However, this wide array of available ectoparasiticides can lead to confusion.

**What is this new class of drugs?**

Fluralaner, afoxolaner, sarolaner, and lotilaner are novel synthetic members of the isoxazoline class of parasiticides showing activity against insects and acarines, including fleas and ticks.

**Which products are currently available in Canada?**

Three oral products are commercially available for oral administration in dogs:

- Fluralaner - Bravecto® (Merck Animal Health)
- Afoxolaner - Nexgard™ (Merial Canada Inc.)
- Sarolaner - Simparica® (Zoetis Canada Inc.)

Fluralaner is also available as a spot-on (Bravecto® Topical Solution, Merck Animal Health) for dogs or cats.

A fourth oral compound for dogs, lotilaner (Credelio®, Elanco), is not yet available in Canada.

**What is the mechanism of action of these drugs?**

Isoxazolines have a novel mode of action and specifically block arthropod ligand-gated chloride channels. They act on the gamma-aminobutyric acid receptor (GABA) and glutamate receptors, inhibiting GABA and glutamate-regulated uptake of chloride ions resulting in excess neuronal stimulation and rapid parasite death.

**What is the main disadvantage of these drugs?**

The main disadvantage of isoxazolines is that fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

**What are the advantages of these drugs?**

Several criteria are judged important for both veterinarians and pet owners: spectrum of action, duration of efficacy, ease of use, safety, and speed of kill.

**The four major advantages of isoxazolines are the following:**

1. Spectrum of action and duration of efficacy
Due to their pharmacokinetic properties, isoxazolines were the first and are currently the only orally administered drugs to provide effective and long-lasting (for a month or more) parasiticidal activity against both fleas and ticks after a single administration.

2. Ease of use
Oral isoxazolines are easy to administer and palatable. This enhances pet owner compliance. They are the therapeutic option of choice in dogs that are bathed or swim frequently.

3. Very good safety profile.
Isoxazolines are not substrates of the P-glycoprotein. These products are generally quite safe. The most frequently reported adverse effects include vomiting, diarrhea, anorexia, lethargy, and flatulence.

4. Great speed of kill.
Speed of kill is also an important criterion for assessing a flea control product, because the more quickly fleas are killed the less likely a pet owner is to observe them on the pet. It also influences flea egg production, and faster speed of kill therefore results in less flea egg contamination of the environment. Isoxazolines kill over 95% of the fleas, starting as early as 4 to 8 hours.

How about the extra-label use of these drugs?

Recently, isoxazolines have received extra-label use for:

- Canine flea allergy dermatitis
- Canine and feline demodicosis
- Canine scabies
- Canine and feline otoacariasis

The results of recently published studies are encouraging because this new treatment modality offers the potential to provide effective and safe control of many parasitic skin diseases of companion animals, with low frequency of administration, while helping prevent and control fleas and ticks.

Online resources

With the In-Clinic Tools, the Canadian Academy of Veterinary Dermatology (CAVD) has made an effort to provide up-to-date and relevant information about certain topics in veterinary dermatology in Canada.

A new members-only CAVD In-Clinic Tool lists and compares the isoxazoline-based products currently available in Canada. You can find all of the CAVD In-Clinic tools at: [www.cavd.ca/resources/in-clinic-tools](http://www.cavd.ca/resources/in-clinic-tools).

This session sponsored by

[ROYAL CANIN]
Demodicosis is a common disease in canine practice caused by a proliferation of *Demodex* mites. Evidence-based clinical consensus guidelines are now available to all veterinarians. Their purpose is to provide veterinary practitioners with a straightforward description of diagnostics and treatment options in dogs.

**Eleven consensus statements on canine demodicosis**

1. Generalised demodicosis is most likely a consequence of temporary immunodeficiency in young dogs and is often associated with an immunosuppressive condition or treatment in older dogs.
2. In young dogs, demodicosis has a genetic basis and most likely multiple genes are involved.
3. In dogs, two *Demodex* species occur, the shorter *D. canis* and the longer *D. injai*.
4. Demodicosis in dogs is characterised by follicular papules and pustules that in more severely affected dogs may develop into alopecia and crusting with secondary bacterial infections and systemic signs. *D. injai* occurs more often in terrier breeds and additionally causes excessive greasiness.
5. Deep skin scrapings (currently the diagnostic method of choice), trichograms, tape strips and examinations of exudate may be useful in identifying *Demodex* mites. More than one mite on any given test is an indication of clinically relevant demodicosis.
6. Dogs with generalised demodicosis should not be bred.
7. Treatment for generalised demodicosis should be monitored clinically and microscopically every month until the second negative skin scraping.
8. In dogs with demodicosis systemic antibiotics will typically not be needed and topical antibacterial therapy combined with good miticidal agents will be sufficient unless severe bacterial infection is present.
9. Weekly amitraz rinses at 0.025-0.05% are effective for canine demodicosis, long-haired animals should be clipped.
10. Oral ivermectin at 0.03-0.06 mg/kg daily, moxidectin at 0.03-0.05 mg/kg daily, milbemycin oxime at 1.0-2.0 mg/kg daily and doramectin injected subcutaneously every week at 0.06 mg/kg are effective therapies for canine demodicosis, but an initial gradual dose increase is recommended for systemic moxidectin and ivermectin to identify dogs sensitive to toxicoses induced by those macrocyclic lactones. Topical moxidectin/imidacloprid should be considered for mild-moderate cases of canine demodicosis.
11. Although not many published studies have evaluated the efficacy of isoxazolines for canine demodicosis in pet dogs, preliminary data is very encouraging and makes this drug class a promising treatment option for dogs with demodicosis.

**Online resources**

With the clinical consensus guidelines, the World Association of Veterinary Dermatology (WAVD) has made an effort to provide up-to-date and relevant information about certain topics in veterinary dermatology, written by international panels reflecting expert opinions from different regions of the world and publish them as open access providing worldwide distribution. For more information, visit: [www.wavd.org](http://www.wavd.org).

This session sponsored by [Royal Canin](https://www.royalcanin.com).
A PRACTICAL APPROACH TO FELINE PRURITUS

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Pruritus (itch) is defined as an unpleasant sensation that, if sufficiently strong, will provoke scratching or the desire to scratch. It is also evidenced by licking, overgrooming, biting, nibbling, and rubbing.

Chronic pruritus is a common reason for consultation in feline dermatology. It is a non-specific symptom associated with a wide variety of causes. A thorough work-up is required to rule out various diseases.

Symptomatic treatment of pruritus can be difficult, and frequently involves a multimodal approach.

Four causes of feline pruritus
The most common causes of pruritus are allergies and ectoparasites, followed by infections, and miscellaneous causes (such as cutaneous neoplasia and pemphigus foliaceus).

Five feline cutaneous patterns
Cats have unique and typical clinical reaction patterns which distinguish them from dogs.

1. Feline miliary dermatitis
2. Feline symmetrical alopecia
3. Feline eosinophilic dermatoses
4. Erosive/crusting dermatosis of head/neck
5. Spontaneously occurring large crusts

Seven steps systematic approach to feline pruritus

1. History
2. Physical and dermatological examinations
3. Minimal database
   - Acetate tape preparation, skin scraping, trichogram (hair plucking)
   - Skin and ear cytology
   - Wood’s lamp examination, PCR (dermatophytes)
   - Dermatophyte culture
   - Flea combing
4. Parasiticidal therapeutic trial (4 weeks)
5. Elimination diet trial (8 weeks)
6. Other diagnostic tests
   - PCR (viral diseases)
   - Skin biopsy/dermatohistopathology
7. Environmental allergy tests (intradermal, serology), and allergen avoidance

Antipruritic therapy

1. Systemic glucocorticosteroids

Avoid long-acting injectable products! Use oral glucocorticoids instead!
Oral glucocorticoids should be commenced at anti-pruritic doses twice daily, and then decreased, as clinical signs abate, to the lowest possible dose and frequency (e.g. once daily to every other day to every 3 days) needed to maintain good quality of life, control of clinical signs and minimal side effects. Oral prednisolone is the superior glucocorticoids choice in cats:

- Dexamethasone 8X more potent on lipid/glucose metabolism
- Decreased hepatic conversion of prednisone (prodrug) into prednisolone
- Decreased gastrointestinal absorption of prednisone vs. prednisolone
- Only 21% of orally administered prednisone appears in blood in prednisolone

The historical use of prednisone in cats may have contributed to the perceived ‘glucocorticosteroid resistance’ (i.e. the perception that cats seem less sensitive to the adverse effects of glucocorticoids than dogs).

2. Oral antihistamines
Various antihistamines have been used in the management of feline pruritus, with variable reported benefit. Their efficacy is unpredictable, and different ones may need to be tried before finding one that works. A fourteen day trial period is recommended.

3. Oral cyclosporine
Oral cyclosporine (Atopica for Cats, Elanco) is labelled for the treatment of feline allergic dermatitis. It should be started at a dosage of 7 mg/kg once daily, and continued at this dosage until a halving or a satisfactory decrease of severity of clinical signs is achieved. After this improvement is reached, the dose should be reduced by either increasing dosage intervals (e.g. going from every day to every other day) or by decreasing the daily dose by half. After a further reduction of signs exceeding approximately 75%, the administration could be reduced to twice weekly or a 75% reduction of the original daily dose.

After beginning cyclosporine administration, the onset of satisfactory clinical benefit normally cannot be expected before four to six weeks. Consequently, the response to this drug should not be assessed, nor dose adjustments be made, for at least one month after commencing therapy.

The most common adverse effects are gastro-intestinal (vomiting, diarrhea/soft stools, weight loss, and anorexia).

4. Behavior-modifying drugs
The use of psychotropic drugs such as amitriptyline, fluoxetine, or paroxetine may be indicated in cats in which a behavioral disorder has been confirmed. It is important to rule out other causes of disease before prescribing these drugs.

5. Allergen-specific immunotherapy
This long-term treatment of feline atopic dermatitis is considered to be safe and effective, with success rates ranges from 60% to 78%. Clinical improvement is usually seen within 3 to 8 months, but can take up to one year in some cats.

This session sponsored by

ROYAL CANIN
Immune-mediated hemolytic anemia (IMHA) is a common cause of anemia in dogs and cats. IMHA can be either primary (idiopathic or autoimmune) or secondary. Primary IMHA, a classic autoimmune disorder with no recognised underlying cause, is the most frequent form of IMHA in dogs and also occurs, albeit less frequently, in cats. The condition typically affects young adult and middle-aged animals, and is most common in cocker spaniels, English springer spaniels, poodles, and, in Australia, old English sheepdogs and Maltese terriers, although there may also be other geographic clusters in other breeds (in Mississippi, for example, we see a bad form of IMHA in Dachshunds). Breed predispositions strongly suggest that there is an inherited susceptibility to IMHA and, in fact, there has been shown to be a strong association between several DLA-79 mutations and multiple immune-mediated diseases, including IMHA, in dogs. Neutered dogs (both male and female) appear to be more prone to IMHA than intact dogs of either sex.

IMHA can also occur secondary to a wide range of infectious, inflammatory or neoplastic processes. Important causes of secondary IMHA in small animals include Feline Leukemia Virus (FeLV) or hemobartonellosis (mycoplasmosis or hemoplasmosis) in cats, and or neoplasia (particularly lymphosarcoma) in dogs. Various medications have also been reported to trigger IMHA. Modified live vaccines have also been implicated, but it is hard to establish causation and, even if vaccines may trigger the occasional case of IMHA, this appears to be an uncommon phenomenon. Secondary IMHA affects animals of any age or breed, and should be strongly suspected in patients with a signalment atypical for primary IMHA, such as geriatric animals. Interestingly, seasonality has been found for IMHA in dogs in some areas (spring and summer in San Diego, for example), potentially suggesting some as yet unidentified environmental or infectious factor. Unlike the dog, IMHA in the cat is (arguably) more commonly secondary, although this varies with location and incidence of infectious diseases that cause secondary IMHA. Distinction between primary and secondary IMHA is therapeutically important because secondary IMHA will often respond poorly to treatment unless the underlying cause is recognized and eliminated.

### Potential Causes of Secondary IMHA

<table>
<thead>
<tr>
<th>Medications</th>
<th>Infectious/Parasitic</th>
<th>Neoplastic</th>
<th>Miscellaneous</th>
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<tbody>
<tr>
<td>Trimethoprim/sulphonamide</td>
<td>Feline leukemia virus infection</td>
<td>Lymphoproliferative disease (esp. lymphosarcoma)</td>
<td>Post-vaccinal</td>
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<tr>
<td>Penicillins</td>
<td>Hemobartonellosis (mycoplasmosis), esp. in cats</td>
<td>Hemangiosarcoma</td>
<td>Elapid snake bites (dogs)</td>
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<td>Cephalosporins</td>
<td>Babesiosis</td>
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<td>Bee stings (dogs)</td>
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<td>Levamisole (dogs)</td>
<td>Bartonellosis</td>
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<td>Pancreatitis (cats)</td>
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<td>Propylthiouracil/methimazole (cats)</td>
<td>Ehrlichiosis</td>
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<td>Non-steroidal antiinflammatories (phenylbutazone)</td>
<td>Dirofilariasis</td>
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<td>Chlorpromazine</td>
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<td>SLE</td>
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<td>Transfusion reactions</td>
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<td>Neonatal isoerythrolysis (esp. cats)</td>
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<tr>
<td>Antilymphocyte globulin (transplantation patients)</td>
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Mechanisms of Red Cell Destruction

The mechanism underlying typical cases of IMHA is antibody-mediated cytotoxic (Type II) destruction of circulating red blood cells (RBCs). Although most cases share this common mechanism, the disease is otherwise very heterogeneous: in primary IMHA, the most studied form of IMHA, both the pattern of immunoglobulin and complement involvement in RBC destruction and the site of antibody attachment to RBC membranes varies widely between patients. Although the most common immunoglobulin type involved in primary IMHA is IgG, less commonly IgM and (rarely) IgA may also be implicated, along with variable involvement of complement, and various combinations of multiple immunoglobulin types (the most common combination is IgG and IgM). Dogs with a combination of both IgG and IgM anti-RBC autoantibodies tend to have lower hematocrits and more autoagglutination. Antibodies have been reported to attach to various components of the RBC membrane, particularly glycophorins and the band 3 anion transport protein, but also a range of other proteins, including calpain, complement component 3, and peroxiredoxin 2. Given the range of different immunoglobins and antigenic targets involved, it is probably overly simplistic to think of IMHA as a single disease syndrome: rather, it is actually a heterogeneous mix of different diseases with different presentations, different prognoses, and different responses to therapy.

Antibody attachment to cell membranes triggers RBC destruction by a number of different mechanisms. With high levels of antibody attachment and, particularly, complement fixation (with involvement of the membrane attack complex), membranes may be so damaged that extracellular water leaks into the cytoplasm, causing swelling and rupture of the RBC while it is still in the circulation, so-called intravascular hemolysis.

