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How to diagnose and treat

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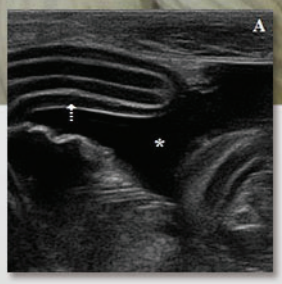

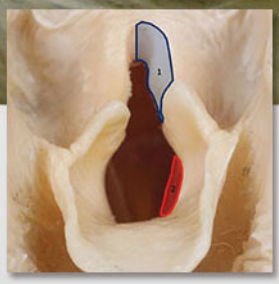

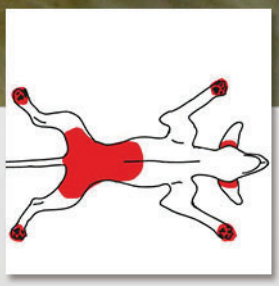

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Ocular abnormalities in pugs, Heartworm infection in a dog in Norway, report on the EuroCongress in Barcelona, veterinary app & book reviews ... and more!

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These refer to the species (in green) of animal or the veterinary discipline (in blue) relevant for the article.


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Ear Nose Throat


Genetics


Internal Medicine


Neurology


Oncology


Opthalmology


Orthopaedics


Practice Management


Surgery


Urogenital

Reprint paper*

Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification

Patrick Hensel[1], Domenico Santoro, Claude Favrot, Peter Hill and Craig Griffin

ABSTRACT

Background: Canine atopic dermatitis (AD) is a common, genetically predisposed, inflammatory and pruritic skin disease. The variation in clinical presentations, due to genetic factors, extent of the lesions, stage of the disease, secondary infections, as well as resemblance to other non-atopic related skin diseases, can complicate a diagnosis of canine AD. A sub-group of the International Committee for Allergic Diseases in Animals (ICADA) was tasked with the development of a set of practical guidelines that can be used to assist practitioners and researchers in the diagnosis of canine AD. Online citation databases and abstracts from international meetings were searched for publications related to the topic, and combined with expert opinion where necessary. The final set of guidelines was approved by the entire ICADA committee.

Results: A total of 81 publications relevant for this review were identified. The guidelines generated focus on three aspects of the diagnostic approach:

1. Ruling out of other skin conditions with clinical signs resembling, or overlapping with canine AD.
2. Detailed interpretation of the historical and clinical features of patients affected by canine AD.
3. Allergy testing by intradermal versus allergen-specific IgE serum testing.

Conclusions: The diagnosis of canine AD is based on meeting clinical criteria and ruling out other possible causes with similar clinical signs. Flea combing, skin scraping and cytology should be performed, where necessary, as part of a thorough work-up. Elimination diet trials are required for patients with perennial pruritus and/or concurrent gastrointestinal signs. Once a clinical diagnosis of canine AD is made, allergy testing can be performed to identify potential causative allergens for allergen-specific immunotherapy.

* This paper originally appeared in *BMC Veterinary Research* (2015) 11:196 (DOI 10.1186/s12917-015-0515-5).
Eur J Comp An Pract (2015), Winter 25(4); p4-19
Go to http://www.ejcap.org to see the online presentation of this paper.

Background

Canine Atopic Dermatitis (AD) has been defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens [1]. Although this definition encompasses many aspects of the pathogenesis and clinical aspects of the condition, it is important to remember that this disease has no pathognomonic clinical signs that permit a definitive diagnosis to be made upon initial owner interview and clinical examination[2]. This is due to the diversity of the clinical presentation, which may depend on genetic factors (breed-associated phenotypes) [3, 4], extent of the lesions

[1] Tierdermatologie Basel, Emil Frey-Strasse 127, Münchenstein, Switzerland. Email: phensel@tierdermatologie.ch

(localised versus generalised), stage of the disease (acute versus chronic), and the presence of secondary microbial infections or other flare factors. Furthermore, some aspects of the disease can resemble other skin conditions that are not related to canine AD. For the above-mentioned reasons, the definitive diagnosis of canine AD can be difficult.

A sub-group of the International Committee for Allergic Diseases in Animals (ICADA) developed, based on extensive searches in online citation databases and abstracts from international meetings, a set of practical guidelines that can be used to assist practitioners and researchers in the diagnosis of canine AD.

These guidelines provide an overview of the diagnosis of canine AD that involves three distinct, but complementary, approaches. These are:

1. Ruling out of other skin conditions with clinical signs that can resemble, or overlap with canine AD. This is traditionally referred to as "the work-up".
2. Detailed interpretation of the historical and clinical features of the condition. A new tool to assist with interpretation of these findings is the application of clinical criteria known as "Favrot's criteria" [5].
3. Assessment of skin reactivity by IntraDermal Testing (IDT) or detection of IgE by Allergen-Specific IgE Serology (ASIS) testing. This is traditionally referred to as "allergy testing".

Use of any one of these approaches in isolation can result in misdiagnosis, so it is important not to rely on any of them as a sole diagnostic principle.

Table 1. Important differential diagnoses for pruritic skin diseases in dogs

Ectoparasitic skin diseases	Fleas Scabies (*Sarcoptes scabiei*) Demodicosis Cheyletiellosis Pediculosis Otoacariasis (*Otodectes cynotis*) Trombiculiasis Nasal mites (*Pneumonyssus caninum*)
Microbial skin infections	Staphylococcal pyoderma *Malassezia* dermatitis
Allergic skin diseases	Flea allergy dermatitis Atopic dermatitis Food intolerance/allergy Insect bite hypersensitivity Contact dermatitis
Neoplastic disease	Cutaneous lymphoma

Ruling out of other skin conditions with clinical signs that can resemble, or overlap with, canine AD.

The evaluation of a pruritic dog requires a step-by-step thought-process and approach that should lead to a definitive diagnosis. The differential diagnoses and role of complicating factors (Table 1) need to be narrowed down using information derived from the history, the findings on physical examination, diagnostic tests (where necessary), and response to treatment. Basic sampling methods and diagnostic tests, which may be required to rule out most of the common differentials are flea combing, skin scraping, hair plucking and cytological examination of skin and ear samples. Depending on the complexity of the case, the following steps may be performed over a series of visits, or all at once.

Step 1 – Consider the possibility of fleas

While the clinical signs in a dog with flea infestation are variable, the location of skin lesions and pruritus associated with flea allergy dermatitis (FAD) are most commonly found at the lumbosacral area, tail base and caudomedial thighs (Fig. 1)[6]. A flea infestation is associated with increased flea counts, whereas in dogs with FAD this may not be the case. In addition, clinicians must be aware that many atopic dogs may suffer from concurrent FAD, which may complicate the clinical diagnosis.

