



LYMPHOMANIA: HOW MUCH CAN YOU TAKE?

In March, Gribbles Veterinary ran a series of free oncology seminars throughout New Zealand, with presentations by Dr Antony Moore of Veterinary Oncology Consultants, New South Wales and Gribbles Veterinary's own Bronwyn Smits and Jon Meyer. The response from companion animal practitioners was unprecedented with more than 300 veterinarians attending across the four venues. Subsequently, we have been inundated with requests for speaker notes and I am pleased to say that Bronwyn's and Jon's notes are included in this edition of Labtalk. Copies of Dr Moore's extensive notes can be obtained by contacting your local Gribbles Veterinary laboratory.

THE LYMPHOCYTE: A BRIEF BIOGRAPHICAL STORY

Bronwyn Smits

During in utero development, stem cells from the foetal liver and yolk sac migrate to the two primary "nursery" organs, the thymus and bone marrow (humans and rodents; possibly the diffuse Peyer's Patches in ruminants), where they undergo a series of changes. This selection and de-selection process is influenced in two ways:

1. The genetic or "nature" aspect of development is a main driver to the immunoglobulin and T cell receptor reassortment that occurs on the cell membrane.
2. The "nurture" environment in which the cells mature (the thymus having epithelial cells, and both marrow and thymus having stromal cells and "nurse" cells which affect the development of the lymphocyte) is the "nurture" side of the growing lymphocyte.

The lymphocyte emerges from the "nursery" with naïve cell surface receptors that have been tested as safe from "self" reaction (i.e. autoimmune

diseases). The cells have been initially programmed to recognise appropriate major histocompatibility antigens and programmed as T helper or T cytotoxic cells (in the case of those from the thymus). They then migrate to their regional distribution centre, which may be as diverse as the tissue-associated lymphoid tissue, for example "SALT, BALT, MALT" (skin, bronchiolar, mucosal etc), lymph nodes or spleen.

At this point the cells have been "patched" or have recognisable receptors that can be picked up by immunocytochemistry (ICC) or immunohistochemistry (IHC) staining, performed on aspirates (the former) or histology sections (the latter). When lymphoma develops from one of the cells there is clonal expansion of that cell and, using ICC or IHC, we can test the cell membrane receptor to determine whether it is a T cell receptor (TCR) or an immunoglobulin family receptor. This test result is reported as a type of Cluster of Differentiation (CD) marker. The CD marker results confirm



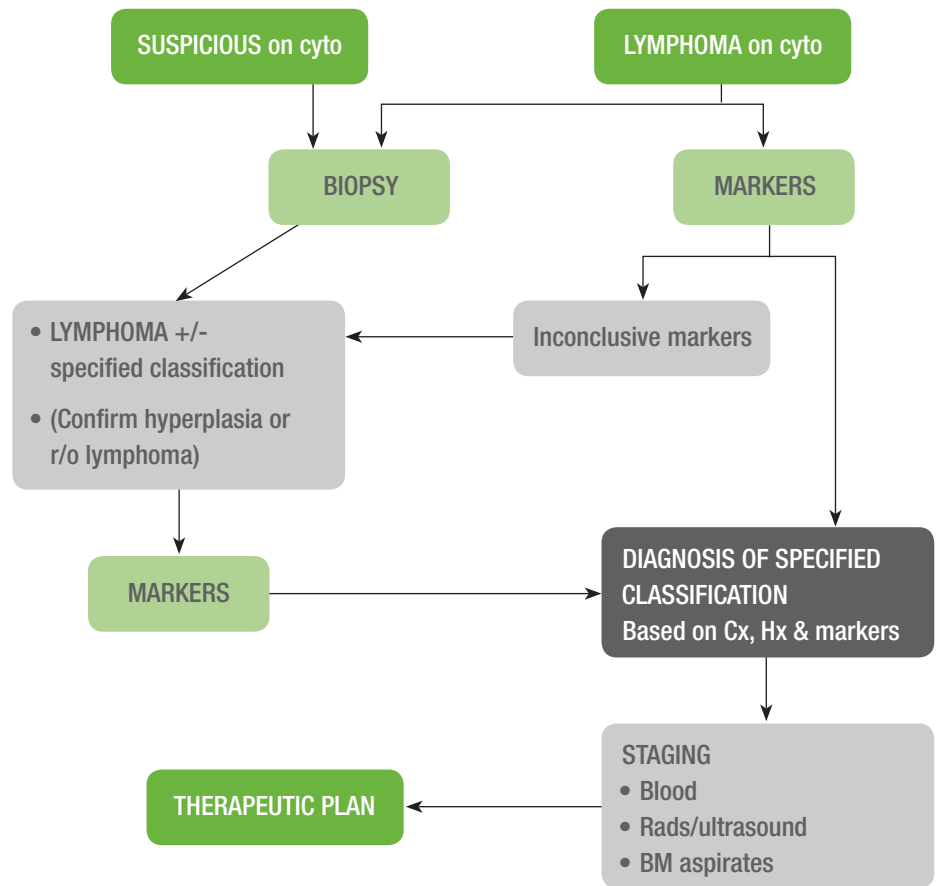
which subtype the lymphoma is, and this in turn helps categorise and give prognoses on the tumour progression and response to treatment.