Mechanism of Intravascular Hemolysis

In the absence of direct RBC lysis, antibody attachment and subsequent cell membrane damage can still lead to an accelerated rate of destruction of affected RBCs by tissue macrophages within the mononuclear phagocytic system (MPS), a process that occurs outside of the circulation (extravascular hemolysis). MPS destruction of RBCs is mediated by Fc receptors on the macrophage surface, receptors which bind the Fc component of the antibodies attached to the RBC membranes. Since the MPS is located throughout the body, extravascular hemolysis can occur in many organs, but typically is most pronounced in the liver and, particularly, the spleen.
Mechanism of Extravascular Hemolysis

In some patients with high levels of anti-RBC antibodies, many individual antibodies can each bind to two or more different RBCs, a process that causes the cells to clump together (agglutinate). Patients that exhibit significant RBC agglutination at body temperature typically have an increased rate of extravascular hemolysis, since clumping of RBC slows their passage through vessels and facilitates their removal by the MPS.

Typically, IMHA is caused by antibodies directed against circulating, mature RBC, with the marrow mounting a healthy regenerative response to the resultant anemia. However, in some small animal patients (perhaps up to about one third), antibodies may also be directed against marrow RBC precursors at any stage in their development. Hemolytic anemia with an inappropriately poor regenerative response ("non-regenerative IMHA") will develop if antibodies are directed against cell membrane components that are present both on mature RBC and their marrow precursors. In contrast, if antibodies are directed against membrane components that are present only on marrow precursors, and not on mature RBC, non-regenerative anemia will develop without peripheral hemolysis, which has been termed "non-regenerative immune-mediated anemia" if there are still immature erythroid precursors in the marrow. Pure red cell aplasia (PRCA), in which all stages of marrow RBC precursor are reduced or absent, may be the most extreme form of this process. Interestingly, in some dogs with IMHA, lack of regeneration appears to be due to a functional iron deficiency (a mechanism comparable to anemia of chronic disease). Acute phase proteins such as C-reactive protein are markedly elevated in some dogs with IMHA and, since anemia of chronic disease is thought to be a functional iron deficiency mediated by acute phase proteins (hepcidin in particular), it should perhaps not be surprising that this mechanism may affect erythropoiesis in IMHA patients.

In primary IMHA, autoantibodies are directed against components of the patient’s own RBC membrane. Although the same process can occur with secondary IMHA, antibodies may alternatively be directed against a foreign antigen (such as a drug or virus) that is attached to the RBC membrane, against normal RBC membrane components that are antigenically similar to non-RBC antigens that are associated with the underlying disease process, or against membrane components that are normally hidden but are exposed by the underlying disease.

Categories of IMHA

Typical IMHA is caused by antibodies that exert their effects at body temperature, so-called warm reactive antibodies. Some animals, however, have anti-RBC antibodies that are only reactive at much lower temperatures. Although such cold reactive antibodies usually cause minimal harmful effects, their presence can potentially cause specific clinical syndromes, and can also lead to a false positive diagnosis of
IMHA if tests such as slide agglutination are performed at cold temperatures. Classically, IMHA has been sub-divided into five main categories based on the thermal reactivity of the anti-RBC antibodies, and their major clinical effects at optimal temperature:

1. **Warm Antibody Type, Agglutination:**

   High levels of antibody lead to detectable autoagglutination of RBC. Agglutination is often associated with acute severe extravascular hemolysis.

2. **Warm Antibody Type, Intravascular Hemolysis:**

   Intravascular hemolysis, usually associated with high levels of antibody and complement fixation, causing severe anemia with detectable hemoglobinemia and hemoglobinuria.

3. **Warm Antibody Type, Incomplete Antibody:**

   Anti-RBC antibodies cause extravascular hemolysis, without autoagglutination or hemoglobinemia. Disease onset can be chronic or sub-acute, and resultant anemia varies from mild to severe.

4. **Cold Antibody Type, Agglutination:**

   Anti-RBC antibodies are only reactive at cold temperatures, and agglutination does not occur at body temperature. Agglutination can however occur within the vasculature of the extremities, particularly in colder weather. Obstruction of the blood supply to the peripheral vasculature due to agglutination can lead to ischemic necrosis of the ear or tail tips, the end of the nose, and the feet.

5. **Cold Antibody Type, Non-agglutinating Hemolysis:**

   Antibodies are again only reactive at cold temperatures, and hemolysis does not occur at body temperature. In cold weather, however, some degree of hemolysis may occur within the extremities, which manifests clinically as transient hemoglobinemia and hemoglobinuria.

Although the above categorization system is derived by extrapolation from people, all five categories of IMHA have been reported in small animals. Clinical manifestations of severe agglutinating and (especially) hemolyzing cold antibody types of IMHA are however uncommon to rare in both dogs and cats, although antibody-mediated agglutination at low (refrigerator) temperatures is quite commonly detected with routine laboratory tests such as slide agglutination and Coomb’s testing. The significance of antibodies that only agglutinate at cold temperatures, without consistent clinical signs, is unknown. Intravascular warm antibody type IMHA is also relatively uncommon.

**Clinical Signs**

Signs typically associated with IMHA reflect the presence of both anemia (lethargy, weakness, pale mucous membranes, and a hemic heart murmur that corrects with transfusion) and compensatory responses caused by tissue hypoxia and stimulation of the sympathetic nervous system (tachypnea, tachycardia, and bounding pulses). Some patients may also show clinical signs of an ongoing immunological or inflammatory process, such as low-grade pyrexia, anorexia and, uncommonly, lymphadenopathy. Surprisingly, since the MPS within the spleen and liver is usually the main site of RBC destruction, organomegaly is only variably present in animals with IMHA. Patients with IMHA of acute onset tend to be very severely affected by their anemia, and are often very depressed, weak or even collapsed.

Hyperbilirubinemia, bilirubinuria and tissue jaundice are often seen during acute severe episodes of IMHA.
Since intravascular hemolysis is relatively uncommon, hemoglobinemia and hemoglobinuria are observed infrequently. Hemoglobinemia can lead to falsely elevated MCHC, because even hemoglobin that is not inside RBCs is measured, leading to falsely increased estimates of intracellular hemoglobin in animals with low RBC counts. Patients with extravascular hemolysis due to sub-acute or chronic IMHA can compensate to some extent for their lack of erythrocytes, and may be remarkably bright despite the presence of severe anemia. In these patients, the liver can often cope with the extra bilirubin released by RBC breakdown, and jaundice does not occur.

Pulmonary thromboembolism (PTE) is a well-recognised complication of IMHA, and is particularly common in those animals with acute severe anemia that are receiving high dose glucocorticoids. Many dogs with IMHA appear to be prothrombotic, as evidenced by high levels of activated platelets (particularly in thrombocytopenic IMHA patients), increased levels of tissue factor and phosphatidylserine positive microparticles, and a range of altered thromboelastographic markers suggestive of hypercoagulability. Pulmonary thromboembolism should always be suspected in those anemic patients that suddenly develop severe and persistent dyspnea, although other causes of dyspnea such as cardiogenic pulmonary edema or acute bacterial pneumonia should also be considered, especially in dogs already receiving glucocorticoid and immunosuppressive therapy. Severe dyspnea in an IMHA patient in the face of normal or subtly altered thoracic radiographs is highly suggestive of PTE, and advanced imaging techniques such as CT pulmonary angiography can be used to confirm the diagnosis if needed. Transient ascites has also been reported, possibly due to cor pulmonale secondary to PTE, or alternatively due to serositis or fluid accumulation associated with extramedullary hematopoiesis. Disseminated intravascular coagulation (DIC) can also complicate IMHA, but clinically significant DIC is probably uncommon to rare. Moderately low platelet counts are relatively common in IMHA patients, and may often reflect platelet consumption within thrombi rather than true DIC. Very low platelet counts may indicate concurrent IMHA and immune-mediated thrombocytopenia, which has been reported in a small sub-set of IMHA patients.

Diagnosis of IMHA

Hematology in patients with IMHA typically reveals a moderate to severe anemia, which is most commonly regenerative, with anisocytosis, polychromasia, a high corrected reticulocyte count and, sometimes, increased numbers of nucleated RBCs. Reticulocyte counts can however sometimes be inappropriately low, either because antibodies are also directed against RBC precursors, or because anemia is peracute (since it takes about 5 days for the marrow to mount a strong regenerative response and, before that, reticulocyte counts will be normal or “pre-regenerative”). White cell and neutrophil counts are often moderately to markedly increased, probably in response to both non-specific marrow stimulation and the inflammatory process associated with RBC breakdown. Occasionally, white cell counts can be high enough to mimic myelogenous leukemia, a reaction sometimes called a “leukemoid response”. Platelet counts are usually normal unless the animal also has immune-mediated thrombocytopenia (IMT) or platelet consumption secondary to PTE or DIC. Concurrent IMHA and IMT (“Evan’s syndrome”), has been reported to affect up to approximately 10% of dogs with IMHA, but the frequency of Evan’s may be overestimated if the effects of PTE or DIC on platelet counts are not considered as an alternative diagnosis to IMT.

Hematology can often also reveal clues that suggest a specific etiological diagnosis:

1. **Spherocytosis:**

   Spherocytes are small spherical erythrocytes that, when present in high numbers, strongly suggest a diagnosis of either primary or secondary IMHA. The absence of spherocytes, however, does not absolutely exclude a diagnosis of IMHA. Spherocytes are formed when tissue macrophages remove a piece of RBC membrane without cell destruction or a significant loss of cytoplasm. Since cytoplasm is not lost, RBC volume (as indicated by MCV) remains normal. Spherocytes can be difficult to recognize in cats, because normal feline RBCs tend to be smaller and less discoid than canine RBCs. Experienced
veterinary clinical pathologists, however, may be able to recognize the presence of spherocytes in the cat. Rare spherocytes can be found in normal dogs and, rarely, young dogs may have hereditary spherocytosis, a spherocytosis that often (unlike IMHA) causes a low MCV.

2. Agglutination:

Examination of blood smears may reveal microscopic autoagglutination (clumping) of RBCs. Such agglutination can form large rafts of RBC that, when a collection tube containing anticoagulated blood is closely inspected, are visible to the naked eye as multiple red speckles. Similar speckles can however be created by rouleaux formation, a phenomenon that can occur in normal or non-anemic sick animals, especially cats. Clinicians should therefore perform a saline dilution (one drop of RBCs to one or two drops of saline in dogs, one drop of RBCs to two or more drops of saline in cats) slide agglutination test to differentiate rouleaux from genuine autoagglutination. True agglutination can be seen grossly as persistent speckles despite dilution with saline, and microscopically as non-linear clumps of RBCs.

A positive slide agglutination result is strongly suggestive of a diagnosis of IMHA, and also suggests that the condition is likely to be acute and severe. Non-immune autoagglutination, sometimes triggered post-collection by anticoagulants such as EDTA and anecdotally, within the patient following exposure to permethrins, toxins or fungi has, however, also been uncommonly reported in dogs and cats. A negative slide agglutination does not rule out IMHA, since in fact a negative result has been reported in some studies to be the most common result in small animals with IMHA because most actually have non-agglutinating antibodies. Recent clinical studies of canine IMHA, however, report a much higher incidence of positive slide agglutination, perhaps reflecting a referral bias as a result of practitioners tending to refer only the more severe cases of IMHA. Cell washing techniques using repeated centrifugation and saline washes have been reported to decrease the diagnostic sensitivity and increase the specificity of slide agglutination.

Automated hematology analyzers sometimes register a clump of agglutinated RBCs as a single cell, often of a size too large to even be recorded as a RBC at all. Resultant erroneous results may include an artifactually high MCV or, if clumped cells are not recognised as erythrocytes, lowering of the calculated hematocrit. Since the hemoglobin within all RBCs is still measured by the analyzer, this leads to an erroneously high estimation of mean corpuscular hemoglobin concentration (MCHC). When agglutination is suspected to be the cause of a lower than expected hematocrit, packed cell volume (PCV), which is not affected by RBC clumping, should be monitored using microhematocrit tube centrifugation rather than an automated analyzer. Even microcentrifugation techniques for measuring PCV can be prone to erroneous results if the EDTA tube is not thoroughly mixed right before centrifugation.
3. **Ghost Cells:**

*RBC ghosts are cells that have ruptured in the circulation as part of intravascular hemolysis, losing their hemoglobin. Residual RBC membranes linger as ghost cells. Ghost cells can be seen with intravascular IMHA, but also with non-immune causes of intravascular hemolysis such as zinc toxicity and, in small numbers, as an artefact, especially in EDTA tubes that are stored or transported to outside laboratories.*

4. **Other RBC Abnormalities:**

*Careful examination of RBC morphology may suggest an underlying cause of either immunological or non-immunological hemolysis. Diagnostically useful RBC abnormalities include detection of parasites such as Mycoplasma haemofelis or haemocanis or Babesia species (which may cause secondary IMHA), Heinz bodies (suggesting hemolysis secondary to oxidative damage) and schistocytosis (suggesting a microangiopathic hemolytic process such as DIC).*

Serum biochemistry and urinalysis are often normal in dogs with IMHA. Potential abnormalities that may be seen in some patients include mild to moderate elevation of liver enzymes (thought to indicate hepatic hypoxia secondary to severe anemia) and variable hyperglobulinemia. Since serum albumin is usually normal, hypoalbuminemia is an unexpected finding that may suggest that anemia is in fact due to occult blood loss rather than hemolysis, or that the patient also has another illness, although hypoalbuminemia has also been reported in one study to be a poor prognostic indicator in dogs with IMHA. Mild to moderate hyperbilirubinemia and bilirubinuria may be seen transiently in animals with acute severe anemia. Since the liver is usually able to cope with all but the transient overwhelming bilirubin loads produced by acute severe hemolysis, severe hyperbilirubinemia or persistence of jaundice for more than 3 to 5 days, even in the markedly anemic animal, usually indicates the presence of concurrent hepatic disease or biliary obstruction. Hemoglobinemia and hemoglobinuria are uncommon, transient events that indicate the presence of severe intravascular hemolysis. Dogs with IMHA, not surprisingly, have been shown to have increased RBC osmotic fragility, although osmotic fragility testing is laborious, and therefore unlikely to attain common usage in practice.