To exclude FAD or flea infestation as a possible cause of pruritus in a particular case, clinicians should apply the following guidelines:

- The prevalence of fleas and associated hypersensitivities depends on the geographical area in which the animal lives. Fleas can be a perennial problem in subtropical and tropical climate zones, seasonal in more tempered climate zones and practically non-existent in arid, high elevation, or cold climates [7, 8]. Even if fleas are considered to be absent from a particular area, clinicians should consider any recent travel history to flea endemic areas or contact with animals from such areas.
- In dogs with pruritus and/or lesions in areas of the body that are not primarily affected by fleas (e.g., the paws or ear canals), FAD may not be the sole cause of pruritus.
- Clinicians should check all pruritic dogs for fleas or flea faeces on direct examination or brushing the hair coat (flea combing). To exclude FAD when fleas or

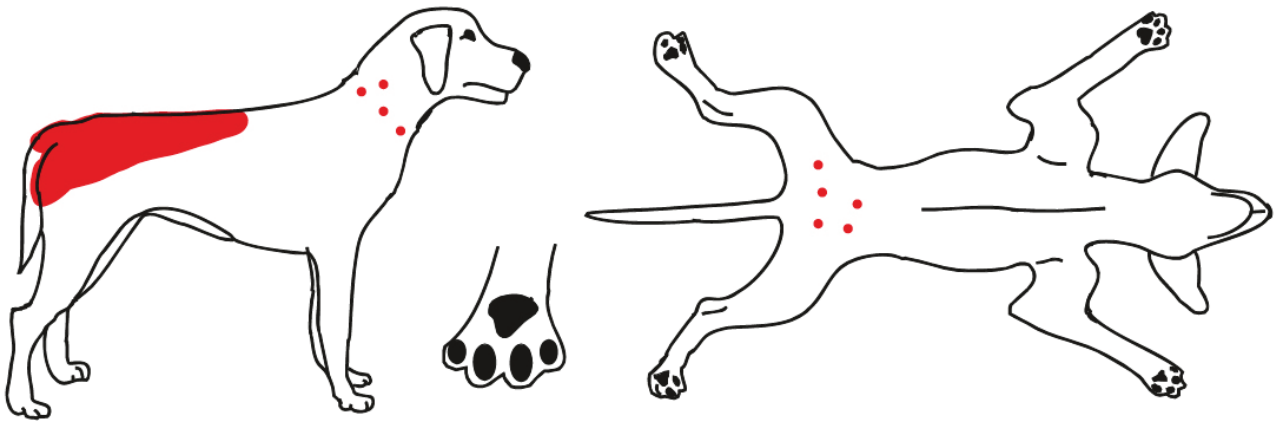

Fig. 1 Distribution of skin lesions and pruritus associated with FAD. Acute lesions: Erythematous macules, papules, crusted papules, hot spots. Chronic lesions: Self-induced alopecia, lichenification, and hyperpigmentation

flea faeces cannot be found, an effective flea control program should be initiated. Clinicians should be aware that none of the current flea preventatives have an effective repellent effect, and that the fleas in the pupal stage can survive up to 174 days [9]. Based on duration of survival it is recommended to maintain consistent flea prevention in flea endemic areas. It is also advised that fast-acting systemic adulticides are used as these may be more effective at reducing pruritus quickly compared to other topically applied flea preventatives [10].

- Cases that are being entered into a study of canine AD should undergo effective flea control prior to study enrollment. Because the duration of flea control, prior to study inclusion, may influence the outcome of such trials, a recent study suggests that dogs should be on flea prevention for at least 3 months prior to study enrollment [11]. In addition, all other dogs and cats in the household need to be on effective flea control as well.

Step 2 – Consider the possibility of other ectoparasites

Besides fleas, other ectoparasites may be associated with pruritus (e.g. sarcoptic mange, cheyletiellosis, pediculosis, trombiculiasis, otoacariasis) or can be found as a concurrent disease (e.g. demodicosis). Although the majority of these parasites favour specific body areas (Figs. 2, 3, 4, 5 and 6), they can be difficult to distinguish clinically.

Prior to an allergy investigation, every attempt should be made to rule out potential ectoparasitic skin diseases. Various sampling methods such as skin scraping, hair combing, hair plucking, ear swabbing and acetate tape impressions can be used to collect specimens. For the identification of these parasites a microscopic examination with a low-power objective (4× or 10×) and low light intensity should be used [12]. The following list indicates which sampling methods are effectively used for various ectoparasites:

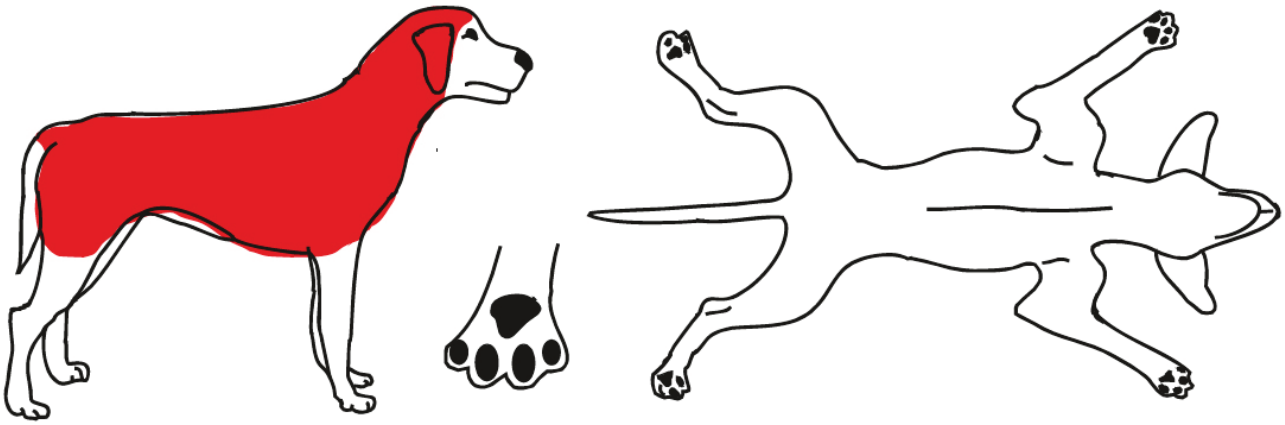

Fig. 2 Distribution of skin lesions and pruritus associated with Lice/Cheyletiella. Lice: No visible lesions, or mild scaling and excoriation. Cheyletiella: Marked dorsal seborrhea