The concept of lymphocyte trafficking is important in understanding the biology and behaviour of lymphoma. It is widely understood that lymphocytes circulate in the blood but, under the concept of lymphocyte trafficking, we now recognise that this circulation

is not in fact a random process. The cells undergo a routine circuit which is pre determined by specific homing receptor/ ligand pairs (chemokine/ chemokine receptors and adhesion molecules), which are present on either lymphocytes or on vascular endothelium, in particular those of the high endothelial venules. The endothelium of these venules provide “exit points”, comparable to highway signs which “tell” the lymphocyte where to get off the vascular circuit in order to move to the tissue or lymph node where they are required for foreign invader surveillance (the usual state of affairs), or for tumour spread (the undesirable state of affairs!). By understanding this concept, we see that neoplastic cells from lymphomas and leukaemias circulate. In the former, the numbers and the cells are often not recognised as abnormal by looking at them in a blood smear. In the latter, the clonal expansion occurs in the bone marrow and the tumour cells invade the blood in large numbers, infiltrating the spleen, nodes, liver and other organs. So whether or not the marrow is involved in lymphoma can be assessed to some extent by peripheral blood examination, and this information is important for case management.

Saying your patient has lymphoma is as useful as telling a car aficionado that you drive a red car: the type of car is what is important in determining how it will perform. Determining the subtype of lymphoma that your patient has allows you to discuss case management and prognosis with the client in a meaningful way.

The determination of CD markers is just part of this process. You also need to determine the World Health Organisation (WHO) stage and, more importantly, the sub-stage. The pathologist also needs this information, as it is part of the new approach to typing lymphomas as clinical entities based on the Revised European and American Lymphoma (REAL) scheme.



Your responsibility as a clinician dealing with possible lymphoma is:

1. Take multiple, good quality smears OR
2. Gently sample biopsy specimens and fix them rapidly, remembering that lymphoma cells are fragile and prone to rupture, which hinders diagnosis. Use a low gauge trucrut or skin punch tool for biopsy or, better still, an excisional or wedge biopsy. **Where case history fits a possible indolent (lazy or slow growing) lymphoma, make your sample an excisional biopsy only. We need to see architecture for these tumours.** AND
3. Supply information on the submission form about the stage and sub stage of the patient. Remember that staging and sub staging is clinical information. Grading is the responsibility of the pathologist.

Histology to confirm un-specified type of lymphoma is charged at \$68.25 (excl.

GST). However, to get more useful and specific diagnoses, we need to look at markers and immunohistochemistry is \$50.00 (excl. GST) per marker. It may be possible to get away with a single marker, based on an “educated guess” by the pathologist looking at the tissue architecture; however, we would generally recommend both B and T-cell markers. As a total laboratory cost “package” to quote to clients, therefore, routine histology plus markers is \$168.25 (excl. GST). The turnaround time for IHC is slightly longer than for immunocytochemistry (about one week), but the final diagnosis for a minority of lymphoma types will be much more accurate than what you can achieve on cytology, and it is the only way to confirm indolent or low grade lymphomas.

Send the samples to your local Gribbles Veterinary laboratory and they will direct specimens onward for further testing or opinion as required. Using history, clinical information and markers, the pathologist should be able to give you a specified classification and grade. With this information you can present the client with all the options they need to make a decision on treatment.

CANINE LYMPHOMA: CYTOLOGY AND IMMUNOCYTOLOGY

Jon Meyer

Lymphadenopathy is a relatively common finding in veterinary practice and may be the result of lymphoid hyperplasia (antigenic stimulation), neoplasia (lymphoma, metastatic disease), infectious agents (bacteria, fungal, protozoal) and immune mediated disease. In dogs, lymphoid hyperplasia and lymphoma are by far the most common causes, and cytological examination of aspirated nodes is usually useful in distinguishing the two.

The reason for this is that reactive and lymphomatous nodes in dogs are usually at opposite ends of the “cytological spectrum”: reactive nodes have a heterogeneous population of lymphocytes, with small cells in predominance and plasma cells are often, but not always, increased in number; whereas, in most cases of canine lymphoma, the lymphoid population is relatively homogeneous, with medium to large lymphoblasts the most predominant cell type. Increased numbers of mitoses are also often reported. This form of lymphoma (intermediate to high-grade) comprises 75 to 90% of all canine cases and is readily picked up on cytology. Intermediate or high-grade lymphomas are usually responsive to chemotherapy.

It is important to note that the other 10 to 25% of canine lymphomas are of the low-grade or indolent type and fall into a somewhat “grey zone” on cytological examination. These are the cases that cytologists may struggle to make a definitive diagnosis on fine needle aspirates. A major shortcoming of cytological preparations is the lack of tissue architecture, a feature crucial in establishing a diagnosis of low-grade/ indolent lymphoma. With low grade, indolent lymphomas an astute cytologist should be able to pick up that the aspirate doesn't quite fit with either a reactive node or intermediate/ high-grade lymphoma, and that a surgical biopsy is required. Further, clinical information regarding the case is vital and should fit with the cytology report received. (For example, golf ball-size lymph nodes are unlikely to be reactive.)

General criteria used for classification of high versus low-grade lymphomas are:

- Tissue architecture
- *Cell size*
- *Mitoses*
- Immunophenotyping*
- *Clinico-pathological information*

Whilst in the past, cytology of aspirates couldn't take into account all of these criteria (only those in italics), it had the benefit of being relatively cheaper for the owner, having a faster turnaround time, and being much less invasive than surgical biopsies.

*An exciting new development is the ability to perform immunophenotyping on cytological preparations through Gribbles Veterinary. After attending a meeting presented by eminent veterinary lymphoma specialists Drs Bill Vernau and Peter Moore (UC Davis) in Australia last year, Gribbles Veterinary has successfully validated a technique to now offer immunocytochemistry on aspirates of canine lymphomas. Immunophenotyping has up until now only been possible on formalin-fixed biopsy specimens.