Many laboratory parameters have been found, in one or more canine IMHA studies, to be suggestive of poor prognosis, including hyperbilirubinemia, hypoalbuminemia, autoagglutination, azotemia, high serum lactate levels, high cardiac troponin 1 levels, cell-free DNA levels, and various inflammatory cytokines such as interleukin-18 and monocyte chemoattractant protein-1, as have non-laboratory indicators like breed (Cocker spaniel), sex (male), need for transfusion, and a high American Society Anesthesiologists (ASA) score, amongst others. None, however, have as yet been consistently shown to reflect prognosis in multiple different studies in dogs with IMHA.
**Immunological Testing**

Specific immunological testing can be used to support a tentative diagnosis of IMHA. The most widely used test is the direct antiglobulin test (DAT) or Coombs’ test, which detects antibodies and/or complement bound to RBC membranes. A standard DAT as provided by many laboratories typically uses a mix of antibodies directed against IgG, IgM (to a variable extent) and complement, and is performed at body temperature. Modifications of the routine screening DAT that have been shown to increase its diagnostic value include running the test at different temperatures and titers, and using individual monovalent antibodies against IgG, IgM, IgA and complement as well as the standard polyvalent antibody/complement mix. Positive DAT results at 4° Celsius, however, may be of dubious diagnostic significance unless the patient has clinical signs consistent with cold antibody type agglutination or intravascular hemolysis.

**Mechanism Underlying Coomb’s Test (DAT)**

![Diagram of Coomb's Test](image)

Gross Agglutination (*Positive DAT*)

Add antiserum with antibodies against IgG and complement

Strictly interpreted, a positive DAT result would support a diagnosis of IMHA, while a negative test result would suggest a non-immunological cause of hemolysis. Numerous studies, however, have shown that a DAT, particularly using polyvalent antibodies, can often be of only mediocre diagnostic accuracy: although sensitivity and specificity undoubtedly improve with meticulous attention to test methodology, the fact remains that even with the best methodologies, both false positive and false negative results probably still uncommonly occur. Veterinarians should therefore be aware that since IMHA can occur in the presence of a negative DAT and, conversely, a positive test does not absolutely prove the presence of IMHA, sometimes a diagnosis may be made based on clinical judgement despite the presence of an apparently discrepant DAT result. Performing a DAT is however still recommended by some hematologists in all patients with suspected IMHA even if criteria such as spherocytosis or a positive slide agglutination already strongly suggest a diagnosis, since a positive DAT will add support to the diagnosis and characterize the disease further by determining the involvement of various immunoglobulin types and complement. Certainly, in cases where the final diagnosis is still in reasonable doubt, a DAT test is strongly recommended.
Various other immunological tests for detecting anti-RBC antibody have been reported, including an enzyme-linked immunosorbent assay, and a direct enzyme-linked antiglobulin test but, although some of these tests may arguably be more sensitive than the DAT, they have not as yet become commonly available. Regardless of whether a DAT or an alternative test for ant-RBC antibody is used, however, clinicians should be aware that a positive result merely records the presence of antibody, and does not determine whether IMHA is primary (AIHA) or secondary.

Very uncommonly, IMHA (with or without IMT) will be merely one component of systemic lupus erythematosus (SLE), a multisystemic immunological disturbance. Measurement of serum anti-nuclear antibody (ANA) is therefore indicated in those patients displaying evidence of the concurrent involvement of more than one body system, such as IMT, glomerulonephritis, polyarthritis, polymyositis or immune-mediated skin disease. In contrast, ANA is not indicated (and is usually negative) in those patients suspected to have uncomplicated IMHA. A high incidence of perinuclear antineutrophil cytoplasmic autoantibodies has been observed in canine IMHA patients, although the prognostic significance of this finding is unknown.

Identification of Underlying Disease

Since IMHA is often secondary, particularly in cats and in dogs with an atypical signalment, confirmation of a diagnosis of IMHA is not necessarily the end of the diagnostic trail. Primary IMHA can only be diagnosed with absolute certainty once potential underlying causes have been thoroughly investigated. Unfortunately, this presents practitioners with a dilemma: although IMHA is unlikely to be treated effectively unless underlying causes have been eliminated, a complete search for such causes can be expensive, time-consuming, invasive and, in the case of primary IMHA, ultimately fruitless. Standard screening tests for underlying disease which ideally should be performed in all animals with IMHA include hematology (including careful examination of a blood smear), serum biochemistry, urinalysis, thoracic and abdominal radiography, abdominal ultrasonography and, in cats, testing for retroviruses (particularly FeLV). Serologic and/or PCR testing for RBC parasites such as hemobartonellosis, now more correctly termed mycoplasmosis (*Mycoplasma haemofelis* in cats, *Mycoplasma haemocanis* in dogs [typically splenectomized dogs]), *Babesia canis* (particularly in greyhounds) or *Babesia gibsoni* (particularly in pit bull terriers, or dogs that have been bitten by pit bull terriers) is also often indicated. Since arguably rickettsial diseases may also predispose to secondary IMHA, testing for *Ehrlichia* species may also be indicated in endemic areas, as may, in dogs, testing for bartonellosis. Further tests that might be considered in some patients, particularly in older animals in which underlying occult neoplasia (especially lymphoproliferative disease) is a real possibility, include lymph node aspiration cytology, and bone marrow analysis.

Bone Marrow Analysis

Bone marrow analysis (aspiration cytology and/or core biopsy histopathology) is indicated in patients suspected to have non-regenerative forms of IMHA. Pure red cell aplasia is characterised by a relative or complete lack of RBC precursors within the marrow, whereas cytological or histopathological evidence of an erythroid “maturation arrest” (preponderance of immature precursors, with an absence of more mature RBC precursors) suggests that, rather than being directed against early stem cells, antibodies are directed against a later stage of marrow RBC development. Marrow cytology and/or histopathology may also reveal macrophages phagocytosing RBCs or RBC precursors. In such patients, when available, techniques such as immunofluorescent or immunoperoxidase staining of marrow samples may confirm the presence of antibodies directed against RBC precursors.
Immune-mediated thrombocytopenia (IMT) is a relatively common cause of bleeding in small animals, particularly the dog. Many differing disease processes may initiate IMT. Despite heterogenous etiologies, most cases of IMT share common pathophysiological features: high levels of platelet-associated antibody, enhanced platelet destruction by the mononuclear phagocytic system (MPS), and markedly decreased circulating platelet life-span. Thrombocytopenia develops when platelet destruction exceeds compensatory platelet production by marrow megakaryocytes.

Pathophysiology

Platelet production (thrombopoiesis) by megakaryocytes maintains circulating platelet numbers that far exceed needs. Spontaneous hemorrhage in dogs (assuming normal platelet function) is extremely uncommon at platelet counts above 50,000/µl, well below the canine reference range of 200,000 to 500,000 platelets/µl. The normal circulating life span of a canine platelet is a little over one week. Senescent (aged) platelets are removed from the circulation and phagocytosed by the MPS, particularly within the spleen.

In IMT, platelet-associated antibody levels are usually increased. Increased antibody binding to platelet membranes enhances destruction of platelets by the MPS, a process mediated by macrophage Fc receptor binding of antibody-coated platelets. The spleen is usually the major organ of immune-mediated platelet destruction, and is also a major source of anti-platelet antibodies. Splenic platelet destruction rates are often markedly increased, up to ten times the rate of normal senescent platelet consumption. The marrow responds to increased platelet consumption by increasing megakaryocyte number and volume: thrombopoiesis can expand up to five times normal in states of excessive platelet destruction.

`Immune-Mediated Thrombocytopenia`
often have a platelet life span of less than one hour. Surviving circulating platelets in IMT patients typically have normal or increased hemostatic function, presumably because of an expanded population of megathrombocytes (young, large platelets).

Immune-mediated thrombocytopenia typically stimulates vigorous thrombopoiesis. Some IMT patients, however, actually have sub-maximal thrombopoiesis, perhaps because anti-platelet antibodies often cross-react with megakaryocytes. Profound megakaryocytic hypoplasia or aplasia (also known as amegakaryocytic thrombocytopenia) is an uncommon finding in canine IMT patients, and is associated with severe bleeding and high mortality rates. As well as affecting platelet numbers, anti-platelet antibodies can also cause platelet dysfunction (thrombopathia). Clinically, the importance of antibody-mediated platelet dysfunction in small animal IMT patients is uncertain. Variations in the degree of thrombocytopenia necessary to induce spontaneous hemorrhage in IMT patients may reflect a balance between the enhanced function of megathrombocytes and the diminished function of antibody-coated platelets.

**Pathogenesis of IMT**

As with immune-mediated hemolytic anemia (IMHA), IMT may be primary or secondary. Primary IMT is a typical spontaneous autoimmune disease, whereas secondary IMT may be initiated by a diverse array of different disease processes that are probably very similar to those processes known to trigger IMHA (see table in Immune-Mediated Hemolytic Anemia lecture notes). Most of the investigations into the pathogenesis of naturally occurring primary IMT have been done in people. Presumably, similar pathogenic processes occur in small animal patients.

Human chronic primary IMT, also called idiopathic or immune-mediated thrombocytopenic purpura (ITP), is a typical autoimmune disease that is clinically very similar to canine IMT. Platelet-associated IgG levels are increased in most patients, and often inversely correlate with platelet count, whereas no consistent correlation has been detected between platelet numbers and platelet-associated IgM, IgA or complement levels. Most primary IMT patients have antibodies directed against platelet membrane glycoproteins such as GP IIb/IIIa and GP Ib/IX. Since these glycoproteins are essential for normal platelet function, the presence of anti-glycoprotein antibodies may explain the platelet dysfunction seen in some patients. Predisposition to develop IMT is thought to be inherited in people, and a genetic predisposition may also explain particular canine breed predilections (including poodle, old English sheepdog and cocker spaniel) for IMT. Primary IMT in cats has been very rarely documented. In the vast majority of instances, IMT in cats is secondary to an underlying disease process.

The pathogenesis of secondary IMT is probably very similar to that discussed in the Immune-Mediated Hemolytic Anemia lecture notes.

**Clinical Signs**

Primary IMT most commonly affects middle-aged female dogs, with an average age of onset of six years. Since IMT is usually secondary in cats, it can occur in cats of any age or sex. Canine IMT typically presents as spontaneous hemorrhage in dogs that previously appeared healthy. Careful questioning, however, may uncover a history of recurrent minor bleeding. Minor trauma or routine surgery may precipitate unexpectedly severe bleeding. Subclinical thrombocytopenia may also be discovered during routine hematology, particularly in cats, since cats seem to be very resistant to significant bleeding despite very low platelet counts. In cases without signs of bleeding, however, it is important to rule out artifact as a cause of a low platelet count. Erroneously low platelet numbers (pseudothrombocytopenia) are very common artifacts seen with hematology analyzer platelet counts, especially in cats.
The hallmark primary lesion in patients with IMT is the petechial (pin-point) hemorrhage. Cutaneous and mucosal petechiae often merge into ecchymotic bruising. Cutaneous bruising typically occurs at sites of either capillary trauma (pressure points) or increased hydrostatic pressure (ventral trunk). Petechiae commonly involve oral, nasal, conjunctival, and urogenital mucosae. Mucosal hemorrhage causes gingival and vulval bleeding, epistaxis, hematemesis, melena, hematochezia and hematuria.

Patients with are often remarkably stable despite marked thrombocytopenia. Cats, in particular, can remain subclinical despite profoundly low platelet counts. Severe thrombocytopenia, however, should always be regarded as a potentially life-threatening disorder. Severe gastrointestinal hemorrhage is the predominant cause of death in canine IMT patients. Less commonly, the loss of even small volumes of blood into a sensitive site such as the eye, brain or spinal cord can cause dramatic clinical signs such as blindness, seizure or paralysis. Nonspecific signs frequently associated with IMT include lethargy, weakness, anorexia, pyrexia, and pale mucous membranes. Splenomegaly is uncommon.

**Diagnosis of IMT**

Routine hematology is the first diagnostic step in patients with suspected IMT. The number of circulating platelets will be reduced, often dramatically (platelet count less than <10,000/μl). Examination of a blood smear may reveal megathrombocytes, indicative of marrow regeneration. Megathrombocytes (also known as “shift” or “stress” platelets) are platelets the same size as, or bigger than, nearby RBCs. Reticulated platelets, immature platelets that are increased in the circulation in conditions causing heightened thrombopoiesis, may also be measured via flow cytometry (when available). Marrow analysis may be indicated if megathrombocyte or reticulated platelet numbers are low, since megakaryocytes may be reduced in number. Anemia (due to hemorrhage or concurrent IMHA) and neutrophilia may be present in IMT patients. Assessment of secondary hemostasis (prothrombin time and activated partial thromboplastin time) will generally reveal no abnormalities.

Primary IMT should be suspected in patients with an isolated severe thrombocytopenia in the absence of any detectable underlying causative disease such as disseminated intravascular coagulation, babesiosis or rickettsial infection. The unequivocal confirmation of suspected IMT then requires the demonstration of anti-platelet antibodies. Reliable tests for anti-platelet antibody, however, are often not readily available, although a sensitive flow cytometric assay is sometimes offered through Kansas State University. The diagnosis of canine IMT in practice often remains a diagnosis of exclusion. In most circumstances, practitioners should feel comfortable with a diagnosis of IMT in patients with isolated moderate or severe thrombocytopenia, reliable indications of increased thrombopoiesis, and no detectable evidence of either multiple hemostatic abnormalities (suggesting DIC) or non-immunologic platelet sequestration, consumption or destruction. Treatment should not be withheld pending measurement of anti-platelet antibody levels.

Microthrombocytosis (presence of small platelet fragments) has previously been reported as a sensitive indicator of the presence of IMT. The technique, however, has not attained common usage.

**Detection of Anti-Platelet Antibody**

Numerous techniques have been developed in people to measure serum levels of anti-platelet antibody. Several of these methods have been modified for application in dogs and cats. The traditional method of measuring serum anti-platelet antibody is the platelet factor-3 (PF-3) immunoinjury technique. Other indirect methods for measuring serum anti-platelet antibody using various radioactive, enzymatic or fluorescent immunoglobulin labels have also been described. Measurement of antibody in serum is convenient for practitioners, because serum may be frozen for storage or transport, and very small volumes are adequate for testing. Unfortunately, the diagnostic utility of testing serum anti-platelet
antibody is limited. Published test sensitivities vary widely depending on the test utilized and the criteria used to define IMT. Many patients with IMT have low serum levels of anti-platelet antibody which do not correlate well with platelet counts. Avid platelet-antibody binding in severely affected animals may effectively remove free antibody from the circulation. Despite low levels of serum anti-platelet antibody, such animals may have profound thrombocytopenia due to high levels of platelet-associated antibody.

The magnitude of antibody binding to platelets or marrow platelet precursors can also be measured. Platelet-associated antibody levels (particularly IgG) appear to consistently inversely correlate with platelet counts. Several techniques for measuring platelet-associated antibody levels in dogs and cats using immunoglobulin labels have been described. Flow cytometric techniques, in particular, hold promise as a means of detecting anti-platelet antibody, even in animals with very few platelets available for measurement because of severe thrombocytopenia. Kansas State University also currently offers flow cytometric measurement of platelet-bound antibodies in suspected IMT cases. Currently available techniques require relatively fresh platelets, necessitating rapid sample handling and transportation. Methods for measuring platelet-associated antibody have not been thoroughly evaluated, and test accuracies are not well determined.