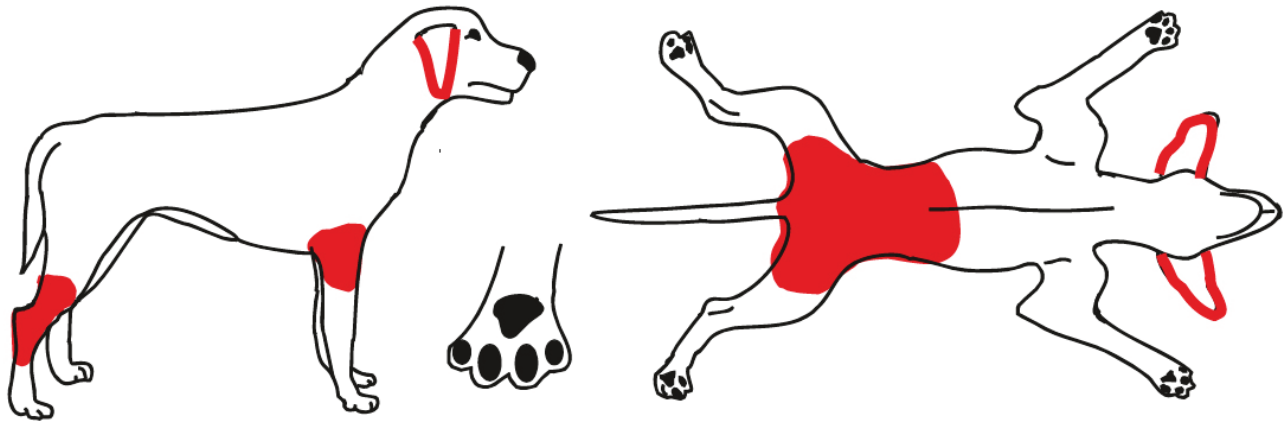

Fig. 3 Distribution of skin lesions and pruritus associated with sarcoptic mange. Lesions include papular eruption, erythema, scaling, excoriations

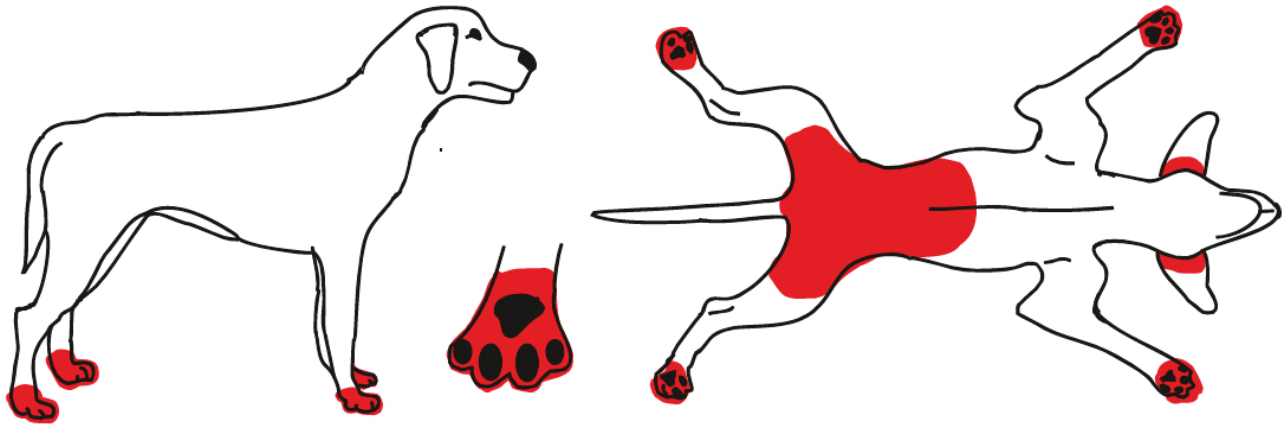

Fig. 4 Distribution of skin lesions and pruritus associated with trombiculiasis. Lesions usually manifest as eruption

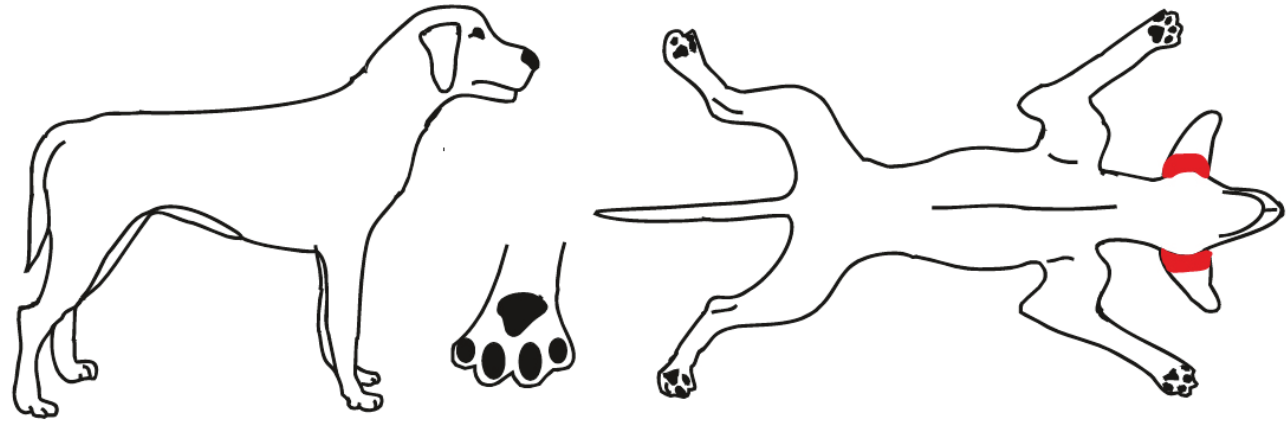

Fig. 5 Distribution of skin lesions and pruritus associated with otoacariasis. Lesions include erythema, dark-brown, coffee-ground-like discharge

- *Sarcoptes scabiei var. canis*: Microscopic examination of multiple superficial skin scrapings, and, where available, blood serum for serology testing (indirect Enzyme-Linked ImmunoSorbent Assay, ELISA). [13, 14] *Sarcoptes* mites can occasionally be found on skin biopsies and fecal flotation [15].
- *Demodex* spp.: Microscopic examination of multiple deep skin scrapings and acetate tape impressions of "squeezed" skin, and hair pluckings [16, 17]. Usually *Demodex* mites are easy to find if multiple affected body areas are sampled. However, sampling infected feet or in breeds with thick skin (e.g. shar peis) may not always be effective and skin biopsies may sometimes be required [18].
- *Cheyletiella* spp., *Trombicula* spp. (chiggers), and lice: Microscopic examination of coat brushings, acetate tape impressions and superficial skin scrapings [15]. *Cheyletiella* spp. and lice also produce eggs, which are attached to hair shafts and can be identified by trichography.