Immunophenotyping is a method of visualising the type of markers that a specific cell expresses on its cell surface. It could be likened to recognising a gang member by the “patch” they wear! A cell that originated in the thymus has a different “patch” to the one from the bone marrow. The T cell marker is known as CD3 and is a good, reliable marker with predictable staining. There are various clones of the B-cell marker CD79a, some of which do not appear to work on canine B-cells. It is therefore important to use validated immunophenotyping protocols to ensure cross-reaction of antibodies with intended species.

What does this mean for you? Well, it markedly enhances a Gribbles Veterinary cytologist's ability to give you relevant information on the subtype of lymphoma more quickly. Although many diagnoses in the long list of recognised subtypes of lymphoma (table 1) can only be determined by using biopsy, the most common subtypes can be diagnosed successfully on cytology. Knowing the phenotype has tremendous prognostic as well as possible therapeutic implications in the management of canine lymphoma. (A fact that was repeatedly emphasized by Dr Tony Moore in his presentation: “.....B is better and T is terrible!”)

When aspirating enlarged nodes in which lymphoma is suspected, it is important to be gentle with the sample as the cells are fragile and may rupture during smearing. Multiple smears (at least 8) should be made and air-dried as normal. Relevant clinical information, such as number of nodes involved, other organ involvement (liver, spleen), size of nodes, how long present, whether the dog is “sick” or not, blood results etc, should be

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supplied on the submission form. We will initially apply routine cytological stains to some of the smears to confirm the diagnosis of lymphoma. Subsequently, we will guide you as to whether the remaining smears can be used for immunocytochemistry, or if a biopsy (for immunohistochemistry) would be more appropriate.

Cytology/ immunocytochemistry and histology/ immunohistochemistry both have their pros and cons in the diagnosis of canine lymphoma. These techniques should not be seen in isolation but rather viewed as complimentary diagnostic techniques, the choice depending largely on the specific clinical situation. There are significant cost

advantages for owners using a combination of cytology and immunocytochemistry, as anaesthesia and surgery to obtain the samples are generally not required. The turnaround time for diagnosis and phenotype will be in the region of a couple of days. Although the turnaround time for histological diagnosis and phenotyping of fixed tissue is slower (about a week), it is important to note that it may be the only way to diagnosis the less common low-grade, indolent forms of canine lymphoma.

The cost for routine cytology is \$39.85 (excl. GST) while immunocytochemistry for each of the markers is \$45.00 (excl. GST). Therefore, a total “package” cost for routine cytology and T-cell and B-cell markers would be \$129.85 (excl. GST).

B CELL NEOPLASMS	T CELL NEOPLASMS
Diffuse large B cell lymphoma – centroblastic, immunoblastic, anaplastic variants	Lymphoblastic T cell lymphoma
T cell/histiocyte rich large B cell lymphoma	Peripheral T cell lymphoma (unspecified)
Marginal zone lymphoma	T-zone lymphoma
Follicular lymphoma	Anaplastic large T cell lymphoma
Mantle cell lymphoma	Enteropathy associated T cell lymphoma
Lymphoblastic B cell lymphoma	Mycosis fungoides / Sezary syndrome
Extramedullary plasmacytoma	Hepatosplenic T cell lymphoma
Multiple myeloma	Subcutaneous panniculitis-like T cell lymphoma
Burkitt-like lymphoma (controversial)	
Lymphomatoid granulomatosis (controversial)	

Table 1: Lymphoma subtypes

EFFECT OF SHORT TERM REFRIGERATED BLOOD STORAGE ON IGG TEST RESULTS

Giti Talebi

The main immunoglobulin (antibody) found in equine colostrum is gamma globulin (IgG), and a number of qualitative and quantitative tests are available for measuring serum IgG to determine if ‘adequate’ quantities have been ingested and absorbed by newborn foals. Such tests are typically not performed until the foal is at least 12-18 hours old, with peak absorption occurring at around 24 hours of age.

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Many veterinarians chose to perform the test when the foal is 12 hours of age so that, if test results indicate a low level of immunoglobulin absorption, there is still time to administer additional colostrum. At 24 hours of age, if the level of absorbed immunoglobulins is low, then the only alternative is intravenous therapy with plasma, which has high levels of immunoglobulins, to help protect against infection.

During the 2009 foaling season, Gribbles Veterinary received an enquiry from a client questioning whether a 5-day old refrigerated blood sample would still be suitable for IgG testing. In response, the laboratory set up a series of experiments to investigate the effect of time on IgG results, and to determine if the sample would have been adversely affected.

Fifteen blood samples were tested for IgG on the day of arrival at the laboratory (day 0) and were then refrigerated for subsequent retesting; serum was

removed from the blood tubes after 1, 2, 3, 5, 6, and 8 days to measure IgG concentrations. Results were then compared with the initial IgG result obtained on day 0.

Over the entire 8 day period, only minor variations between measured IgG concentrations were observed (-5.8% and 4.5%). These variations could have been due to calibration factors, daily instrument variation and/ or the storage effect. Quality control samples (tested as per protocol each day of testing), also showed a fluctuation of a similar magnitude to the test samples and were within acceptable parameters. The coefficient of variation and the uncertainty of measurement for both levels of quality control sample tested were less than 7%.

The results of this small study indicate that a sample can be safely taken from a foal at 24 hours post gestation, refrigerated at the clinic for up to 8 days before IgG testing is performed, and a reliable result can still be obtained. This could have important implications where IgG results are required for insurance purposes.