Detection of megakaryocyte-associated antibodies can also provide indirect evidence of concurrent platelet-associated antibodies. High levels of megakaryocyte-associated immunoglobulin have been demonstrated by fluorescent labeling of marrow aspirates from canine IMT patients. Feline primary IMT has also been documented by immunoperoxidase labeling of megakaryocytes in formalin-fixed marrow biopsies. Marrow immunolabeling techniques have, however, not yet been clinically evaluated in large numbers of IMT patients. Immunolabeling will not be possible in those uncommon patients in which megakaryocytic hypoplasia precludes megakaryocyte collection. Adjunct immunodiagnostic testing may sometimes be indicated: patients with SLE may have positive serum ANA, and if IMHA is suspected, a Coombs test should be performed.

No current test for anti-platelet antibody has indisputable diagnostic accuracy and clinical utility. Results of anti-platelet antibody tests should therefore not be the sole basis for clinical decision making. Confirmation of anti-platelet antibody usually does not assist clinical differentiation between primary and secondary IMT. Additionally, many disorders causing thrombocytopenia, although not usually classified as IMT, do have an immune-mediated component, and may therefore cause positive anti-platelet antibody tests. Positive tests may be detected, for example, in dogs with rickettsial infections, and in cats with thrombocytopenia associated with feline leukemia virus or antithyroid medications.

Identification of Underlying Disease

As with IMHA, primary IMT can only be diagnosed with certainty after underlying causes have been investigated. Screening tests for underlying disease which ideally should be performed in all animals with IMT include hematology, serum biochemistry, urinalysis, thoracic/abdominal radiography and, in cats, testing for retroviruses. Serologic or PCR testing for rickettsial infection is also indicated in endemic areas, as is a treatment trial with doxycycline, and testing for babesiosis is indicated in at-risk breeds such as greyhounds and pit bulls. Tests that may be considered in older animals in which IMT with underlying neoplasia is a possibility include abdominal ultrasonography, lymph node aspiration, and marrow analysis.
A number of established immunosuppressive agents have been used in small animal medicine for many decades. Some have justifiably fallen out of favor whereas, for others, new and promising uses have been described in the recent veterinary literature. This lecture will discuss some “old favorites”: cyclophosphamide, chlorambucil, azathioprine, danazol and vincristine.

Cyclophosphamide

Cyclophosphamide, a cell-cycle nonspecific nitrogen mustard derivative alkylating agent, was one of the first major chemotherapeutic agents approved by the FDA over 50 years ago, and has since become very well-established in human medicine as both an antineoplastic drug and as an immunosuppressive agent. Within a few years of FDA approval in the late 1950s, the use of cyclophosphamide for the prevention of transplant rejection in experimental models and for the treatment of both neoplasia and immune-mediated diseases was described in both dogs and cats. Cyclophosphamide has persisted to this day as one of the core drugs used in many small animal cancer chemotherapeutic protocols. In contrast, after many years as one of the most commonly immunosuppressive drugs utilized to treat immune-mediated diseases in cats and dogs, the use of cyclophosphamide as an immunosuppressive agent in small animal patients has in the past two decades essentially faded away. The reasons for the steadily diminishing popularity of cyclophosphamide as an immunosuppressive agent are myriad, and include a high incidence of unacceptable side effects, the development of other immunosuppressive agents that are generally safer and more convenient to administer, and the publication of a number of papers a decade or so ago that suggested that cyclophosphamide was associated with poor outcomes when used to treat conditions such as immune-mediated hemolytic anemia.

Cyclophosphamide is a prodrug that is metabolized by the hepatic cytochrome P450 enzyme system to eventually form active metabolites such as 4-hydroxycyclophosphamide, 4-hydroperoxycyclophosphamide, and aldophosphamide. These metabolites can enter cell cytoplasm, where they are ultimately metabolized to phosphoramide mustard and acrolein. Phosphoramide mustard is an alkylating agent that replaces a hydrogen atom with an alkyl group on the guanine base of DNA, which interferes with nuclear DNA replication and cytoplasmic RNA transcription by forming crosslinks both within and between nucleotide strands. Cyclophosphamide has long been reported to be a potent immunosuppressive that inhibits humoral and cell-mediated immunity, including inhibition of primary and secondary immune responses, reduction of antigen trapping in lymph nodes, and inhibition of local inflammatory responses, although in a number of experimental studies in dogs, cyclophosphamide often appears to be relatively mildly immunosuppressive compared to other drugs.

Cyclophosphamide shares the toxicity profile of most alkylating agents, with common side effects including gastrointestinal signs, myelosuppression, and hair loss. Gastrointestinal signs are relatively common, and include nausea, anorexia, vomiting and diarrhea. Dose reduction and antiemetic agents are often enough to control gastrointestinal signs, but occasionally persistent gastrointestinal side effects will prevent ongoing use of the drug, especially in cats. Myelosuppression appears to be dose dependent, and is associated with both the use of high drug doses and the use of lower doses over a sustained period of time. Moderate to severe neutropenia is the most potentially life-threatening feature of cyclophosphamide myelosuppression, but moderate thrombocytopenia and mild anemia may also occur. Neutropenia is typically reversible with drug dose reduction or discontinuance, but can occasionally persist for weeks or
even months, particularly after chronic cyclophosphamide therapy. Recombinant granulocyte colony-stimulating factor can be used to hasten recovery in dogs with severe cyclophosphamide-induced neutropenia. Alopecia is most common is susceptible breeds such as poodles and old English sheepdogs. Interestingly, cyclophosphamide has been used in the past to “chemically shear” sheep.

A major side effect of cyclophosphamide that is (to a large extent) unique to this drug is the development of sterile hemorrhagic cystitis. Cystitis is mediated by urinary excretion of the cyclophosphamide metabolite acrolein. Cystitis is often severe and debilitating to the patient, and will not resolve until the drug is discontinued. Unfortunately, distressing signs of cystitis can sometimes persist for days or even weeks after drug discontinuation. Cystitis is more likely to develop after long-term therapy with cyclophosphamide, which can present a particular problem for patients with immune-mediated diseases because such diseases are often persistent, and tend to relapse if immunosuppressive therapy is discontinued prematurely. The incidence of cystitis can be reduced significantly by the concurrent administration of furosemide or sodium 2-mercaptoethane sulfonate (mesna), a sulfhydryl donor that binds acrolein, by ensuring ready access to water, and by taking canine patients for regular walks. The chronic local bladder inflammatory effects of cyclophosphamide have also been reported to predispose to the development of irreversible bladder wall fibrosis and transitional cell carcinoma.

Over the years, a number of immune-mediated and inflammatory diseases in dogs and cats have been treated with cyclophosphamide, including immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia, megakaryocyte hypoplasia; pure red cell aplasia, systemic lupus erythematosus, immune-mediated polyarthritis, inflammatory bowel disease, glomerulonephritis, noninfectious inflammatory meningoencephalitis, immune-mediated vasculitis and pemphigus. For many years, cyclophosphamide was considered a “big gun” to be used in dogs with severe or life-threatening IMHA. However, in the late 1990s and early 2000s, a number of case studies were published that suggested that, at best, cyclophosphamide was not better than glucocorticoids alone for the treatment of IMHA and, at worst, associated with a higher than expected mortality rate. Given the known limitations of retrospective studies, including the associated potential for “case selection bias” (that is, the dogs with the most severe IMHA may have been given cyclophosphamide because it was the drug perceived to be most potent), it is hard to know with any real certainty whether cyclophosphamide actually worsens prognosis in dogs with IMHA. Nevertheless, there is no doubt that, since publication of these papers, the use of cyclophosphamide to treat conditions such as canine IMHA has markedly decreased.

Compared to many immunosuppressive agents, cyclophosphamide has been relatively cheap, with a generic 25 mg tablet costing under $2, and a 50 mg tablet costing under $4, although there has been a recent (hopefully temporary) marked surge in drug prices, with a 50 mg tablet now costing over $10. One of the major problems associated with using cyclophosphamide as an immunosuppressive agent is that it is difficult to dose accurately, and even more difficult to taper, especially in smaller patients. Cyclophosphamide is available as 25 or 50 mg tablets that composed of an active inner tablet surrounded by an inert outer flecked tablet. Because of uneven distribution of the drug through the tablet, cyclophosphamide tablets cannot be split or crushed without a risk of major dosing inaccuracies. Without drug compounding, cyclophosphamide doses must therefore be in multiples of 25 or 50. Published immunosuppressive doses for cyclophosphamide in dogs include 50 mg/m² or 1.5 to 2.5 mg/kg every second day or daily on a “4 days on, 3 days off” weekly protocol. In cats and small dogs, similar total weekly doses can be used, but “pulsed” at infrequent dosing intervals that ensure that the total weekly dose is equivalent to seven times the calculated daily dose. Since myelosuppression can occur at any time during chronic cyclophosphamide therapy, complete blood counts must be performed regularly throughout the course of drug treatment. Cyclophosphamide is available in an intravenous form as well as an oral form, and a recent study in dogs confirmed that equivalent oral and intravenous doses of cyclophosphamide achieved comparable blood levels of the active metabolite 4-hydroxycyclophosphamide. Intravenous cyclophosphamide may therefore be a viable treatment option in vomiting animals that are unable to tolerate oral immunosuppressive agents.
Chlorambucil

Chlorambucil is a nitrogen mustard derivative cell-cycle nonspecific alkylating agent that has, for many decades, been used in both human and veterinary medicine predominantly as an antineoplastic agent for the treatment of cancers such as lymphoid leukemia, lymphoma, mast cell tumors, multiple myeloma and polycythemia vera. Antineoplastic cytotoxicity is derived from inappropriate cross-linkage of cellular DNA and RNA by insertion of alkyl radicals on the purine base, guanine. Chlorambucil also has immunosuppressive properties, and has occasionally been used in human medicine to treat immune-mediated and inflammatory conditions such as glomerulonephritis. More than 30 years ago, some veterinary clinicians began suggesting the use of chlorambucil as an immunosuppressive agent for our small animal patients. Since then, the use of chlorambucil for the treatment of a number of feline inflammatory skin conditions, such as pemphigus and eosinophilic granuloma complex, and for treatment of diseases such as immune-mediated thrombocytopenia and refractory inflammatory bowel disease, has become very well established, primarily because of a paucity of viable alternative medications that could be accurately dosed with safety in cats. The use of chlorambucil as immunosuppressive agent in dogs has been slower to evolve, but its use has been described for the treatment of pemphigus, glomerulonephritis and, most recently, inflammatory bowel disease. It is somewhat surprising that chlorambucil has not attained more common usage as an immunosuppressive agent in dogs, since it appears to have much the same mechanism of action as cyclophosphamide with significantly less onerous side effects (specifically, chlorambucil does not cause sterile cystitis), and comes in a more convenient tablet size.

Chlorambucil is metabolized predominantly in the liver, primarily to the active metabolite phenylacetic acid mustard. Compared to other alkylating agents, chlorambucil is relatively well tolerated, especially at immunosuppressive doses, but does occasionally cause gastrointestinal side effects such as vomiting and diarrhea, and/or myelosuppression with neutropenia, thrombocytopenia and non-regenerative anemia (anemia is usually mild). Alopecia and poor hair growth are sometimes reported in susceptible dog breeds, such as poodles. Neurologic side effects are reported with chronic chlorambucil use in people, and chlorambucil-associated neurologic signs (including myoclonus, twitches and seizures) have been reported in cats. Recently, acquired Fanconi syndrome has also been reported in cats on chlorambucil.

Chlorambucil is available as a coated 2 mg tablet that cannot feasibly be divided, and dosing recommendations in smaller patients are therefore typically provided in multiples of two, and/or “pulsed” at infrequent dosing intervals (given at an interval that ensures the overall weekly dose is equivalent to seven times the calculated daily dose) in order to avoid overdose. For immunosuppressive therapy, chlorambucil is almost always given in combination with an oral glucocorticoid. In dogs, recommended starting oral immunosuppressive chlorambucil doses (with a glucocorticoid) range from 0.1 to 0.2 mg/kg (or, alternatively, 4 to 6 mg/m^2) every one to two days, with dosing individualized based on patient size and disease severity. In cats (and small dogs) with inflammatory or immune-mediated disease, a starting oral chlorambucil dose of 2 mg every second day (with a glucocorticoid), tapered to every 3rd or 4th day, is my preferred dosing regime, although a number of other tapered dosing protocols are also available. Lower daily doses of chlorambucil, comparable to dog dosing regimes, can also be used in cats if the drug is compounded, but the effects of compounding on drug efficacy have not been established. Complete blood counts must be monitored regularly (weekly at first) and, since myelosuppression tends to be dose-dependent rather than idiosyncratic, doses can be tapered “to effect” rather than discontinued completely. Myelosuppression, provided it is detected promptly, is typically reversible.

Compared to many other immunosuppressive agents, chlorambucil has until recently been moderately priced. Unfortunately, the patent on the only available chlorambucil product, Leukeran®, recently expired, leading to a change in ownership of the company responsible for distributing the drug, and the US price of chlorambucil has doubled as a result, to over $10 for a 2 mg tablet. There are currently no other US generic alternatives, apart from compounded products. The efficacy of compounded chlorambucil in dogs and cats
Azathioprine has not been established, although anecdotally veterinarians have reported success when switching from the proprietary to the compounded product.

**Azathioprine**

Azathioprine has been used as an immunosuppressive agent in dogs for over 50 years. The drug was initially primarily used in studies that utilized dogs as a model for investigations of organ transplantation and the effects of immunosuppression on various body systems. Within a few years, azathioprine was also being used to treat naturally occurring diseases in dogs. Despite almost half a century of cumulative clinical and research experience on the use of azathioprine in dogs, however, there have been remarkably few studies that actually elucidate the precise effects that azathioprine has on the canine immune system. Most of our understanding of the mechanism of action of azathioprine in dogs is extrapolated from work in other species.

Azathioprine is a prodrug for the active metabolite 6-mercaptopurine, and the primary mechanism of action was long believed to be inhibition of the synthesis of the purines adenine and guanine by blockage of enzymes such as amidophosphoribosyltransferase, with resultant production of nonfunctional nucleic acid strands. Disruption of purine synthesis inhibits DNA and RNA synthesis, thereby inhibiting the proliferation of fast-growing cells such as lymphocytes. In the past few decades, however, multiple other mechanisms of action mediated by various azathioprine metabolites have been proposed, including blockage of T cell activation and stimulation of T cell apoptosis. Azathioprine has long been reported to be more effective against T cell function than B cell function, although strong evidence supporting this is lacking, and recent work in our laboratory demonstrated that azathioprine inhibited both B and T cell proliferation.