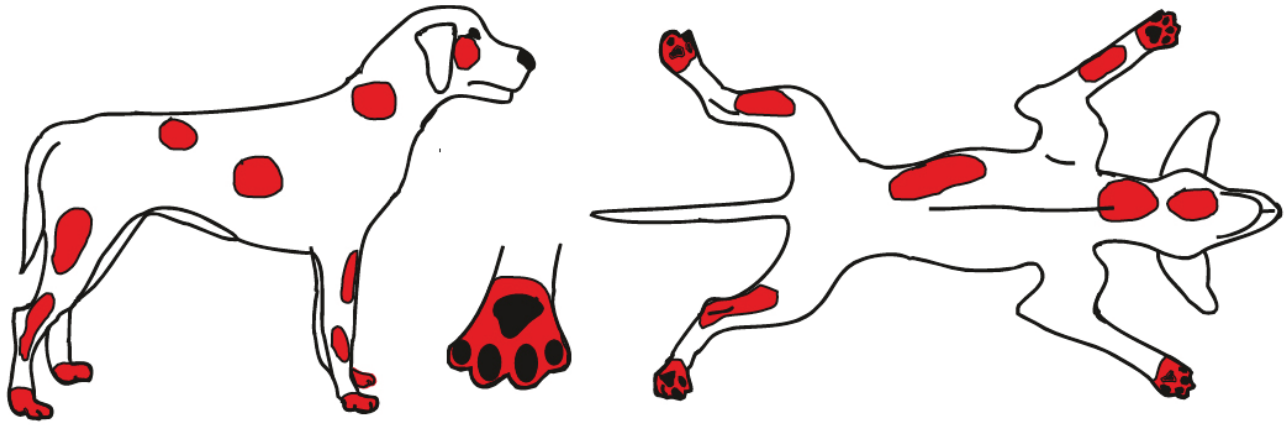

Fig. 6 Distribution of skin lesions and pruritus associated with demodicosis. Lesions include focal, multi-focal or generalised alopecia, scaling, erythema, follicular casts, comedones, Furunculosis

- *Otodectes cynotis*: Microscopic examination of aural discharge. The discharge often appears dark brownblack and crumbly (coffee ground-like) and the mites are white, very mobile and light shy. Occasionally ear mites can be found on superficial skin scrapings at other body sites [19].

Sarcoptes scabiei var. canis and *Cheyletiella* spp. can be difficult to find [15,20]. For this reason a response to an antiparasitic trial treatment (e.g. selamectin, moxidectin, ivermectin, amitraz, lime sulfur) may be necessary to rule out these parasites. A positive pinnal pedal reflex has been associated with Sarcoptes and justifies trial therapy [21]. Especially in the light that Sarcoptic mites are able to cross-react with house dust mites (HDM) in allergy testing, a trial treatment in very pruritic patients is strongly recommended [22, 23].

Step 3 – Consider the possibility of Staphylococcal infection and *Malassezia* overgrowth

Pyoderma

Bacterial skin infections caused by *Staphylococcus pseudintermedius* (SP) are common in dogs with AD. The typical lesions of superficial pyoderma, such as papulopustular eruption and epidermal collarettes, are often distinctive enough to make a clinical diagnosis on gross appearance alone. However, the initial diagnosis should be confirmed by examining cytological samples, stained with Diff-Quik®, taken from the skin by impression smears or acetate tape impressions [12, 24]. Samples from pricked pustules will most likely yield definitive results, while samples from papules and epidermal collarettes may be less rewarding. Aerobic bacterial culture and sensitivity testing is not indicated in every case, but if particular conditions are fulfilled (e.g. previous history of antibiotic treatment, initial appropriate antibacterial treatment has not been effective, high prevalence of meticillinresistant SP in the area, etc.), a bacterial culture with antibiogram should be performed [25]. Bacterial cultures can be performed while the dog is currently being treated with systemic antibiotics [26].

Staphylococcal pyoderma is in most cases a secondary problem associated with underlying pruritic and nonpruritic diseases such as canine AD, but also other allergies as well as endocrinopathies. The pyoderma often causes a change in the overall level or distribution pattern of the pruritus. In these cases, eliminating the pyoderma will determine if the primary disease is itself pruritic, and what its severity and distribution pattern may be. In addition to typical pyoderma lesions, dogs with AD can develop bacterial overgrowth that can complicate other lesion types. Hence, it is wise to sample a variety of lesions to characterise the extent of bacterial involvement and manage the infection appropriately. This should certainly be done whenever cases are poorly responsive to "anti-allergy" therapies, or if studies on canine AD are being performed.

Malassezia dermatitis

The most effective diagnostic test for the identification of *Malassezia* organisms is skin cytology from affected areas such as skin folds, areas with lichenification and oily seborrhea (Fig. 7)[12, 24]. *Malassezia pachydermatis* is a budding yeast organism (3–5 µm in diameter) with a characteristic oval, peanut or "Russian doll" shape, allowing easy identification. In general, clinical signs associated with the cytological presence of yeasts

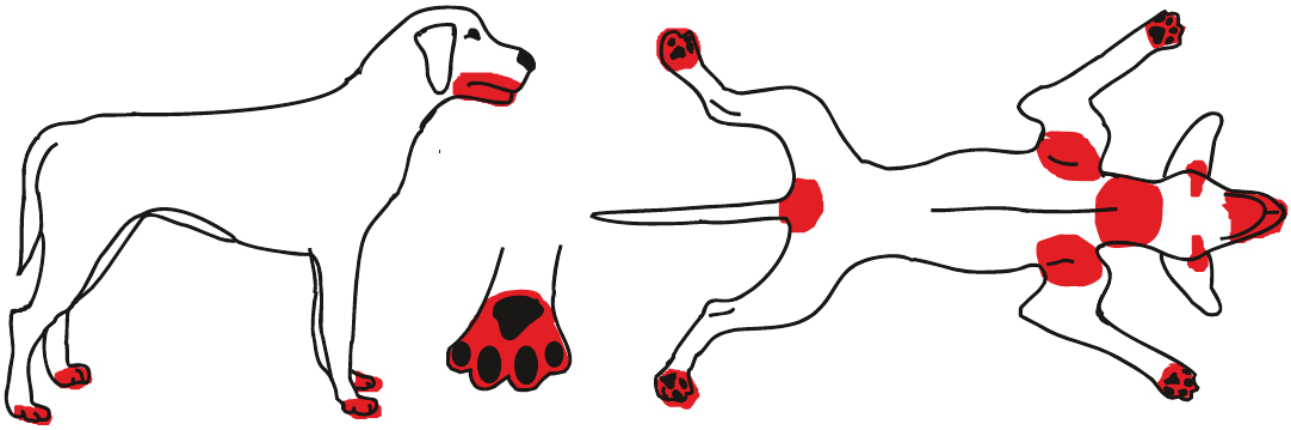

Fig. 7 Distribution of skin lesions and pruritus associated with Malassezia dermatitis. Lesions include erythema, yellowish or brownish greasy scale, hyperpigmentation