THYROID FUNCTION AND THE LABORATORY DIAGNOSIS OF HYPOTHYROIDISM IN THE DOG

Janice Thompson

Physiology of thyroid hormone synthesis

Thyroid hormone synthesis is under the control of the hypothalamic-pituitary-thyroid axis, with the thyroid gland responsible for producing the two metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3).

Thyroglobulin is a large glycoprotein that is secreted by the thyroid cells and contains iodotyrosine molecules that serve as a precursor for thyroid hormone synthesis. Thyroid hormone synthesis commences with the binding of iodine molecules to acceptor proteins on the thyroglobulin protein to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). This is mediated by the enzyme thyroid peroxidase. These compounds are coupled, again by the enzyme thyroid peroxidase, to form T4 (DIT +DIT) and T3 (DIT + MIT) that, at this stage, are attached to the thyroglobulin and are subsequently stored extracellularly in the colloid within the follicular lumens. Following stimulation of secretion, the colloid containing the thyroglobulin-T4-T3 complexes enters the thyroid follicle cell by endocytosis. Here the thyroglobulin is digested releasing the T4 and T3 that are then secreted by exocytosis from the thyroid gland into circulation. The majority of hormone secreted by the thyroid gland is T4. A small amount of thyroglobulin may also enter the circulation and this amount is increased when thyroid cells are damaged, as occurs with lymphocytic thyroiditis.

Thyrotropin (thyroid stimulating hormone, TSH), secreted



by the thyrotrophs in the anterior pituitary, stimulates the synthesis and release of the thyroid hormones by the thyroid gland. TSH is under negative feedback control by the circulating thyroid hormones, via modulation by Thyrotropin releasing hormone (TRH). Other hormones will also alter TSH formation and secretion. Glucocorticoids, growth hormone, somatostatin, dopamine and androgens all decrease TSH secretion.

TRH is secreted by the paraventricular nucleus of the hypothalamus into the hypophyseal circulation, from where it causes direct stimulation of the thyrotrobes of the anterior pituitary and the secretion of TSH.

The majority of thyroid hormone in circulation is bound to a number of plasma proteins, namely thyroid hormone-binding globulin, thyroxine binding pre-albumin, albumin and some lipoproteins. Only the unbound hormone (free T4 and free T3) is free to enter the cells and to exert its effects on metabolism, and only free hormone has feedback effects and regulates the axis. Less than 1% of the total T4 (TT4) and total T3 (TT3) circulates in the unbound state, while the bound forms act as a reservoir for the unbound forms. While the majority of hormone secreted is T4, the most biologically active hormone is T3. The majority of T3 (80%) is produced by peripheral tissues such as liver and kidney which deiodinate T4 to form T3 or the inactive reverse T3. The thyroid hormones are degraded in the liver and kidney and excreted via the biliary system or urine. Thyroid hormones have an effect on many metabolic systems including carbohydrate, protein and lipid metabolism, erythropoiesis, cardiac function, respiratory centres, and bone turnover.

Primary hypothyroidism

The most common form of hypothyroidism arises from dysfunction of the thyroid gland (primary hypothyroidism) as a result of lymphocytic thyroiditis or, less commonly, idiopathic atrophy.

1. Lymphocytic thyroiditis

Lymphocytic thyroiditis is thought to occur in about 50% of hypothyroid dogs, and is characterised by inflammatory infiltrates within the glands. The cause is not known but there may be a genetic predisposition as there appears to be in humans. Environmental factors combined with a genetic predisposition may trigger the destructive process of the gland. It is also suggested there may be viral or bacterial antigens mimicking thyroid antigens that trigger the condition. Antibodies to thyroglobulin, thyroid peroxidase, colloid, Na/I transporter molecule, and TSH have all been detected in various species of animal affected with lymphocytic thyroiditis. In dogs, the thyroglobulin auto-antibody has been the major antibody detected in about 50% of hypothyroid dogs and this is consistent with the numbers of hypothyroid dogs thought to suffer from lymphocytic thyroiditis. Lymphocytic thyroiditis is characterised by the gradual destruction of the thyroid gland accompanied by lymphocyte infiltration. The pathogenesis in dogs is under investigation, but at present is not yet fully understood. It is thought that there is a gradual destruction of the thyroid gland, with a period of months to up to 3 years in which dogs have preclinical disease. At this preclinical stage, thyroglobulin auto-antibodies may be measured,

and while histologically there is lymphocyte infiltration, there is no overt disease. When there is destruction of >60–70% of the thyroid gland, an increase in serum TSH concentration occurs that stimulates the remaining portion of the gland to increase secretion of thyroid hormones. The serum concentrations of TT4 and TT3 are normal in the preclinical phase of the disease. This stage is followed by overt disease in which there is increased serum TSH and decreased TT4 concentrations. Clinically affected dogs are also positive for thyroglobulin auto-antibody (TgAA).

2. Idiopathic atrophy

Idiopathic atrophy is characterised by fibrous and adipose tissue replacement of the thyroid glands. The cause is not known, but there is thought to be degeneration of individual follicular cells. There is progressive reduction of the size of cells and replacement with adipose tissue. This can be distinguished histologically from atrophy as a result of decreased TSH secretion. Alternatively, atrophy may also be the end stage of lymphocytic thyroiditis, although with these cases there is usually a residual inflammation present. Cases of idiopathic atrophy are generally TgAA negative; however, it has been shown that auto-antibodies to thyroglobulin and thyroid hormones also decrease as lymphocytic thyroiditis progresses and may become negative, suggesting that there is disappearance of the initiating antigens.