One of the key enzymes involved in azathioprine metabolism and inactivation is thiopurine methyltransferase (TPMT). Individual human patients (about one in 300 people) inherit a marked deficiency in the TPMT enzyme that renders them highly susceptible to azathioprine toxicity, particularly life-threatening bone marrow suppression. Interestingly, cats have also been shown to have a marked deficiency in enzyme activity, which may explain why azathioprine causes marked myelosuppression in cats at standard canine doses. Although the use of azathioprine at a very reduced dose rates has previously been published in cats, given the narrow margin for safety it is probably wisest to recommend that azathioprine never be used in cats at any dose, especially considering the availability of other immunosuppressive agents that appear to be much safer in cats, such as chlorambucil and cyclosporine. Although TPMT expression in dogs is widely variable, severe deficiencies in enzyme activity of the magnitude seen in cats and some people have not been commonly reported, and TPMT deficiency does not appear to be associated with the severe drug toxicities sometimes seen in dogs.

The standard azathioprine starting dose in dogs is 2 mg/kg orally once daily. This dose is usually well-tolerated and, although gastrointestinal side effects such as nausea, anorexia, vomiting and diarrhea are occasionally reported, they are typically mild and self-limiting. Although, in dogs, marked myelosuppression is uncommon, chronic azathioprine usage sometimes causes mild to moderate poorly regenerative anemia. Since anemia is a possible outcome in dogs receiving azathioprine, and is typically very well tolerated (that is, sub-clinical), mild to moderate anemia alone should not be mistaken as evidence of either drug overdose or treatment failure. Azathioprine can also cause profound myelosuppression or severe hepatotoxicity in dogs. Marked myelosuppression and hepatotoxicity appear to be idiosyncratic non-dose-dependent drug reactions (Type B reactions), and are typically reversible if the problem is recognized early enough and azathioprine is discontinued. Myelosuppression is uncommon, but hepatotoxicity (typically characterized by a rise in reversible rise in ALT in the absence of clinical signs) occurs in about 15% of dogs. Anecdotally, some veterinarians believe that hepatotoxicity is more common with the generic forms of azathioprine compared to the proprietary product. Complete blood counts and serum biochemistry panels (especially ALT) should be monitored regularly during initial azathioprine
therapy. Several individual case reports have also reported pancreatitis in dogs receiving azathioprine, but cause and effect has not been established.

Azathioprine has, over the years, become well-established as an “add on” immunosuppressive agent to be considered for the treatment of many different immune-mediated and inflammatory conditions when glucocorticoids alone are ineffective or poorly tolerated, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, inflammatory bowel disease, chronic hepatitis, glomerulonephritis, immune-mediated polyarthritis, myasthenia gravis, non-infectious meningoencephalitis, immune-mediated skin diseases, and anal furunculosis. Despite decades of azathioprine usage, evidence supporting immunosuppressive efficacy for many of these common diseases is remarkably limited. Interestingly, because (despite a relative paucity of evidence) azathioprine has commonly been recommended as the standard immunosuppressive drug of choice for many conditions, the efficacy of newer drugs for the treatment of these conditions is sometimes compared to a parallel group receiving azathioprine. One perceived “limitation” of azathioprine compared to other immunosuppressive agents, that it can take many weeks or even months to exert its effects, is based on limited and dated data derived predominantly in humans. In my experience, azathioprine in a clinical setting exerts its immunosuppressive effects in dogs about as rapidly as most other comparable agents, and recent research in our laboratory also demonstrated inhibition of canine lymphocyte proliferation within 2 weeks of commencing azathioprine.

Compared to most other immunosuppressive agents, azathioprine is relatively inexpensive, which is an important consideration with long-term immunosuppressive therapy, especially in large dogs. While the proprietary product (Imuran® or Azasan®) typically still costs over $5 per 50 mg tablet, the generic equivalent can be obtained for less than $1 a tablet. Recently, however, it can sometimes be difficult to locate the cheaper generics because of some production shortages. The smallest tablet size is 50 mg (although tablet scoring permits a 25 mg dose), which can present dosing problems in small (under 20 lb) dogs.

**Danazol**

Danazol, a synthetic androgen with weak (“impeded”) androgenic effects, has in the past been suggested for the treatment of canine immune-mediated hemolytic anemia and immune-mediated thrombocytopenia, in combination with glucocorticoids, in order to reduce the dose of steroid that is needed. Danazol is derived from the synthetic steroid ethisterone, a modified progestogen. Danazol’s most important mechanism of action is probably to reduce macrophage Fc receptor/antibody binding affinity. Danazol also competes with glucocorticoids for combination with steroid-binding globulin, consequently increasing the availability of active unbound glucocorticoid. Concurrent danazol therefore enables significant glucocorticoid dose reduction. Danazol may also reduce the degree of binding of antibody and complement to the red blood cell or platelet surface. Side effects are uncommon, and include hepatotoxicity and masculinization of female dogs. However, although some dogs with refractory IMHA and IMT have been reported to benefit from danazol, the drug fell out of favor a few decades ago, probably because it was very expensive at the time, and response to therapy was sluggish and highly unpredictable.

Reported oral danazol doses in dogs with IMHA or IMT, in combination with glucocorticoids, range from 5 to 15 mg/kg daily, either given as a single dose or 2-3 divided doses. Danazol comes in 50 mg, 100 mg, and 200 mg capsules. Danazol currently costs about $3 for a 50 mg capsule, and not much more for the 200 mg capsule.

**Vincristine**

The vinca alkaloids are biologically-active dimeric alkaloids derived from the Madagascar (or rosy) periwinkle plant, Catharanthus roseus. Vincristine, a naturally-occurring vinca alkaloid, were originally characterized phytochemically more than fifty years ago. The diverse biological effects of vincristine have traditionally been attributed to drug-induced disruption of various intracellular microtubules. Microtubules
Microtubules are composed predominantly of complex helical polymers of the structural protein tubulin. Vinca tubulin binding include the mitotic spindle in dividing cells, the neurotubules in neurons, and the cytoskeletal microtubules in platelets. Vinca may also exert biological effects that are independent of disruption of intracellular microtubules, such as inhibition of RNA, DNA and protein synthesis, and modification of prostaglandin production.

Vincristine is a cell-cycle-specific cytotoxic agent. Vincristine disrupts microtubules within the mitotic spindle of dividing cells, thereby arresting chromosomal separation in metaphase. Vincristine at standard therapeutic doses is minimally myelotoxic, and is therefore commonly used in combination with more myelosuppressive chemotherapeutic agents. Vincristine is frequently used in veterinary cancer chemotherapy, both as a single agent for the treatment of canine transmissible venereal tumors, and as a component of combination protocols for the treatment of acute leukemia, lymphoreticular neoplasms, mast cell tumors, and various carcinomas and sarcomas.

Vincristine is usually administered intravenously as a sulfate salt, which is chemically more stable than its corresponding free base. Inadvertent subcutaneous or intramuscular administration causes severe local tissue irritation and necrosis. Oral absorption of vincristine is poor. Plasma disappearance of vincristine following intravenous administration is markedly biphasic, with a short initial half-life and a prolonged terminal half-life. The short initial clearance phase reflects extensive extravascular drug redistribution due to a combination of both avid binding to intracellular tubulin and rapid biliary excretion. The prolonged terminal clearance phase is due to the gradual release of vincristine bound to circulating plasma proteins and intracellular tubulin. Platelets demonstrate a remarkable ability to concentrate vincristine from plasma, and are therefore the principal circulating cellular carriers of the drug.

The degree of immunosuppression induced by vincristine at intravenous therapeutic doses is minimal compared to that induced by glucocorticoids, cyclophosphamide or azathioprine, and vincristine therefore is not used as an immunosuppressive agent for the treatment of most immune-mediated or inflammatory diseases in dogs and cats. The one exception is immune-mediated thrombocytopenia (IMT), where vincristine has become a mainstay of treatment.

During early clinical trials in human cancer patients, it was observed that the administration of vincristine was frequently associated significant but transient increases in circulating platelet numbers. A similar phenomenon has since been reported in dogs, both in research animals and in cancer patients. This effect appears to be due to increased megakaryocytopenia and thrombopoiesis, although the precise mechanisms of vincristine-associated thrombocytosis are still uncertain. Circulating platelet life-span does not appear to be significantly affected by standard low doses of vincristine in healthy animals.

The serendipitous discovery that vincristine induced thrombocytosis in human cancer patients with normal pre-treatment platelet numbers prompted conjecture that a similar outcome could be obtained in thrombocytopenic patients. Following publication of several anecdotal reports describing prompt, marked increases in circulating platelet numbers after administration of vincristine to people with IMT, vincristine gained favor with some hematologists as the treatment of choice for chronic refractory IMT. Vincristine frequently induces partial or complete remission of thrombocytopenia within one week of commencing therapy, although such remissions are typically transient. Only a relatively small proportion of human chronic refractory IMT patients achieve complete sustained remission with vincristine therapy.

Rapid drug clearance from plasma reduces the therapeutic efficacy of a standard intravenous bolus of vincristine. Several alternate methods of vincristine administration have therefore been used in human IMT patients in order to sustain therapeutic plasma concentrations. Constant intravenous vincristine
infusion (over six to eight hours) effectively maintains therapeutic plasma concentrations the drug throughout the period of administration. Alternatively, the ability of platelets to concentrate vinca alkaloids from plasma has been utilized to enhance therapeutic efficacy via transfusion of vincristine-loaded platelets. Incubation of donor platelets in high concentrations of vincristine (vinca loading) prior to transfusion maximizes intracellular vinca-tubulin binding. Following transfusion, circulating vinca-loaded donor platelets gradually release vincristine into the recipient’s plasma, thereby sustaining therapeutic plasma drug concentrations. Both constant rate infusion with vincristine and transfusion with vinca-loaded platelets induce sustained remissions in some human chronic IMT patients previously refractory to single intravenous boluses of the drug.

Vincristine, typically in combination with prednisone, has been reported to similarly facilitate remission of thrombocytopenia in many canine patients with IMT. Original case reports demonstrating an apparent rapid response to vincristine in dogs with IMT have been supported, decades later, by evidence obtained from prospective studies. Circulating platelet numbers increase markedly within three to five days of vincristine administration in responsive dogs, and the addition of vincristine to standard immunosuppressive therapy in dogs with IMT appears to shorten hospitalization time by several days. Most authors currently recommend an intravenous vincristine bolus dose of 0.02 mg/kg for the treatment of canine IMT. Vincristine boluses may subsequently be repeated weekly if thrombocytopenia recurs. Apparent rapid clinical response to vincristine-loaded platelets has been reported in one dog with refractory IMT. Vincristine has been used in cats with IMT, although evidence of clinical efficacy is lacking. One significant advantage of vincristine compared to other therapeutic options for IMT (such as human intravenous globulin) is that vincristine is inexpensive (a 1 ml vial of 1mg/ml vincristine sulfate costs around $20).

The pathogenesis of vincristine-induced remission of thrombocytopenia in IMT patients is uncertain. Clinicians initially assumed that remissions were due to increased megakaryocyte production and release of platelets, the principal mechanism assumed to underlie the vinca-induced thrombocytosis seen in healthy animals and cancer patients. However, since IMT patients typically already have high levels of circulating thrombopoietic factors and maximal thrombopoiesis, platelet precursors may be refractory to further stimulation by vincristine. Furthermore, studies in people suggest that the main therapeutic effect of vincristine in IMT patients is not increased thrombopoiesis. Post-treatment average platelet life-spans are significantly prolonged in human IMT patients that respond to vincristine, suggesting that remission is due to reduced platelet destruction rather than increased platelet production. Since platelets are the major circulating cellular carriers of vincristine, researchers have speculated that antibody-coated platelets selectively deliver vincristine to those phagocytes within the mononuclear phagocytic system that are actively involved in platelet destruction. This proposed mechanism explains why, despite being an ineffective immunosuppressive agent for the treatment of most conditions, vincristine can still be very effective for the treatment of IMT.

During electron microscopic studies of platelet ultrastructure, it was discovered that prolonged incubation of platelets in vincristine solutions caused marked disruption of cytoskeletal microtubules. Laboratory investigations have since demonstrated that as well as disrupting platelet structure, exposure to high concentrations of vincristine also significantly impairs platelet function. Based on the in vitro evidence that exposure to vincristine impairs platelet function, hematologists expressed concern that using the drug in patients with IMT could similarly induce platelet dysfunction. Subsequent studies revealed that vincristine affected platelet function (aggregation) in dogs with lymphoma, but not in healthy dogs. Since several recent prospective studies showed no significant increase in bleeding in IMT dogs receiving vincristine, the effect of vincristine on platelet function, if it occurs, does not appear to be severe enough to be clinically significant.

Neurotoxicity, although uncommon, is the most frequent significant side-effect associated with therapeutic doses of vincristine in dogs and cats. Reversible vincristine-induced neurotoxicity in the dog
has been reported with chronic cancer chemotherapy, but is not likely to be an issue with the single doses used to treat IMT. Other side-effects such as gastrointestinal disorders (including megaesophagus and gastric hypomotility) and alopecia, occur less frequently and are typically mild and temporary. Vincristine at doses used for IMT typically causes minimal myelosuppression in dogs, although dogs with the ABCB1-1Δ (MDR1) gene mutation and some Border Collies have been reported to be more susceptible than other dog breeds to myelosuppression, especially at antineoplastic vincristine doses. In affected Border Collies, this effect appears to sometimes be independent of the MDR1 gene mutation reported in this breed. Genetic testing prior to vincristine is recommended in breeds at high risk of the MDR1 gene mutation, such as Collies and Australian Shepherds, and drug doses should be reduced by 50% in homozygous affected dogs, and by 25% in heterozygous affected dogs. Temporary erythrodysplasia of erythroid precursors in the bone marrow and peripheral blood smears, featuring bizarre mitotic figures, abnormal nuclear configurations, and Howell-Jolly bodies, can be observed after administration of vincristine in dogs, but is of little clinical significance. An unusual transient pulmonary toxicity has been reported in a cat receiving chemotherapeutic doses of vincristine. Vincristine has no known mutagenic or carcinogenic potential.

Given the high price of many human immunosuppressive agents, and also given the dosing difficulties associated with giving human tablet and capsule sizes to our smaller patients, veterinarians are often tempted to instead use compounded equivalents of these drugs. Compounded versions of many of these drugs can be found at on-line pharmacies that cater to the veterinary market at attractive prices and convenient dosing sizes. However, the bioavailability and clinical efficacy of most of these products in our patients is not established and, with the few drugs where the compounded version has been evaluated (cyclosporine, for example), drug bioavailability was markedly variable and often led to subtherapeutic blood concentrations. Using these products in our patients, especially in those animals with life-threatening disease, therefore represents a major gamble. Generic equivalents of proprietary products, in contrast, are probably likely to be comparable in efficacy to “brand name” products, and are sometimes significantly cheaper.