reflect a yeast overgrowth or infection. However, in dogs with *Malassezia* hypersensitivity, few organisms may elicit pruritus and associated skin lesions. For this reason a diagnosis of *Malassezia* dermatitis should be based on the clinical and cytological findings and confirmed by a response to antifungal therapy [27]. Fungal culturing can be performed as well, but is not used routinely for the diagnosis of *Malassezia* dermatitis, because false negative culture results have been reported [28, 29]. Therefore, in studies of canine AD, the presence of any number of *Malassezia* organisms should warrant a trial therapy to determine what role, if any, low numbers of *Malassezia* are playing in causing the dog's pruritus.

Step 4 – Consider the role of cutaneous adverse food reaction (CAFR)

Food-related pruritus can be caused by two different mechanisms, one a non-immune mediated reaction (food intolerance), the other immune mediated which includes IgE-mediated hypersensitivity (food allergy)[30]. Because reactions to food components can present clinically as canine AD, or serve as a flare factor in canine AD, dogs with CAFR may be indistinguishable clinically from canine AD [31–33]. The presence of gastrointestinal signs, such as diarrhoea, vomiting, tenesmus, soft stools, flatulence, and increased number of bowel movements is more typically seen with food-induced canine AD [5, 33]. In any canine AD case that has year-round clinical signs, CAFR can only be ruled out by effective strict elimination diet trials, since accurate diagnostic commercial tests are not currently available. This is especially important in trials evaluating drugs for the treatment of canine AD since food-induced AD may not respond well to those drugs, as shown for corticosteroids [5]. Unfortunately, there are no diets that have been shown to be effective in all cases of CAFR. Therefore in some cases, especially when gastrointestinal signs are present, multiple different diet trials may be needed until a sufficient control of the clinical signs has been achieved.

Ideally an elimination diet trial should be performed with a diet to which ingredients the dog has never been exposed before. Unfortunately, most commercially available diets contain a wide range of ingredients and byproducts, making the selection of an appropriate diet difficult. Most over-the-counter diets as well as some prescription elimination diets may be contaminated with traces of other food components [34, 35]. Although hydrolysed diets are offered as an alternative option, the protein source is based on either chicken or soy. For this reason some dogs allergic to chicken and/or soy may not respond to such diets [36]. The most common food allergens in dogs are: beef, dairy, chicken products and wheat, and to a lower degree soy, lamb, pork, fish, and corn [37].

A diet trial is performed by instituting a strict trial with a diet containing commercial or home-cooked novel (e.g. rabbit, kangaroo, venison, horse, etc.) or hydrolysed protein ingredients. The use of these novel proteins is becoming more problematic because several of these novel proteins are now available in over-the-counter commercial diets. A study in humans has also shown that venison does cross-react in vitro with bovine IgG [38], while another study reported that up to 85 % of food allergic dogs may adversely react to venison [39]. Any strict elimination diet trial should be fed exclusively for a minimum of 8 weeks to achieve complete clinical remission in most cases [40]. If the condition improves, the diet should be continued to determine if there is

complete or only partial control of the clinical signs. If a dog is not responding to a commercial elimination diet a second attempt with a home-cooked diet should be performed [34]. Home-cooked diets are considered the most limited-ingredient diets if done properly. All diet trials should be continued until the veterinarian examines the dog. This is important as some owners may not recognize a partial response or be aware of lesions still present when a dog appears to have improved. Dietary involvement is confirmed if there is a relapse of clinical disease when the original diet is re-introduced. Clinicians should be aware that poor owner/patient compliance is a common problem. Typical pitfalls during a diet trial are: feeding table food, raw hides, treats, "hiding" medication in food, using flavoured tooth paste, giving medication in gelatine capsules, using flavoured drugs (e.g. NSAIDs, antibiotics, chewable heartworm or flea preventative), and dogs eating other animals' faeces. Clients need to realize that very small amounts of other foods or food additives ingested, even intermittently, can prevent a favourable response [41]. Crumbs on the floor and even licking another pet's empty bowl may result in a poor outcome. The client's job is to make sure the dog ingests nothing but the prescribed diet and water.

Once steps 1–4 of the diagnostic work-up has been completed, a clinical diagnosis of canine AD should be considered if the pruritus is still present.

Detailed interpretation of the historical and clinical features of canine AD

The initial clinical feature of canine AD is pruritus, which can include scratching, rubbing, chewing, excessive grooming or licking, scooting, and/or head shaking. Depending on the allergens involved, the pruritus may be seasonal (e.g. pollen) or non-seasonal (e.g. dust mites, food) [42]. At the beginning the pruritus may be alesional or associated with primary skin lesions such as erythema and occasionally papules (Table 2) [43, 44]. The face, concave aspect of the ear pinnae, ventrum, axillae, inguinal area, perineal area and distal extremities are most commonly affected in canine AD (Fig. 8) [43], but breed-associated variations of body sites affected by canine AD have been identified (Table 3, Fig. 9)[3]. In more chronic stages secondary skin lesions (Table 2) will occur due to self-trauma, chronic inflammation and secondary infections. Typical secondary skin lesions are excoriations, alopecia, lichenification, hyperpigmentation, crusting, and seborrhea (Fig. 10a-c).