3. Follicular cell hyperplasia

Some hypothyroid dogs have small follicles with small amounts of colloid present. There is follicular cell hyperplasia and no significant inflammatory response. They are TgAA negative. Changes resemble those seen in young dogs with iodine deficiency but many of these dogs are on diets adequate in iodine. One possible explanation is follicular cell dysfunction (dysmorphogenesis). Follicular cell hyperplasia may also develop in response to increased TSH secretion, as a result of thyroid hormone deficiency. Excessive iodine (toxicity) also causes inhibition of iodine uptake and organification, and decreased thyroid hormone secretion by follicular cells. This results in increased TSH secretion.

4. Neoplastic destruction

Infiltrative tumours may destroy the thyroid gland resulting in hypothyroidism.

5. Other causes

Treatment for hyperthyroidism in cats may result in hypothyroidism.

6. Congenital defects

These defects may occur but are thought to be lumped into the fading puppy syndrome. Thus the majority if present will not be recognised.

Secondary hypothyroidism

Defects within the pituitary gland may result in decreased TSH secretions and a secondary decrease in thyroid hormone synthesis. Potential causes include genetic defects, e.g. pituitary dwarfism, and pituitary malformation, e.g. cystic Rathkes pouch. Destruction of the pituitary may result from neoplasia and trauma.

Tertiary hypothyroidism

Dysfunction of the hypothalamus results in decreased TRH secretion and tertiary hypothyroidism. This has not been reported in dogs.

Other causes

There may also be very rare conditions in which there is a failure of conversion of T4 to T3 and defects in iodine organification.

Factors that may affect serum thyroid hormone concentrations

There are a number of factors that may alter the hormone concentrations either by altering formation, metabolism, excretion or plasma binding. Some of these are detailed below with specific emphasis on T4 measurement as T3 concentrations are variable and considered to be of less importance diagnostically:

1. The “Euthyroid Sick Syndrome” (non thyroidal illness)

Total T4 is decreased due to alterations in binding, metabolism and excretion. Severe non thyroidal illness may also be associated with TSH and TRH stimulation results that mimic hypothyroidism. Euthyroid dogs with severe systemic concurrent illness may have serum thyroid hormone concentrations that are suggestive for hypothyroidism. Severe renal, hepatic disease and diabetic ketoacidosis are among conditions that are associated with lower TT4 and free T4 concentrations. These conditions may also increase serum TSH concentrations into hypothyroid ranges. With severe non thyroidal illness, such as ketoacidotic diabetes mellitus, evaluation of thyroid hormones should be done after the condition has been controlled.

Cushing's disease is often associated with lower TT4 concentrations, and treatment for Cushing's will reverse the apparent hypothyroidism. Serum TSH concentrations are variable. While borderline TT4 concentrations may occasionally occur in dogs with dermatologic disease, cases of pyoderma, flea dermatitis and allergic dermatitis do not typically cause low thyroid hormone values. Serum thyroid hormone concentrations return to normal with treatment of the concurrent illness.

2. Drugs

Many drugs will affect thyroid hormones, either by

affecting secretion, metabolism or protein binding of the thyroid hormones. These include corticosteroids, sulphonamides, NSAIDs, phenobarbital, tricyclic antidepressants and thyroid supplementation.

3. Breed

Sighthounds (including Greyhounds, Wolfhounds, Afghans, Whippets and Salukis) have lower TT4 concentrations than other breeds of dog. Total T4 tends to be higher in small breeds than medium or large breeds.

4. Age

Young dogs less than 6 weeks of age have higher mean TT4 concentrations compared to dogs >6 weeks of age. As dogs age there is a slight decrease in TT4 concentrations. Total T4 concentrations may be very high immediately after birth.

5. Obesity

Total T4 concentrations are increased in fat non hypothyroid dogs and are thought to result from increased calorie intake rather than the actual obesity. Obesity is not thought to mask hypothyroidism.

6. Hypoproteinaemia

Decreases in TT4 concentrations are seen due to decreased plasma protein binding.

7. Hormones/gender

In the bitch, progesterone but not oestrogen affects TT4 concentrations. Increased TT4 concentrations were seen in di-oestrous bitches when compared with anoestrous, pro-oestrous and lactating bitches.

8. Thyroid hormone auto-antibodies

Auto-antibodies to T3, T4 or thyroglobulin (T3AA, T4AA and TgAA) may be found in circulation of either normal or hypothyroid dogs, and are markers for lymphocytic inflammation and predictors of lymphocytic thyroiditis. However, assays to measure these antibodies are not function tests. T3AA and T4AA are subsets of TgAA which cross react with TT3 and TT4 immunoassays respectively and which may cause false increases in measured T3 and T4 levels.

9. Miscellaneous

Fasting/temperature/diurnal variation: Random samples from a euthyroid dog may occasionally give results that suggest hypothyroidism or hyperthyroidism. Similarly, random samples from a hypothyroid dog may occasionally be within the reference range. While it has been suggested that dogs have a diurnal rhythm with a peak at about midday, this has not been definitely proven.

Dogs fasted for up to 36 hours had no changes in TT4 concentrations. Total T4 concentrations, but not free

T4, were significantly lower in cachexic dogs when compared to dogs of normal weight.

In humans and rats, temperature may affect thyroid hormone concentrations, but the effects in dogs are not known. The season of the year also does not appear to have an effect on thyroid hormone concentrations. The effects of hypothermia and hyperthermia may affect TT4 and TSH concentrations, but it is not known if these changes result from factors associated with the euthyroid sick syndrome.

Tests for thyroid function

Thyroid function tests may be used to identify either a dog that may become hypothyroid, actual hypothyroidism or, to a much lesser extent, hyperthyroidism.