In animals that are difficult to give pills to, it is tempting to use a liquid compounded formulation. However, many of the common immunosuppressive agents are potentially mutagenic, carcinogenic and teratogenic. In fractious animals that end up with more medication on their whiskers and fur than in their mouth, given liquid suspensions has the potential to significantly increase the level of owner exposure to these potentially dangerous drugs.
Several potent immunosuppressive drugs developed over the past few decades in human medicine have recently made the leap to our small animal patients, and our use of them is growing. This lecture will discuss cyclosporine, leflunomide and mycophenolate.

Cyclosporine

Cyclosporine is a potent immunosuppressive drug indicated for the treatment of inflammatory and immune-mediated diseases, and for organ transplantation. Cyclosporins are cyclic polypeptide macrolides originally derived from the soil fungus *Beauveria nivea* (*Tolypocladium inflatum*), but are also produced by other fungal organisms. Cyclosporine A is the molecule developed for commercial use as an immunosuppressive agent. Discovered in the 1970s, the use of cyclosporine as an immunosuppressive agent was first described in humans to prevent rejection of renal allografts. Within a decade, cyclosporine had become the cornerstone of immunosuppression for organ transplantation. In veterinary medicine, oral cyclosporine capsules received FDA approval in 2003 for the treatment of canine atopy, and were more recently also approved for allergic skin disease in cats. Cyclosporine has been used in an extra-label fashion for many years for renal transplantation in dogs and cats, and for the treatment of a variety of inflammatory and immune-mediated conditions.

Cyclosporine’s primary immunosuppressive mechanism of action is inhibition of T lymphocyte function. Cyclosporine acts to inhibit calcineurin, an intracellular protein phosphatase that activates gene transcription factors through dephosphorylation. In the untreated patient, activation of T cells results in activation of calcineurin, which dephosphorylates inactive nuclear factor (NFAT). NFAT translocates into the nucleus, where it upregulates transcription of genes coding for several important cytokines, including IL-2, IL-4, TNF-α, and INF-γ. Production of IL-2 in particular plays a key role in the activation and proliferation of T cells. Calcineurin inhibitors, including cyclosporine, act by binding to intracellular cyclophilins, which are proteins that facilitate protein folding. Binding of cyclosporine to cyclophilin A creates a complex with high affinity for calcineurin. Through inhibition of calcineurin, cyclosporine specifically inhibits T cell function and thus, cell-mediated immunity, but has little immediate impact on humoral immunity. Decreased IL-2 expression in CD4+ Th1 cells associated with cyclosporine therapy leads to inhibition of proliferation and activation of both T-helper and T-cytotoxic lymphocytes, and blunting of the immune response. Cyclosporine has also been shown to have many other local anti-inflammatory and immunosuppressive effects, especially in the skin.

Cyclosporine is a large lipophilic molecule which must be solubilized prior to intestinal absorption. Commercial cyclosporine is available as two very different types of oral formulations. Cyclosporine was initially approved in humans as a vegetable-oil based preparation (*Sandimmune®*), but variability in oral bioavailability caused marked variability in blood drug concentrations. A more recent formulation, an ultramicronized (“modified”) preparation approved in 1996 (*Neoral®*), forms a microemulsion upon contact with aqueous fluids, resulting in more consistent and predictable absorption. Oral bioavailability of the microemulsion is improved by up to 50% compared to the oil-based preparation. Because of the marked variability in bioavailability of the non-ultramicronized (*Sandimmune®*) preparation, it is not recommended for oral use in small animals.

Cyclosporine has a high binding affinity for red blood cells and plasma lipoproteins. Because up to 50% of the drug in blood is located in red cells, whole blood is recommended for therapeutic drug monitoring.
Once in the circulation, cyclosporine distributes widely, accumulating in the skin, liver, kidneys, and fat of dogs, resulting in a large volume of distribution. Tissue levels exceed levels in serum by a factor of 3 to 14. Peak blood concentrations generally occurring approximately 2 hours after oral administration of cyclosporine. Blood concentrations then rapidly decrease over the remainder of the dosing interval, reflecting a relatively rapid half-life as the drug is cleared from plasma.

Extensive metabolism of cyclosporine by the hepatic cytochrome P-450 system yields many different metabolites, some of which may retain therapeutic efficacy. In dogs, several drugs that inhibit P-450 enzymes have been given concurrently with cyclosporine in order to decrease the dose needed to maintain adequate blood drug concentrations. Ketoconazole, in particular, has been used to decrease oral cyclosporine dosages in dogs by as much as 75 percent, although individual responses are variable.

The complexities of cyclosporine disposition in normal animals, coupled with confounding factors associated with disease and differences in drug preparation, may contribute to markedly variable blood drug concentrations both between patients and even within the same patient. Therapeutic management may therefore be facilitated by monitoring blood cyclosporine concentrations. Unfortunately, however, the process of adjusting drug doses based on monitoring cyclosporine blood concentrations is clinically complex, and not necessarily associated with the desired clinical outcome. Currently available methods for TDM include HPLC, a specific monoclonal RIA, and a dimersion cyclosporine immunoassay. HPLC has the advantage that the parent drug can be discriminated from metabolites, although most methods detect only the parent compound. Both RIA and dimersion cyclosporine immunoassay, in contrast, measure metabolites as well as the parent drug, and blood cyclosporine concentrations will therefore be higher by a factor of 1.5 to 1.7 compared to the same sample analyzed using HPLC. Although HPLC is considered the gold standard for cyclosporine assays, HPLC is labor intensive and not routinely offered for patient monitoring. TDx and RIA have been the methods most often employed in clinical situations, with the laboratory performing the assay typically providing recommendations regarding ideal target blood drug concentrations. Some laboratories have adjusted target blood concentrations upward to reflect the fact that TDx and RIA results will be approximately double HPLC assay results. Other laboratories have not made this adjustment, with the rationale that the cyclosporine metabolites measured by the TDx and RIA assays may arguably be pharmacologically active and contribute to overall immunosuppressive effects. Much study has gone into determining the most appropriate sample collection time in patients receiving cyclosporine. In human medicine, trough blood concentrations were the initial basis for adjustment of drug dosages. However, multiple studies in people have since suggested that area under the plasma drug concentration time curve (AUC) or 2 hour peak drug concentrations are preferred. With measurement of peak cyclosporine concentrations requiring only a single sample, adjusting drug doses to attain target peak drug levels has become the single best blood concentration measurement for use during human organ transplantation. In veterinary medicine, measurement of trough cyclosporine concentrations also prevailed for many years based on initial work done in canine and feline renal transplant studies. Recommendations from laboratories offering TDM have often involved measurement of both peak and trough cyclosporine blood levels, although target peak concentrations have not been well established. Individual laboratory recommendations depended on the target ranges determined by each laboratory as well as the assay used to measure cyclosporine concentrations. Currently, the Auburn University Clinical Pharmacology Laboratory is the only veterinary pharmacology laboratory routinely offering cyclosporine blood level assays.

Pharmacodynamic assays investigate a drug’s effect on target cells. Several pharmacodynamic biomarkers of the immunosuppressive effects of cyclosporine have been studied in human medicine, including lymphocyte proliferation, calcineurin enzyme activity, lymphocyte surface antigen expression, and intracellular cytokine quantification. Through pharmacodynamic monitoring, human studies have shown individually distinct degrees of calcineurin inhibitor sensitivity in patients. Pharmacodynamic monitoring shows great promise for optimizing cyclosporine therapy and delivering individualized therapy. At Mississippi State University, we have conducted extensive investigations into the pharmacodynamic
evaluation of cyclosporine in dogs. We initially measured activated T cell expression of IL-2, IL-4, and IFN-γ via flow cytometry in dogs administered two different oral cyclosporine dosages. The dogs were first administered a high dose of cyclosporine (10 mg/kg orally twice daily), with doses adjusted upwards as needed to attain a target trough drug concentration greater than 600 ng/mL as measured via HPLC, a dosing protocol known to be sufficiently immunosuppressive for canine organ transplantation. With high dose cyclosporine, activated T cell expression of IL-2 and IFN-γ was significantly suppressed. The dogs were then administered the FDA-approved dose of cyclosporine used to treat canine atopy (5 mg/kg orally once a day), a dose which has been considered to be low enough to avoid predisposing to immunosuppression-associated infection. Even with this low dose of cyclosporine, however, T cell expression of IFN-γ and IL-2 was still markedly suppressed in some dogs. Subsequent studies evaluating activated T cell mRNA IL-2 and IFN-γ expression utilizing molecular methods have demonstrated that results using a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay are comparable to flow cytometry, and that the technique shows promise as a pharmacodynamic assay in dogs. One advantage of the qRT-PCR assay compared to flow cytometry is that it can be performed on blood samples mailed in by practitioners. This assay has now been offered to practitioners for several years through our Mississippi State University Pharmacodynamic Laboratory, and assists veterinarians in adjusting oral cyclosporine doses in dogs to optimize systemic immunosuppressive effects. Cyclosporine has been shown to have much the same effect on T cell cytokine production in cats as it does in dogs, but a pharmacodynamic assay based on this effect in cats is not yet commercially available.

Cyclosporine is FDA-approved for the treatment of canine atopic dermatitis and feline allergic skin disease, and has also been used to prevent transplant rejection and to treat sebaceous adenitis, pemphigus foliaceus, anal furunculosis, feline stomatitis, inflammatory bowel disease (IBD), myasthenia gravis, non-infectious inflammatory meningoencephalitis, pure red cell aplasia, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (IMT), and immune-mediated polyarthritis in dogs and cats. Pharmacodynamic research evaluating T cell responses to cyclosporine in dogs has confirmed that canine responses are comparable to the response profile that is well recognized in people: that individual responses to cyclosporine are extremely variable from dog-to-dog, both in dogs receiving the same standard oral dose, and in dogs with oral doses adjusted to attain comparable blood levels. Given that a high degree of variability of individual responsiveness to cyclosporine has been established in dogs, cyclosporine dosing protocols should be tailored to allow for this patient-to-patient variability. In my opinion, recommended dosing protocols in dogs with chronic, non-life-threatening inflammatory skin and gastrointestinal diseases should be quite different from the protocols used in dogs with more acute and life-threatening immune-mediated diseases.

In chronic inflammatory diseases that are typically not immediately life-threatening, such as skin conditions, anal furunculosis, and mild IBD, cyclosporine is often effective at a standard, relatively low starting dose. Cyclosporine therapy is typically delivered long term, with drug doses adjusted upwards if needed “to effect”, based predominantly on clinical signs. Most commonly, however, starting doses do not need to be increased and, in the long-term, the cyclosporine dosage is typically tapered to the lowest effective dosage needed to maintain disease remission. Currently recommended starting cyclosporine doses in dogs are 5 mg/kg once daily for most skin diseases and IBD, and 5 mg/kg once to twice daily for anal furunculosis. In cats with skin conditions such as allergic skin disease, eosinophilic granuloma complex and pemphigus foliaceus, a starting cyclosporine dose of around 5-8 mg/kg daily is recommended. Cyclosporine blood concentrations are usually not necessary for treatment of these conditions, as remission of disease is the main criterion used to decide whether adequate cyclosporine therapy is being delivered. In fact, for many of these conditions, cyclosporine blood concentrations have been shown to have minimal correlation with disease remission, perhaps because the drug is selectively concentrated in tissues such as the skin. Recent pharmacodynamic studies, however, have shown that, even at standard low FDA-approved doses, some dogs can still develop significant suppression of certain T-lymphocyte biomarkers of immunosuppression despite very low trough cyclosporine concentrations. This could explain the phenomenon reported by dermatologists, that individual dogs treated for atopic dermatitis can
develop severe secondary infections, although the “atopy” cyclosporine dose was originally not thought to cause clinically significant immunosuppression. Therefore, even in dogs on low cyclosporine doses, clinicians should remain vigilant for potential signs of systemic infection.

In canine patients suffering from more acute and immediately life-threatening diseases such as severe IMHA and IMT, in contrast, cyclosporine must be targeted to attain effective immunosuppression as rapidly as possible. These animals are somewhat comparable to patients that have recently undergone organ transplantation, in that any delay in attaining effective immunosuppression can lead to a disastrous outcome. In these patients, starting cyclosporine at a low dose and adjusting doses upwards “to effect” is not recommended. Attaining effective oral doses as rapidly and accurately as possible is essential for ensuring adequate immunosuppression whilst avoiding overdosage with associated adverse effects and expense. Currently recommended starting cyclosporine doses for life-threatening diseases range from 5 mg/kg to 10 mg/kg twice daily. Subsequent measurement of blood cyclosporine concentrations and/or assessment of activated T cell mRNA IL-2 and IFN-γ expression using qRT-PCR within one week of commencement of treatment, with dose adjustments as needed, are the best methods that are currently routinely available to assess adequacy of therapy, and are strongly recommended in patients with life-threatening diseases.

Side effects are uncommon with cyclosporine therapy in dogs and cats, with the exception of gastrointestinal side effects such as vomiting, diarrhea, anorexia and nausea. Administering the medication frozen and/or with food can reduce gastrointestinal side effects, although there is a risk that such measures will also alter drug absorption profiles. Uncommonly, cyclosporine can cause an idiosyncratic hepatotoxicity, which does not seem to be dose dependent. Gingival hyperplasia and hypertrichosis have also occasionally been reported with cyclosporine therapy. Chronic cyclosporine therapy may also predispose to neoplasia such as lymphoma. One advantage of cyclosporine as an immunosuppressive agent is that it is not myelosuppressive. Experimentally, oral cyclosporine has been shown to increase platelet thromboxane production, which may be a concern in patients with IMHA, where hypercoagulability and resultant pulmonary thromboembolism can be a major contributor to patient mortality. However, to date, it has not been demonstrated whether this phenomenon is clinically relevant in IMHA patients with naturally occurring disease. Furthermore, recent work in our laboratory has shown that, when cyclosporine and low-dose aspirin are given concurrently, the aspirin nullifies the surge in thromboxane seen in dogs that are receiving cyclosporine alone.