Table 2 Key dermatologic features for canine pruritic skin diseases

Alesional Pruritus	May be seen in the early stages of allergy or when seasonal disease begins. This finding of pruritus in areas with no lesions can occur in canine AD cases at any point in the disease process, especially in cases that have recurrences or come out of remission.
Primary skin lesions	
Erythema	Can be seen with most of the above differentials, but lice and *Cheyletiella* do not usually cause erythema. Demodicosis is highly variable – the skin may or may not appear to be inflamed.
Papules	Seen with flea bites, scabies, trombiculiasis, insect bite hypersensitivity, staphylococcal pyoderma, atopic dermatitis, cutaneous adverse food reaction, and contact dermatitis. Dogs with AD may have small non-crusted papules unless there are concurrent diseases.
Pustules	Most commonly associated with staphylococcal pyoderma
Secondary skin lesions	
Epidermal collarettes	Most commonly associated with staphylococcal pyoderma
Crusting	Most commonly associated with secondary infections and excoriations
Salivary staining	Indicates excessive licking and often associated with *Malassezia*
Excoriations	Self-induced trauma from scratching due to severe pruritus
Alopecia	May be due to self-trauma or folliculitis (superficial pyoderma, demodicosis and dermatophytosis)
Lichenification	Indicates chronic pruritus, inflammation and commonly associated with secondary infections
Hyperpigmentation	Indicates chronic pruritus. Allergies and *Malassezia* are the most common causes and result in dark discoloration of the skin. Blue-grey pigmentation is seen with demodicosis in some cases.

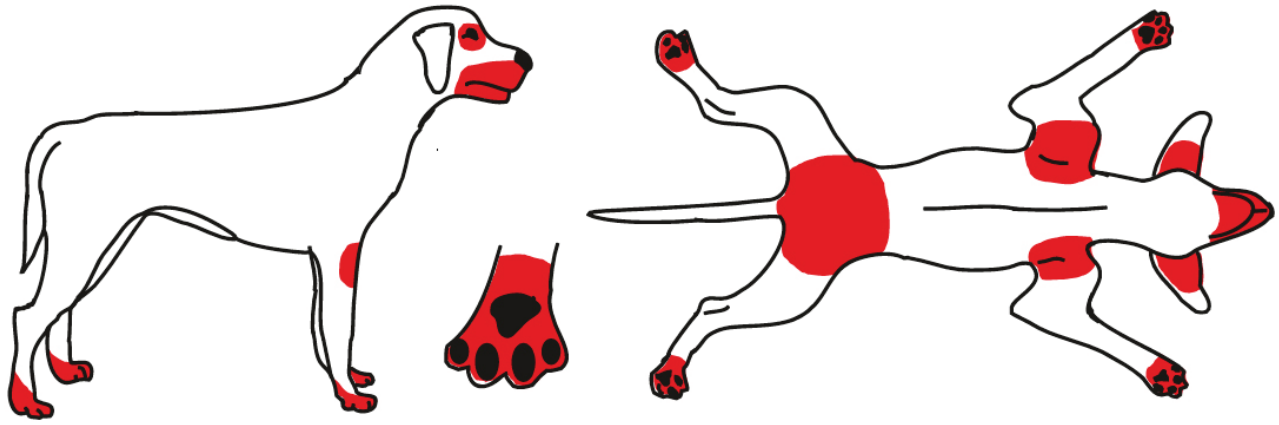

Fig. 8 Common distribution of clinical lesions and pruritus associated with canine AD and food allergy

Table 3 Additional body sites involved in canine AD in certain breeds [3]

Dalmatian	Lips
French bulldog	Eyelids, flexure surfaces
German shepherd dog	Elbows, hindlimbs, thorax
Shar-pei	Thorax, flexure surfaces, dorso-lumbar area
West Highland white terrier (WHWT)	Dorso-lumbar area, lips, flexure surfaces
Boxer	Ears

A new tool to assist with the interpretation of the clinical findings when confronted with a pruritic dog is application of clinical criteria known as "Favrot's criteria" (Table 4) [5]. These include a set of criteria that have been developed from a large case series of confirmed cases of canine AD. The use of complex statistical analysis allowed a set of clinical features to be identified that had maximum association with canine AD. The analysis revealed two sets of criteria, which yield varying levels of sensitivity and specificity for the condition. Clinicians can use whichever set best serves their needs. For example, use of a set of criteria that yields the highest specificity is more likely to ensure that a particular case actually has canine AD. However, this set would exclude some pruritic dogs that were suffering from the disease. A set yielding the highest sensitivity is more likely to capture cases of canine AD, but it could allow some dogs with other conditions to be classified as atopic when in fact they were not. Further guidance about application of these criteria sets is shown in Table 4.

It is crucial to remember that these criteria should not be used in isolation as a "diagnostic test" for canine AD. They should be applied alongside the other guidelines outlined in this review. In other words, the accuracy of using these criteria will be greatly enhanced if the dog has been subjected to a careful work-up as described in the previous section.

Allergy testing

Once a clinical diagnosis of canine AD has been made several factors may play a role in the decision-making whether an allergy test is necessary or not. Severe clinical signs, duration of clinical signs for more than 3 months per year, and insufficient management with symptomatic therapy, due to side effects to the drugs used and/or poor owner compliance, justify in most cases allergy testing. These can be performed by IDT and ASIS. Both tests are not recommended as screening tests and should only be used to confirm the clinical diagnosis of canine AD. The results of these tests are also used to identify the offending allergen(s) in order to formulate an allergen-specific immunotherapy (ASIT).

Although IDT is considered the preferred diagnostic method among dermatologists, ASIS has several advantages over IDT, such as: no patient risk (no sedation required), less traumatic (no repeated injection required), more convenient (no clipping needed, less time consuming), and lower risk of drugs interfering with test results (concurrent anti-inflammatory/antipruritic therapy) [45, 46]. However, ASIS only measures circulating allergen-specific IgE, does not take into account other allergic pathways and often shows positive reactions in non-allergic dogs [47, 48].

IDT and ASIS are still lacking standardization and it is suspected that false positive and false negative results do