It has been suggested that the possibility of hypothyroidism developing may be detected in dogs with lymphocytic thyroiditis. These are dogs that are often clinically normal but may have an unknown genetic predisposition to develop hypothyroidism. Dogs with lymphocytic thyroiditis may be TgAA positive. Thyroglobulin auto-antibodies may, therefore, be able to predict the onset of hypothyroidism before it occurs and, if known in a breeding situation, may prevent the use of dogs that will develop the condition in the future.

It is not until the majority of the thyroid gland has been destroyed that thyroid hormone levels start to alter. Initially there may be increases in T3 relative to T4 as the animal tries to maintain homeostasis. Clinical signs of hypothyroidism develop when > 75% of the thyroid gland is non functional.

Diagnosis of hypothyroidism in dogs

Diagnosis of hypothyroidism may be difficult and multiple tests are often required for an accurate diagnosis. It is necessary to rule out the above factors causing decreased TT4 concentrations from true hypothyroidism. This involves further testing and also a complete database including routine clinical pathology. In New Zealand, the tests commercially available are total T4 (TT4), free T4 by equilibrium dialysis (free T4 [ED]), thyroglobulin auto-antibodies (TgAA) and endogenous serum TSH (cTSH).

1. History and clinical examination

The history is extremely important and the prior use of drugs needs to be considered, along with the presence of non-thyroidal disease, e.g. hepatic, renal disease, diabetes mellitus, etc. Also examine for clinical signs of hypothyroidism, such as coat and skin changes, bradycardia, weight gain, lethargy, neurological signs, etc. Thyroid hormones affect many body systems and thus a wide variety of clinical signs may be seen.

2. Routine clinical pathology

Routine haematological and biochemical examinations should be carried out to rule out other non thyroidal illness. Hypothyroidism may cause a mild non-regenerative normocytic, normochromic anaemia. Serum cholesterol and triglyceride concentrations are often increased, and there may be mild increases in ALT and AP due to hepatic lipidosis. Urinalysis is usually normal.

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Recommendations for the Evaluation of Thyroid Disease in Dogs

- 1) Full history, clinical examination, and results of routine blood work (CBC and chemistry) and urinalysis.
- 2) Initial single screening tests include baseline serum total T4, or free T4 by equilibrium dialysis [ED].
- 3) Treatment trials may be indicated if serum T4/free T4 [ED] are low and clinical signs/ history are supportive of hypothyroidism.
- 4) Additional testing (endogenous canine TSH [cTSH] and thyroglobulin auto-antibodies [TgAA]) if T4 concentrations are normal but clinical signs/ history are supportive of hypothyroidism.
- 5) Screening protocols using two tests include either serum T4 or free T4 [ED] and serum cTSH.
- 6) Treatment is not indicated if serum free T4 [ED] is normal and cTSH is increased. Tests should be repeated in 8-12 weeks.
- 7) Testing TgAA is indicated if serum T4 is normal, cTSH is increased and clinical signs/ history are supportive of hypothyroidism.
- 8) A full thyroid panel should include serum T4, free T4 [ED], cTSH and TgAA.
- 9) Treatment is indicated if all test results are abnormal and clinical signs/ history are supportive of hypothyroidism, regardless of TgAA result.
- 10) Treatment is not indicated if all test results are normal and clinical signs/ history are not supportive of hypothyroidism, regardless of TgAA result. Positive TgAA supports possible presence of lymphocytic thyroiditis and requirement to monitor thyroid parameters every 3-6 months.
- 11) If discordant thyroid test results are obtained, the decision to treat should be based on evaluation of the dog (clinical signs/ history), clinician's index of suspicion for hypothyroidism, and evaluation of each test result. Serum free T4 [ED] is the most accurate test of thyroid function.

(adapted from Feldman & Nelson, Canine and Feline Endocrinology and Reproduction, 3rd edition, 2004)

3. Measurement of circulating thyroid hormones

Total T4 concentration is the hormone most commonly measured when hypothyroidism is suspected. While this may give an indication of hypothyroidism, values may be decreased depending on some of the above factors. A single low TT4 measurement should not be accepted as proof of hypothyroidism. A normal TT4 value will in most cases rule out hypothyroidism but if a decreased value is obtained the possibility of non thyroidal illness and/or drug administration needs to be ruled out.

Free T4 is the form of T4 that is directly available to the cell to influence metabolism. It is less altered by the above factors but low values may still be obtained with the usage of some drugs and also severe non thyroidal illness.

Measurement of TT3 is of minimal value for the diagnosis of hypothyroidism because there is considerable overlap in the mean and range between normal dogs, euthyroid sick dogs and hypothyroid dogs. Reportedly, 90% of hypothyroid dogs may have normal TT3 concentrations.

Following measurement of a single low TT4 concentration, further testing is essential for an accurate diagnosis.

4. Endogenous serum TSH (cTSH) measurement

The majority of cases of hypothyroidism are caused by defects of the thyroid gland. These defects will theoretically cause decreased feedback inhibition of the pituitary and increased serum TSH concentrations. In practice, this is often the case but some cases of hypothyroidism (25–40%) may also have normal/non-increased serum TSH concentrations, and some dogs (10–20%) with normal thyroid function may have increased serum TSH concentrations. There is, therefore, overlap in serum TSH concentrations between normal and hypothyroid dogs. Reasons for normal TSH concentrations in hypothyroid dogs include random fluctuations, secondary hypothyroidism, concurrent drug administration and disease suppressing TSH secretion, and chronic hypothyroidism eventually causing decreased TSH production due to down regulation of pituitary thyrotrobes. When there is destruction of 60-70% of the thyroid gland there is an increase in TSH secretion but initially TT4 and free T4 concentrations are normal. Serum TSH concentrations, therefore, need to be considered in conjunction with routine clinical pathological results, clinical signs and history, in addition to TT4 and free T4 concentrations.