Cyclosporine is an expensive drug, particularly at higher immunosuppressive doses, and clinicians are therefore tempted to explore cheaper forms of the drug. In human medicine, there are many approved human generic microemulsion (“modified”) preparations similar to the Neoral® formulation, and these generic preparations have been shown to have therapeutic equivalency in people. Studies investigating the pharmacokinetic properties of these generic preparations in dogs have not been performed, and it is not safe to assume that a generic formulation is therapeutically equivalent to the approved canine product (Atopica®). Clinically, there appears to be some variability seen in individual dogs in the oral bioavailability of these generic products. Use of generic products may therefore have the potential place our patients at risk of either therapeutic failure or toxicity although, if blood drug levels or pharmacodynamics assays are used to monitor therapy, this risk is minimized. The proprietary human microemulsified cyclosporine product, Neoral®, currently costs around $2 for a 25 mg capsule and $6 for a 100 mg capsule, while the generic equivalent equivalents cost around $2 and $3 for the 25 mg and 100 mg capsules accordingly. The veterinary product, Atopica®, tends to be priced comparably to the human proprietary products, but has the advantage of being FDA-approved and available in a range of capsule sizes that are convenient for dosing accuracy in our small animal patients (10 mg, 25 mg, 50 mg and 100 mg), as well as a 100 mg/ml oral suspension. Non-modified (Sandimmune® or equivalent) cyclosporine has highly unreliable bioavailability, and should not be used. Nor should compounded cyclosporine, because compounders usually do not specify if the product is modified or non-modified. Unfortunately, transdermal cyclosporine has been shown to be inadequately absorbed in cats.
Leflunomide

Leflunomide is an isoxazol derivative immunosuppressive drug that was developed within the past two decades, initially for treatment of rheumatoid arthritis and prevention of transplant rejection. Leflunomide is a prodrug for its primary active malononitriloamide metabolite, A77 1726 (also known as teriflunomide). Malononitriloamides reversibly inhibit the mitochondrial enzyme dihydroorotate dehydrogenase, a key enzyme in pyrimidine synthesis, with resultant inhibition of the pyrimidine ribonucleotide uridine monophosphate (rUMP), and decreased DNA and RNA synthesis and G1 cell cycle arrest. Leflunomide inhibits B and T cell function, suppresses antibody production and has anti-inflammatory effects, possibly via inhibition of de novo pyrimidine biosynthesis and cytokine-associated and IL-2-stimulated tyrosine kinase activity.

Prior to commercial development, leflunomide was made available for small animal transplant research to Dr. Clare Gregory’s group at the University of California, Davis. Because of the drug’s availability to this group, a small number of canine patients with refractory naturally-occurring inflammatory and immune-mediated diseases such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, non-infectious inflammatory meningoencephalitis, systemic histiocytosis, immune-mediated polymyositis, immune-mediated polyarthritis, and pemphigus foliaceous were also treated, typically with promising success rates. Unfortunately, when these initial promising results were reported at the ACVIM Forum and in the veterinary literature in the late 1990s, the drug was not commercially available. When leflunomide did become available, as the proprietary product Arava®, the drug was so prohibitively expensive that its use was very limited in small animal clinical studies. Even after the generic equivalent was approved in 2005, leflunomide remained expensive for several more years. Only recently did the generic drug become more affordable and, as a result, anecdotal and preliminary reports of leflunonide’s use in small animal patients are beginning to surface. There are therefore currently very few published reports discussing the use of leflunomide in dogs and cats. Recently, a case series describing the use of leflunomide in 14 dogs with immune-mediated polyarthritis reported a high response rate with minimal side effects, and a recent abstract also reported a high success rate, with few side effects, in a series of about 100 dogs with various immune-mediated and inflammatory diseases.

One of the most promising features associated with the early usage of leflunomide in dogs was that it appeared to be very well tolerated. However, as anticipated with most drugs only recently introduced to veterinary medicine, as the drug attained more common usage, occasional more serious side effects have been recognized. The most common side effect reported with leflunomide use in dogs was occasional inappetence, lethargy and vomiting, but a recent study in dachshunds with inflammatory colorectal polyps also reported a relatively high incidence of hepatopathy (about half the treated dogs, although it was difficult to elucidate the impact of concurrent glucocorticoids) and several instances of significant myelosuppression. Serious side effects occasionally reported in people, and thus with the potential to appear in our veterinary population with more common usage, include myelosuppression, cutaneous drug reactions, lung disease and hepatotoxicity. In humans, traces of the active metabolite teriflunomide can persist for months or even years after drug discontinuation, and in the instance of severe drug reactions, cholestyramine or activated charcoal is needed to rapidly reduce drug levels. In dogs, the terminal half-life of teriflunomide is much shorter than in humans, so the potential for persistent side effects is probably significantly less. Complete blood counts and serum biochemistry (especially ALT) should be regularly monitored in small animal patients on leflunomide.

The initial recommended starting oral dose for leflunomide in dogs is 2-4 mg/kg daily, with doses adjusted to attain a plasma trough A77 1726 level of 20 µg/ml within a few weeks of commencing therapy. For most dogs, a dose of 2 mg/kg daily appears to be sufficient to control most immune-mediated or inflammatory diseases. For cats with immune-mediated polyarthritis, a leflunomide dose 10 mg (total dose) orally, once daily, in combination with methotrexate, has been suggested, with dose reductions to effect. Measurement of blood drug levels (teriflunomide) is available through the Auburn University Veterinary
Clinical Pharmacology Laboratory. One advantage of leflunomide is that it comes in tablet sizes (10 mg and 20 mg) that are convenient for dosing our smaller patients. Leflunomide as the proprietary product Arava® currently costs around $40 for a 10 mg tablet and, interestingly, $40 for a 20 mg tablet, although it is rumored to soon be discontinued. The generic leflunomide equivalent was until recently priced at around $1 for a 10 mg tablet and $1.50 for a 20 mg tablet although, regrettably, that price just went up to $6 and $12 respectively (although good deals can still variably be found through GoodRx.com, which provides discount coupons that are accepted by some, but not all, pharmacies). Drug availability, unfortunately, has recently been patchy. Leflunomide generics, as with many commercially available generic problems, have an ‘AB’ rating by the FDA, meaning that the generic is “equivalent” to Arava®. However, since “equivalence” is often determined by pharmacokinetic data in healthy individuals, an AB rating does not guarantee identical performance in clinical patients.

Mycophenolate

Mycophenolate mofetil is the synthesized prodrug form of mycophenolic acid, a selective and reversible inhibitor of inosine monophosphate dehydrogenase, an enzyme that controls the rate of synthesis of guanine monophosphate in the de novo pathway of purine synthesis. Mycophenolate mofetil is a fermentation product derived from fungi in the *Penicillium* group. Mycophenolic acid inhibits B and T cell proliferation, and decreases antibody production. Mycophenolate mofetil is primarily used in human medicine for prevention of rejection of transplanted organs, although it also used to treat immune-mediated diseases such as systemic lupus erythematosus, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia and pemphigus vulgaris. Mycophenolate mofetil is often used in the place of azathioprine in human medicine and, since they have similar mechanisms of action, the two drugs should not be used together.

The original proprietary mycophenolate mofetil product, CellCept®, and the closely related mycophenolate sodium product, Myfortic®, were expensive, and as a result the products only achieved limited usage in small animal medicine. However, more recently, the availability of much cheaper generic alternatives has led to a greatly increased usage of mycophenolate mofetil in small animal patients. A single 250 mg CellCept® capsule currently costs around $9, whereas the equivalent generic 250 mg capsule costs less than 50c. An oral suspension version of mycophenolate mofetil (200 mg/ml) is available for more convenient dosing in smaller patients. Successful usage of mycophenolate mofetil in a small animal patient with naturally-occurring disease was first described in a dog with acquired myasthenia gravis. Much of the subsequent anecdotal usage of mycophenolate mofetil for a variety of different immune-mediated diseases was similar to the dosing reported in this original paper. Mycophenolate mofetil is also available in an injectable form, and the intravenous use of the drug has been described during the successful initial stabilization of three dogs with acquired myasthenia gravis that could not tolerate oral medications. Ironically, a more recent case report of 15 dogs with acquired myasthenia gravis treated with mycophenolate mofetil reported that the drug was ineffective at attaining clinical remission. Recent papers reporting the use of mycophenolate mofetil in dogs with IMHA or IMT have shown variable results: while individual dogs appear to respond to therapy, overall response rates are no better than (and, often, worse than) those seen with more established drugs, and gastrointestinal side effects can often limit clinical usefulness of the drug. Recently, a retrospective case series reporting the use of mycophenolate mofetil for the treatment of meningitis of unknown origin in dogs showed promising results: interestingly, mycophenolate has also shown some promise in treating multiple sclerosis in people, where it is believed to have a neuroprotective effect. In my opinion, however, the clinical effectiveness of mycophenolate for treating most immune disease in dogs has not yet been well-established. The extensive protein binding of the drug, which can vary widely from patient to patient, may explain variable and unpredictable responses to drug, as could variations in drug metabolite profiles produced in individual profiles. Promisingly, recently completed work in our laboratory has established that mycophenolate mofetil at maximally tolerated doses (in individual dogs, this dose varies between 10 mg/kg and 20 mg/kg twice daily) does significantly
inhibit lymphocyte proliferation in normal dogs, although this effect is not observed until two weeks into therapy, suggesting a delayed response to the drug.

A recommended starting dose for mycophenolate mofetil in dogs is 10-20 mg/kg twice daily, although often gastrointestinal signs (particularly vomiting and, especially, severe diarrhea) at the higher end of the dose rate will necessitate dose reductions. Recent work by our group has found that, often, diarrhea doesn’t develop until after approximately one week of therapy, and so it shouldn’t be assumed that higher doses will be well-tolerated until 1-2 weeks of therapy have passed without gastrointestinal side effects. In stable patients, a low end starting dose of 10 mg/kg twice daily is probably advisable. Mycophenolate mofetil appears to have variable oral bioavailability in dogs, so variability in response to therapy should probably be expected. An older pharmacodynamic study in dogs measuring inosine monophosphate dehydrogenase enzyme activity suggested that mycophenolate mofetil would best be dosed three times daily, but this recommendation has not entered common usage. Mycophenolate mofetil has not been used widely enough in veterinary medicine to establish the frequency of serious side effects but, in people, gastrointestinal signs and, less commonly, marked myelosuppression and a rare and fatal neurologic disease (progressive multifocal leukoencephalopathy) have been reported. Based on the human side effect profile, complete blood counts should probably be regularly monitored in dogs receiving mycophenolate mofetil. In humans, gastrointestinal side effects can be reduced by replacing mycophenolate mofetil with mycophenolate sodium, but this does not appear to help reduce gastrointestinal side effects in dogs. Mycophenolic acid in humans is primarily excreted conjugated to glucuronide and, since cats lack the glucuronyl transferases responsible for glucuronidation of drugs such as mycophenolate mofetil, concern has been expressed that the drug should used with caution, if at all, in this species. However, the use of mycophenolate mofetil has been described at a dose rate of 10 mg/kg twice daily, with no obvious side effects, in two cats with IMHA. Recent work at Washington State University, reassuringly, has found that cats seem to be able to metabolize mycophenolate rapidly, by actually utilizing glucosidation rather than glucuronidation.

One ‘side effect’ that is common to all immunosuppressive agents, both established and new, is that they can cause significant immunosuppression. This is highly desirable when treating severe life-threatening diseases, but comes with the significant associated risk that immunosuppression also predisposes to infection. Severe infection and even, occasionally, infection-associated deaths have been associated with most of the immunosuppressive agents used in dogs and cats. This is especially true when high doses of potent drugs are used, or when multiple drugs are used in combination, such as the kinds of protocols that are used to prevent transplant rejection. As well as bacterial infections, immunosuppressed patients can develop all kinds of unusual infections, including toxoplasmosis, mycobacteriosis, nocardiosis, and generalized demodecosis.

Although the risk of infection is always going to be present when immunosuppressive agents are used, a few general guidelines can help to reduce this risk:

- Avoid using powerful immunosuppressive therapy to treat minor diseases that are not life-threatening, and instead save the “big guns” for more severe illnesses.
- Use the lowest effective drug doses that are possible.
- Avoid using combinations of multiple different immunosuppressive agents unless absolutely necessary.
- Screen patients very carefully for underlying infectious disease before commencing immunosuppressive therapy, especially when infection can mimic immune-mediated disease (Babesia gibsoni masquerading as IMHA, for example).
- Watch patients on immunosuppressive therapy closely for signs of new infection.
Cannabis (marijuana) toxicosis is becoming an increasing concern for veterinarians as pets are exposed to a variety of recreational and medical marijuana products. Previous studies have shown an increase in accidental exposures in both veterinary and human medical practice, associated with the legalization of cannabis.  

**Cannabis**

The *Cannabis sativa* plant produces chemical compounds called cannabinoids, the most notable being delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the major psychoactive cannabinoid and is primarily responsible for the signs encountered in cannabis toxicity. The concentration of THC in each product can vary greatly and depends on the strain of plant, cultivation techniques, what part of the plant is used, and how the plant is processed after harvest.

THC acts on cannabinoid receptors, in particular the CB1 receptor in the brain. It is highly lipid soluble and accumulates rapidly in adipose tissue, which results in a prolonged half-life. Excretion of THC and its metabolites occurs primarily via the biliary route (with enterohepatic recirculation) and the rest is excreted in the urine.

**Routes of exposure**

Cannabis exposure in dogs can occur by many routes, but by far the most common is by ingestion of recreational or medical cannabis products. Exposure by inhalation or dermal routes is possible but signs of toxicity are less common and less severe.

Ingestion of “joints” or marijuana plant material are common sources of exposure in dogs and can occur in environments away from the home (eg parks, sidewalks). The more concerning ingestions in terms of toxicity come from concentrated cannabis products (eg hashish, hash oil, shatter, wax) where THC is extracted and can reach concentrations of 80% or higher. These concentrated cannabis products can then be incorporated into “edibles” such as cookies, butter, brownies and candies, and pose a life threatening risk to pets and children if left unattended. The edible products also pose another potential threat if they contain other toxins like chocolate.

**Signs of Toxicity**

Signs of cannabis toxicity often occur within 30-60 minutes of ingestion but can vary greatly in severity depending on the product ingested, THC concentration, age of patient, body weight, and underlying medical conditions.

The most common signs include depression, ataxia, mydriasis, urinary incontinence, bradycardia, hypothermia, and hypersensitivity to light and sound. Gastrointestinal upset can also occur, especially if a large amount of dry plant material was ingested or if the edible product contained butter, other fatty/rich foods, or chocolate. Signs of severe toxicity include severe tremors, seizures, coma, tachycardia, hyperthermia, loss of airway protective reflexes, and hypoventilation.
**Diagnosis**

Diagnosis of cannabis toxicity in dogs is largely by the owner’s account of witnessed, or suspected, ingestion. A high index of suspicion is warranted for patients presenting with acute neurologic signs and potential for exposure (in the household or neighbourhood).

Human urine drug screens are available and can be used to support a diagnosis of marijuana ingestion. These drug tests have not been validated for use in dogs and this must be kept in mind when interpreting results. False negatives can occur if ingestion was recent and it is possible that metabolites produced by dogs are not detected by the test.

**Treatment**

Decontamination with emesis +/- activated charcoal can be attempted in dogs with recent ingestion as long as they are asymptomatic, or mildly effected. This should not be attempted in dogs with altered mentation, tremors, severe hypersensitivity, or respiratory concerns due to the risk of aspiration. The anti-emetic effect of cannabis may make emesis induction unsuccessful in some patients.

Treatment for symptomatic cannabis toxicosis is entirely supportive. There is no antidote for THC overdose.