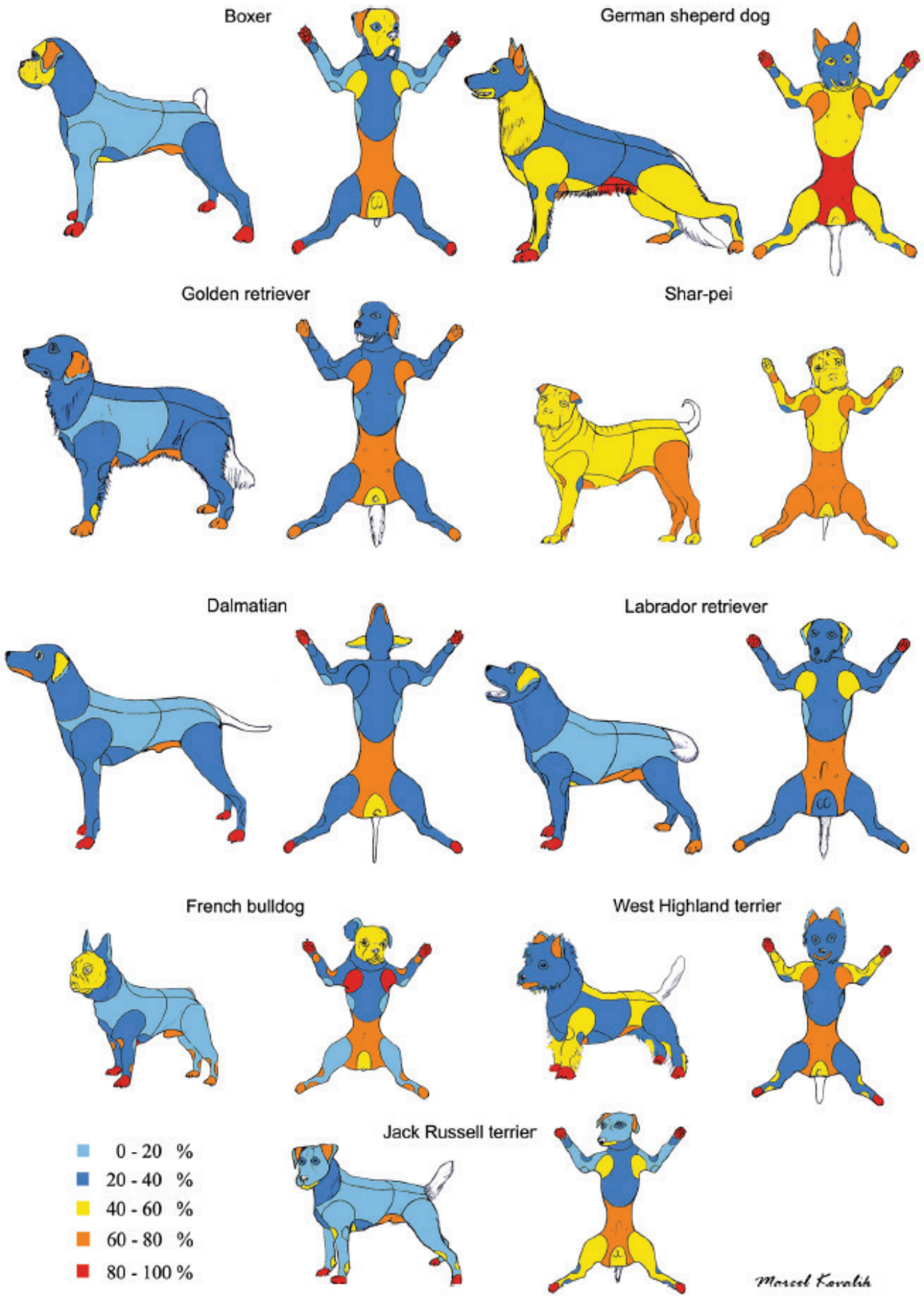

Fig. 9 Silhouettes of atopic boxers, German shepherd dogs, golden retrievers, shar peis, Dalmations, Labrador retrievers, French bulldogs, West Highland white terriers and Jack Russell terriers (in this order). Each colour corresponds to the percentage of affected animals (Reproduced with permission from Wilhelm et al. Breed-associated phenotypes in canine atopic dermatitis. Veterinary Dermatology 2011; 22: 143-149, Wiley; 2010 The Authors' Journal compilation 2010 ESVD and ACVD).

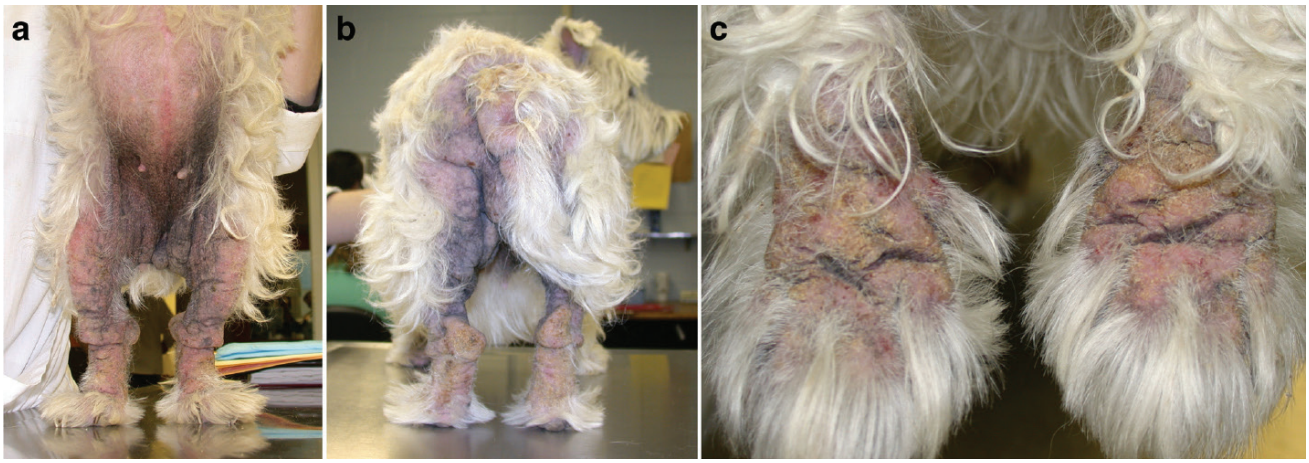

Fig. 10 a, b, c Typical distribution of secondary skin lesions in a West Highland white terrier

Table 4 Favrot's criteria [5]

	Use	Reliability
Set 1: 1. Age at onset <3 years 2. Mostly indoor 3. Corticosteroid-responsive pruritus 4. Chronic or recurrent yeast infections 5. Affected front feet 6. Affected ear pinnae 7. Non-affected ear margins 8. Non-affected dorso-lumbar area	• Use for clinical studies and adapt required criteria based on the goal of the study. • If higher specificity is required, 6 criteria should be fulfilled (e.g., drug trials with potential side effects) • If higher sensitivity is required, 5 criteria should be fulfilled (e.g., epidemiological studies)	**5 criteria:** Sensitivity: 85.4 % Specificity: 79.1 % **6 criteria:** Sensitivity: 58.2 % Specificity: 88.5 %
Set 2: 1. Age at onset < 3 years 2. Mostly indoor 3. "Alesional" pruritus at onset 4. Affected front feet 5. Affected ear pinnae 6. Non-affected ear margins 7. Non-affected dorso-lumber area	• Use to evaluate the probability of the diagnosis of canine AD • 5 criteria should be fulfilled • Do not use alone for diagnosis of canine AD, and rule out resembling diseases	**5 criteria:** Sensitivity: 77.2 % Specificity: 83 % **6 criteria:** Sensitivity: 42 % Specificity: 93.7 %

occur. It is estimated that between 10 and 30 % of dogs with a clinically confirmed canine AD may show a negative IDT [49, 50]. This high percentage of false negative results can be due to several factors including improper technique, too low test concentration of allergens [51,52], drug interference [46], intrinsic host factors, incorrect selection of allergens, IDT performed too long after (>60 days) or during the peak allergy season, and presence of a condition called atopic-like dermatitis [49].