Measurement of serum TSH alone is not recommended for the diagnosis of hypothyroidism. It should always be interpreted with total T4 and/ or free T4 measured on a concurrent serum sample.

5. Response tests

Since TSH and TRH are no longer readily available in New Zealand, response tests have effectively become redundant.

6. Auto-antibodies

Thyroglobulin auto-antibodies, if present with low TT4 and increased serum TSH, may indicate lymphocytic thyroiditis. Approximately 34-59% of hypothyroid dogs are reported to have circulating thyroglobulin auto-antibodies and thus a negative TgAA result does not rule out hypothyroidism, but may indicate idiopathic atrophy of the thyroid gland. In one US study, it was shown that only 20% of dogs that tested positive for thyroglobulin auto-antibodies went on to develop hypothyroidism at a later stage.

7. Histopathology

Thyroid biopsy may differentiate lymphocytic thyroiditis or idiopathic atrophy. Skin histopathology may also be useful. A biopsy from the flank is suggested as showing underlying hormonal problems and compared to one from the groin that will be more likely to demonstrate perivascular dermatitis.

An accurate diagnosis of hypothyroidism should be made on the basis of multiple test results, clinical signs and history, and not on the basis of a single test/ sign alone.

Canine Thyroid Tumours

Dogs rarely demonstrate hypothyroidism secondarily to thyroid tumours. The majority of thyroid tumours in dogs are also non-functional and, hence, TT4 concentrations will be normal in most cases. Cases of functional thyroid tumours with secretion of thyroid hormones will demonstrate clinical signs similar to those seen in hyperthyroid cats, namely weight loss, tachycardia, premature contractions, restlessness and gastrointestinal signs. They will also usually have large obvious masses that may cause physical obstructions and pressure on adjacent structures, and clinical signs related to this.

Thyroxine supplementation

If thyroxine has been supplemented to a dog on the basis of a low TT4 result then it will act on the pituitary to cause decreased TSH secretion and, therefore, decreased endogenous T4 and T3 secretion. Prolonged treatment will result in atrophy of the pituitary thyrotrobes and the thyroid gland. If further examination of thyroid function is to be carried out after treatment/ supplementation of T4 has been commenced, particularly if that treatment is prolonged, then it is recommended that the supplementation be discontinued for at least 8 weeks, or longer, to allow for recovery of the axis. In humans such recovery may take over one year.

Thyroid evaluation in Cats

A diagnosis of obvious hyperthyroidism in cats can be made when the TT4 level is increased, along with

appropriate clinical signs. Most hyperthyroid cats will have elevated TT4 but some cats may have signs of early hyperthyroidism with high normal or only slightly increased levels of thyroid hormone. Cats with non thyroid illness may also have TT4 in the high normal range. However, TT4 concentrations may also be significantly decreased in cats with non thyroidal illness, and this is reported to correlate inversely with mortality. Thyroid hormone levels can vary over time so it may be necessary to check blood levels several times or perform a T3 suppression test on cases with equivocal results.

Free T4 [ED] may be useful to differentiate those cats which have TT4 levels in the high normal reference range

but which are suspected of having hyperthyroidism. A mid to high normal TT4 with elevated free T4 [ED], along with appropriate clinical signs, is consistent with hyperthyroidism. Low TT4 and elevated free T4 [ED] levels are likely to be associated with non thyroid illness.

Free T4 [ED] is more sensitive than TT4, but is less specific in that a small number of sick euthyroid cats (5% in one study) have been shown to have false positive results for hyperthyroidism with free T4 [ED]. Therefore, free T4 [ED] must be interpreted with caution if used as the sole diagnostic criterion for confirmation of hyperthyroidism in cats.

References

A full reference list is available upon request.

BOVINE ABORTIONS STATISTICS 2009

Fraser Hill

Introduction

Data was extracted from Gribbles Veterinary Palmerston North laboratory information system where either a foetus for necropsy or a full range of samples was submitted to allow a comprehensive abortion investigation. Forty four cases met the criteria and were analysed in depth. Analyses included one or more of the following tests: necropsy on foeti; microbiology and mycology on stomach contents; histopathology; and serology (dam and/ or foetal) for leptospirosis, BVD and neospora.

Results

DIAGNOSIS	NUMBER	2008 %	NUMBER	2009 %
Aspergillosis	8	15	4	9
Neosporosis	6	11	14	32
Listeria monocytogenes	3	6	1	2.3
Bacillus licheniformis	2	3	0	0
Bacillus cereus	1	2	1	2.3
Salmonella Typhimurium	1	2	0	0
Streptococcus uberis	1	2	1	2.3
Streptococcus bovis	1	2	1	2.3
Campylobacter jejuni	1	2	0	0
Mortierella wolfii	1	2	0	0
Leptospira hardjo	1	2	2	5
Leptospira pomona	0	0	1	2.3
Arcanobacter pyogenes	0	0	1	2.3
Escherichia coli	0	0	1	2.3
Neoplasia - nephroblastoma	0	0	1	2.3
Toxin - macrocarpa	0	0	1	2.3
Foetal inflammation - no aetiology	10	19	11	25
No diagnosis made	17	32	4	9
TOTAL	53	100	44	100

NB

1. The diagnosis of leptospirosis was made by positive serology on the dams (titres > 1:1600) or leptospira titres in foetal fluid
2. Foetal inflammation included thrombosis and neutrophilia of various tissues with or without suppuration and necrosis in the placenta

Comments

If the correct samples are submitted an aetiological diagnosis was made in 90% of cases.