Mild cases can often be managed at home or in the hospital for observation. Treatment is focused on maintaining normal body temperature and hydration, as well and confinement to a safe area until ataxia and hypersensitivity have resolved. Antiemetics and subcutaneous fluids may be of benefit while signs of toxicity are wearing off. It is important to keep in mind that some cases may appear mild initially but can progress with time, especially if products with high THC content were ingested.

Moderate toxicity cases may require admission to the hospital for monitoring and supportive care. Heart rate, temperature, blood pressure, respiratory rate/effort, and neurologic status monitoring is warranted. IV fluid are often started to maintain hydration and for cardiovascular support until the patient is alert enough to eat and drink. Antiemetics are useful, especially if the patient is drooling or appears nauseous. Sedatives like diazepam may be needed to calm tremors and anxiety/agitation.

Cases of severe cannabis toxicity require hospital admission and potentially intensive care. Supportive care is provided including IV fluids and monitoring of vital signs, with particular attention given to the patient’s neurologic and respiratory status. If protective airway reflexes are lost, the patient needs to be intubated. If respiratory rate/effort are inadequate (visual or by elevated ETCO₂ or arterial/venous CO₂), the patient may need assisted ventilation. Symptomatic treatment with antiemetics, thermal support, anti-convulsants etc are administered as needed.

Lipid emulsion is another treatment option for severe cannabis toxicity. It is generally reserved for life-threatening cases and/or for cases that are progressing rapidly and financial limitations will prohibit ICU care. A reference is provided below with more detailed background on lipid use for toxicities in veterinary medicine and potential risks.³

- Check for lipemia before starting, especially if fatty food was ingested with cannabis. Check hematocrit tube or spin down plasma/serum.
- 20% lipid emulsion – bolus dose 1.5 mls/kg over 1-2 minutes then 0.25 mls/kg/min for 30 minutes.
- Dose can be repeated in 4-6 hours if needed AND if no signs of lipemia.
Prognosis
The overall prognosis for cannabis toxicity is good and most patients can be treated with outpatient care or a short stay in the hospital. Significant improvement in typically noted within 12–24 hours of ingestion, but signs can persist for days in some cases. Toxicity has the potential to be fatal in extreme cases, either due to life threatening complications of toxicity, or due to financial limitations prohibiting intensive care.

Prevention
Pet owner awareness becomes increasingly important as cannabis legalization approaches in Canada. Clients should be aware of all medications and potential toxins in the house and take care to keep these away from pets and children. Clients also need to be vigilant when away from the house as marijuana exposure is common in public settings.


Hyperglycemic Emergencies

Hyperglycemic diabetic crises are complications of unregulated diabetes mellitus and are often precipitated by underlying disease. They are due to an imbalance between insulin (absolute or relative deficiency) and counter-regulatory hormones, which, in turn, disrupts normal glucose utilization. Successful treatment requires prompt diagnosis and intervention directed at restoring hydration/perfusion, acid-base/electrolyte balance, normal glucose handling, and treatment of underlying/concurrent disease.

Diabetic Ketoacidosis (DKA)

Pathophysiology

- Ketone bodies are formed from fatty acids in the liver and used as an alternate energy source when intracellular glucose is inadequate
- There are 3 ketone bodies: beta-hydroxybutyrate (dominant ketone), acetoacetate, and acetone.
- Metabolic acidosis results from accumulation of ketone bodies and is compounded by factors such as dehydration, azotemia, vomiting.
- Counter-regulatory hormones (cortisol, glucagon, catecholamines, growth hormone) are often increased due to concurrent disease.
- Common concurrent diseases: urinary tract infection, pancreatitis, hyperadrenocorticism (dogs), chronic kidney disease, neoplasia, acromegaly (cats), hepatic lipidosis (cats).

Diagnostic Evaluation

Initial work-up can differentiate between uncomplicated diabetes mellitus, diabetic ketosis, and diabetic ketoacidosis, and identify underlying diseases that may alter the treatment plan or prognosis.

- **D (hyperglycemia, glucosuria)** – initial blood glucose (BG) is often too high to read on a glucometer or in house chemistry analyzer. Dilute the sample to get a number if possible.
- **K (ketosis)** - urine dipstick detects acetoacetate NOT beta-hydroxybutyrate, making false negatives possible. Serum or plasma ketones can also be detected using the urine dipstick. Serum beta-hydroxybutyrate can be measured at a reference lab or using a portable meter.
- **A (metabolic acidosis)** - look at the HCO₃ (usually < 15 mmol/L) and the pH (usually < 7.35)

- **Electrolytes**
  - Potassium – can be low, normal, or high on baseline lab work
    - Hypokalemia is expected during treatment and can be profound, especially after starting insulin therapy.
  - Sodium – usually low on baseline lab work
    - Pseudohyponatremia - high BG causes fluid to shift from the intra to extracellular space and dilutes out the Na.
- Na decreases approx. 1.6 mEq per 5.6 mmol/dL increase in BG from normal
  - Phosphorous - can be low, normal, or high on baseline lab work
    - Hypophosphatemia often develops during treatment; can cause hemolysis if severe.
  - Magnesium – often normal initially but can become hypomagnesemic during treatment.
- Underlying disease - CBC, chemistry profile, urinalysis and culture +/- radiographs, abdominal ultrasound, spec PLi etc

**Treatment**

1) Fluid Therapy
   - Isotonic crystalloid – eg Normosol R, PlasmalyteA, Lactated Ringers, 0.9% NaCl.
   - Correct hypovolemia with fluid bolus(es) if needed to restore cardiovascular parameters. NO additives in this fluid bag.
   - Rehydration and maintenance fluid plan to correct dehydration over 12-24 hours and keep up with ongoing losses (gi losses, osmotic diuresis etc). Electrolyte additives and dextrose can go in this fluid bag.
   - Monitor BG, as it is likely to drop with initial fluid therapy.

2) Insulin
   - Insulin restores normal glucose utilization and prevents ongoing ketoacid formation.
   - Start approximately 4-6 hours after initiation of fluid therapy.
   - Goal is to decrease BG slowly (< 2-5 mmol/L/hour).
   - Check BG every 2 hours; more frequently if rapid decline or other concern.

   **Intermittent IM Technique** - Regular (Toronto) insulin (U-100)
   - 0.1-0.2 U/kg initial IM injection
   - 0.05-0.2 U/kg subsequent doses every 1-4 hrs, based on BG and rate of decrease
   - Add 2.5-5% dextrose to maintenance IV fluids if BG < 14

   **CRI technique** – Regular (Toronto) insulin (U-100)
   - See chart below. Check BG and adjust rate every 2 hours.
   - Supplement with dextrose if BG < 11, to allow continued insulin administration.
   - Run 20-50 mls of insulin solution through IV line before administering; insulin adheres.

   Many alternate protocols noted in references - glargine SQ and IM (cat), glargine SQ and regular insulin IM (cat), lispro CRI (dog and cat), insulin aspart CRI (dog).

### CRI Technique for Regular (Toronto) Insulin. U-100.

<table>
<thead>
<tr>
<th>Blood Glucose mmol/L</th>
<th>Dextrose (in maintenance fluids eg NormR, LRS, P-lyteA)</th>
<th>Regular Insulin CRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 14</td>
<td>0%</td>
<td>10 mls/hr</td>
</tr>
<tr>
<td>11–14</td>
<td>2.5%</td>
<td>7 mls/hr</td>
</tr>
<tr>
<td>8-11</td>
<td>2.5%</td>
<td>5 mls/hr</td>
</tr>
<tr>
<td>5.5 - 8</td>
<td>5%</td>
<td>5 mls/hr</td>
</tr>
<tr>
<td>&lt; 5.5</td>
<td>5%</td>
<td>discontinue insulin CRI</td>
</tr>
</tbody>
</table>
3) Electrolytes
   • **Potassium**
     o Supplement with potassium chloride (KCl) when starting insulin, if not sooner.
     o 20-40 mEq/L to start (if not hyperkalemic); may need to increase to 80 mEq/L or more if severe/refractory hypokalemia.
     o Do not exceed 0.5 mEq/kg/hr – in extreme cases where necessary to exceed this amount, the patient must have constant monitoring including continuous ECG.
     o Adjust as needed based on serial blood electrolytes. Check at least every 6-12 hours.
   • **Phosphorous**
     o 0.01-0.12 mmol/kg/hr using potassium phosphates (Kphos); often start at 0.03 mmol/kg/hr
     o Remember to account for K in Kphos when calculating total K in fluids.
     o Check at least every 12-24 hrs.
   • **Magnesium**
     o 0.5-1 mEq/kg/day for hypomagnesemia, Magnesium sulphate IV CRI.
     o Check every 12-24 hrs.
   • **Sodium**
     o Hyponatremia usually corrects itself with initial treatment as BG decreases.
     o Can use 0.9% NaCl for maintenance fluid if low Na is not improving as expected.
   • **Bicarbonate**
     o Rarely needed. Consider if severe acidemia (pH < 7) after initial fluid therapy.
     o Replace 1/4 to 1/3 deficit. NaHCO₃ IV over 30-60 min. Re-evaluate blood gas.
     o $\text{HCO}_3^-$ Deficit = 0.3 x weight x (15-patient $\text{HCO}_3^-$)

4) Treat Underlying Disease
   • Antibiotics if indicated, antiemetics/promotility agents, analgesics, nutritional support etc.

5) Transition to long-acting insulin
   • Criteria for transition to long-acting insulin:
     o Ketones negative (or at least trace)
     o Clinical improvement and underlying disease improved
     o Patient is eating well (or supplemented with a feeding tube) and transitioned to regular feeding schedule (or close to).
   • Discontinue insulin CRI/IM injections and dextrose at least 4 hours before the patient is due for long-acting insulin dose.

Prognosis
   • Depends on the severity of DKA crisis and concurrent illness but overall prognosis is fair (70%).
   • Risk of recurrent DKA in lifetime.
   • Diabetic remission is still possible in cats that have previously been treated for DKA.

Hyperglycemic Hyperosmolar Syndrome

Pathophysiology
   • Small amount of insulin and hepatic glucagon resistance inhibit ketone formation.
   • Osmotic diuresis leads to severe hyperglycemia, hyperosmolality and dehydration (including cerebral dehydration); reduced GFR must be present to result in such profound hyperglycemia.
   • Severe hyperosmolality induces idiogenic osmoles in the brain.

Criteria
• Blood glucose > 30-33 mmol/L
• Ketones usually absent but can be trace or small
• Serum osmolality > 325-350 mOsm/L
  o 2(Na+K) + BUN mmol/L + glucose mmol/L = calculated serum osmolality
  o 2(Na) + glucose = effective osmolality

Treatment – Treatment for HHS is much the same as for DKA discussed above, but the focus is on correcting hypovolemia/dehydration and less on early insulin administration.  Changes from DKA protocol noted below.

1) Fluid Therapy
   • 0.9% NaCl is the initial fluid of choice.  Na in NaCl replaces glucose and helps prevent a rapid shift in osmolality.
   • Correct hypovolemia with fluid bolus(es) if needed to restore cardiovascular parameters. NO additives in this fluid bag.
   • Rehydration and maintenance fluid plan to correct dehydration over 24-48 hrs (slower than DKA).  Isotonic crystalloid eg Norm-R, P-lyteA, LRS, 0.9% NaCl etc.  Keep up with ongoing losses (gi losses, osmotic diuresis etc). Electrolyte additives and dextrose can go in this bag.
   • Monitor BG, as it will drop with initial fluid therapy. Goal to drop BG < 2-4 mmol/L/hr.
   • Monitor neuro status carefully – risk of cerebral edema if rapid decrease in BG.
   • Supplement and monitor electrolytes as per DKA protocol above.

2) Insulin
   • Start after at least 4-8 hours of fluid therapy AND decline in BG has slowed to < 3 mmol/L/hr
   • Regular (Toronto) insulin IV CRI or IM protocol as per DKA but may need to decrease dose/rate by half or more to achieve slower decline in BG.
   • Goal to decrease BG by < 2-4 mmol/L/hr to avoid sudden change in osmolality.

Prognosis
   • Carries a more guarded prognosis than DKA due to severity of HHS illness and severity of underlying disease.  Poor prognosis in cats.

Hypoglycemic Emergencies

Hypoglycemic diabetic crises are frequently encountered complications of diabetes mellitus.  Mild cases often go unnoticed by pet owners or may be easily managed at home by providing an extra meal.  Severe hypoglycemia can rapidly become life threatening and requires emergency veterinary treatment.

Pathophysiology
   • Exogenous insulin overdose is common in diabetic dogs and cat. Paraneoplastic insulin/insulin-like hormone release is a possible cause of hypoglycemia in diabetics but is less common.
   • Neuroglycopenia – hypoglycemia of the central nervous system.  The brain relies on glucose for energy and symptoms develop when peripheral BG is inadequate to maintain CNS glucose needs.
   • Clinical signs of neuroglycopenia and severity of signs vary with degree of hypoglycemia, rate of BG decline, and chronicity.  Some patients with chronic hypoglycemia have acclimated to the low BG and show no, or relatively mild signs, due to upregulated cerebral glucose uptake.

Diagnostic Evaluation
   • Blood glucose – glucometers can read falsely low with hemoconcentration or at the lower reference interval and chemistry analyzers can read falsely low if samples have been sitting > 30
min before serum/plasma is separated. Recheck a fresh sample on chem analyzer if needed to confirm hypoglycemia, especially if clinical signs are not present.

- Fructosamine can help determine recent BG control if concern for chronic insulin overdose or remission. Patients experiencing a Somogyi effect (hypoglycemia induced hyperglycemia) may have a high fructosamine despite chronic insulin overdose.
- Systemic work-up if concern for underlying disease affecting/changing insulin needs.

**Treatment** - depends on degree of hypoglycemia and severity of clinical signs. The goal is to alleviate signs of neuroglycopenia and maintain normal, or near normal, blood sugar while the effects of insulin wear off.

- **Oral sugar support** – extra meal, karo/pancake syrup or honey on gums.
- **IV dextrose bolus** – 0.5-1ml/kg of 50% dextrose IV over a few minutes. Dilute at least 1:2 to reduce chance of phlebitis from hypertonic solution.
- **IV dextrose CRI** – 1.25-5% dextrose in isotonic crystalloid. Higher concentrations (>5%) best given through a central line to avoid phlebitis in peripheral veins.
- **Glucagon CRI** – for refractory hypoglycemia. Stimulates glycogenolysis and gluconeogenesis. Has a very short half-life that allows fine-tuning of BG by adjusting dose.
- **Wean dextrose/glucagon** as tolerated as exogenous insulin wears off.
- **Restart insulin injections** at reduced dose (usually decrease 25-50%) once sufficient hyperglycemia develops and the patient is eating. Will need follow-up to re-establish maintenance insulin needs (dose, insulin type, schedule).
- **May need seizure prophylaxis** (at least short term) if seizures persist after hypoglycemia is resolved.

**References**