The recommended standard abortion protocol is:

- stomach content microbiology and mycology;
- histopathology;
- leptospira and neospora serology on dam sera;
- BVD antibody (foetus > 150 days gestation) and neospora IFAT on foetal sera.

BVD antigen testing on autolysed foetal tissues (spleen, skin, foetal fluid) is not recommended, as autolysis may interfere with the test (perhaps due to the production of peroxidases) leading to false positive results. PCR testing for BVD virus in autolysed tissues is also not recommended as tissue destruction can lead to false negative results. Serum from freshly dead foeti would be suitable for testing by either method.

Diagnosis of BVD in a herd is a well-documented procedure with numerous other effective tests

available. Control of BVD in the herd will eliminate BVD as a cause of abortion. We recommend efforts should be focused there rather than in using foetal tests to investigate the BVD status of a herd.

Neospora was diagnosed much more commonly this year than last year, although this year's figures are probably more typical of the long term trend. The total number of neospora positive diagnoses was from cases where disease was diagnosed. Within some of the cases up to twelve individual, recently aborted dams had neospora IFAT titres of >1:2000.

Leptospira hardjo was confirmed as a cause of abortion in two cases.

Recommendations

Samples to collect include:

- Foetus and placenta
- Dam sera

If conducting a foetal necropsy collect:

- Fixed brain, lung, liver, kidney, heart, skeletal muscle, conjunctiva and placenta;
- Fresh stomach contents, foetal fluid (thoracic) or heart blood, placenta.

For a full diagnostic work up, select the following tests:

- Microbiology and mycology on stomach contents and/ or placenta;
- Histopathology on all tissues, including placenta;
- BVD antibody serology on foetal fluid, foetus only if foetus is > 150 days of age (CR length >38 cm);
- Neospora and leptospirosis serology on dam sera.

FOETAL BODY LENGTH (CM)	APPROXIMATE GESTATIONAL AGE (DAYS)
1	30
2.5	42
5	56
6	58
7.3	63
10	70
11	75
14	80
14.5	90
18	98
19.5	102
20	107
24	110
27	118
31	130
35	142
38.7	152
41.25	160
44	168
46	170
48	174
62	206
72.5	220
73	230
87	260
90	271
95	274
105	275

Table 1: Gestation age estimation.

Reference

Rexroad CE, Casida LE, Tyler WJ. Crown-rump length of foetuses in purebred Holstein-Friesian cows. Journal of Dairy Science, 57, 3, 346-7, 1974



OLD BUT STILL DEADLY: ARSENIC POISONING IN YEARLING CATTLE

Alan Julian

Arsenic, a relatively ubiquitous element in soil and water, produces cellular damage through a variety of mechanisms. It reacts intracellularly with sulphhydryl groups, inhibiting the sulphhydryl enzyme systems necessary for oxidative phosphorylation. The disrupted cellular metabolism affects the brain, lung, liver, kidney and alimentary mucosa.

The clinical signs and post mortem findings with arsenic poisoning depend on the form of arsenic, its solubility and the dose ingested. In peracute cases, no signs may be observed. In acute cases, there may be intense abdominal pain manifested by teeth grinding, salivation and grunting. Diarrhoea, ataxia and signs of colic may also be seen. Death can occur within 24 to 48 hours of the onset of signs. Alternatively, illness up to 8-10 days may occur with affected animals showing persistent, dark and putrid diarrhoea. Chronic cases may develop dermatitis with multiple abscess formation and wasting accompanied by brick-red discoloration of mucus membranes.

History

Five yearling heifers died within five hours of one and other on a property in Galatea. Two others appeared unwell. The mob had been grazing the same paddock, containing oats and Tama (2nd grazing), for five days. The animals had access to a derelict shed which contained an old sack of fertilizer and a drum of oil. There was also an empty fertilizer bag outside the shed that had been chewed. No known poisonous plants were noticed.

The mob was moved to another paddock leaving behind an ill steer. This steer was in dorsal recumbency and had dark red blood. It was given methylene blue in case it had nitrate poisoning but did not improve. It was ataxic when it stood and subsequently died overnight. An ill heifer would get

up when approached but was very ataxic. A third cow died overnight.

Postmortem Findings

Three dead animals were post mortemed. The mucus membranes were deep purple-red and the eyes were sunken. One had blood-tinged foam around the nostrils and one had profuse diarrhoea. One had reddening of the abomasum and rumen walls.

A limited range of tissues from three cows was examined. One cow had moderate chronic facial eczema damage in the liver. The small intestine from this cow and one other had advanced autolysis of the superficial mucosa. There were numerous lymphoid cells and eosinophils in the deeper mucosa. Moderate numbers of degenerate or necrotic cells were present in the crypts. These appeared to be a mixture of epithelial cells and inflammatory cells. One cow had increased numbers of white blood cells in the red pulp of the spleen, some of which were degenerate. The brain from one cow had mild generalized congestion and scattered small haemorrhages. Lead concentrations in liver samples from three animals were <0.5 mg/kg; the arsenic concentration in the liver sample of one animal was 56mg/kg.

Samples of fertilizer, floor material and oil that were in the shed were sent for arsenic analysis. The floor sample contained 296g/kg of arsenic. This level correlates well with that

found in old arsenical sheep and cattle dips (20% soluble arsenic and 3% insoluble arsenious sulphide). The site has subsequently been decontaminated by a professional pesticide disposal agency.

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