Canine atopic-like disease is clinically identical to canine AD, but IgE response to environmental or other allergens cannot be documented [1]. However, in a recent study the condition has been associated with a lymphocyte-mediated reaction to food [53]. Although it is well known that in people age and season may influence ASIS [54], this information has not been well established in dogs. Both testing methods are very different and not standardized, which inevitably results in poor correlation between both tests [55]. Nonetheless the success rate of ASIT based on ASIS vs. IDT is not significantly different [56]. Finally, it is important to remember that, although little information is available, cross-reactions between related allergens, e.g. house dust and storage mites, have been reported [57–59].

Based on this problem it is important to determine if a dog is really exposed to the allergen(s) it reacted to. The proper interpretation of these test results, in conjunction with the clinical history and clinical presentation, can be complex and time-consuming. For this reason, referral to a veterinary dermatologist is recommended.

Table 5 Recommended IDT concentrations for most allergen suppliers

Allergen	Recommended allergen dilution for IDT [49]	Revised recommended allergen[a] dilution for IDT[51, 52, 60]
Histamine	1:100,000 w/v	1:10,000 w/v
Pollens and moulds	1,000 PNU/mL	1000 to 8000 PNU/mL
Individual dust mites	250 PNU/mL or 1:50,000 w/v	100–200 PNU/mL (*Dermatophagoides pteronyssinus*) 75 PNU/mL (*D. farina, Tyrophagus putrescentiae*, and *Lepidoglyphus destructor*) 50 PNU/mL (*Acarus siro* and *Blomia tropicalis*)
Epidermal extracts (hair, wool, feathers and dander)	250–500 PNU/mL	At least 1,250 PNU/mL 300 PNU/mL (human dander)
Insects	1,000 PNU/mL	At least 1,750 PNU/mL
Whole flea extract	1:1,000 w/v	1:500 w/v

PNU: Protein Nitrogen Units; w/v: weight to volume; a: Allergens from Greer Laboratories Inc., Lenoir, NC, USA

Intradermal testing

IDT is an indirect measure of cutaneous mast cell reactivity due to the presence of IgE [2]. The appropriate selection of allergens to test is fundamental to obtain reliable IDT results. In fact, allergens, mainly pollens, are subject to a great geographic variability. Thus, it is important for veterinarians performing IDT to identify the allergens present in the regional location where the patients live.

Information about relevant allergens can be obtained by contacting veterinary dermatologists, veterinary and medical schools, allergy laboratories, textbooks, local human allergists, weather bureau as well as National Allergy Bureau (http://www.worldallergy.org/pollen/) [49]. From time to time the overall IDT results should be assessed and allergens, which do not exhibit a reaction may be replaced with other important allergens [49]. Intradermal test concentration may also be adjusted since different test concentrations have been suggested over time (Table 5) [49, 51, 52, 60].

Allergens are relatively stable once diluted and can be stored in glass vials up to 8 weeks and in plastic syringes for up to 2 weeks at 4°C [49]. The test solutions should be removed from the refrigerator just prior to the IDT long enough to reach room temperature. As mentioned before, the selection of test allergens should be made based on the prevalence of the allergens in a specific geographical region. However, the selection of test allergens is often based on personal preference and experience and can vary significantly among dermatologists even within the same geographical region [61].

Intradermal injections for IDT are most commonly performed on the lateral thorax, after the hair has been gently clipped and the injection sites marked (minimum 2 cm apart). Typically a volume of 0.05–0.1 ml of each test concentration is injected intradermally and evaluated after 15–20 min. The reaction at each injection site will be compared between those of the positive (histamine phosphate) and negative (saline with phenol) controls. The reaction can be read subjectively and/or objectively. In the first case, assessment of the intensity and/or size of the erythema, turgidity and/or wheal formation will be considered, while for the objective evaluation, measurement of mean diameter of the area of erythema or wheal formation is measured. However, no significant differences were seen where the two methodologies have been compared with each other [62]. By convention, an allergen reaction is positive when the wheal formed is at least equal or greater than halfway between the negative and the positive control reaction. If the subjective evaluation is used, the positive control will assume a conventional grade of 4, whereas the negative control will be graded as 0. A reaction to an allergen is considered positive if it is graded as 2 or greater [49].

Many positive controls have been tested for IDT in dogs; of those the most reliable is histamine phosphate. Histamine has been used at 1:10,000 w/v (0.1 mg/mL) in Europe and 1:100,000 w/v (0.01 mg/mL) in the USA; nevertheless it has been suggested that the more concentrated solution (1:10,000) may yield a more consistent positive skin reaction [51, 63]. The negative control should consist of the solution, which is used to

dilute the allergens for the IDT; this is generally sterile saline with phenol as preservative.

Allergen-specific IgE serology testing

Several assays, mostly based on solid phase ELISAs, have been tested for serum IgE in both human and veterinary medicine. These assays are used to detect specific IgE antibodies against a panel of allergens (e.g. pollen, mould, HDM and epidermal allergens) considered relevant for the patient. In the past decades, the detection of serum IgE has been done using monoclonal, mixed monoclonal or polyclonal anti-canine IgE. However, due to the higher sensitivity and specificity of a monoclonal antibody, the use of polyclonal anti-canine IgE antibodies has decreased markedly [64, 65]. Another veterinary assay using a unique recombinant fragment of the extracellular portion of the human high affinity IgE receptor alpha-subunit (FcεRIα) has shown a strong affinity for canine IgE and a lack of cross-reactivity with IgG [66, 67]. Two versions of in-clinic immunodot assay, Allercept E-screen© (Heska Corp, Ft Collins, CO, USA) has been validated to detect allergen-specific IgE in canine sera [68, 69]. This test has been used as screening test to guide the veterinarian to determine the possibility to perform a full panel ASIS or IDT using mixtures of flea, HDM and pollen allergens. The Allercept E-screen© immunodot assay was able to predict with high probability whether an IDT and/or ASIS would be negative or positive[68]. However, this test is a screening test using mixed allergen, which does not allow the identification of the individual offending allergen, and so does not replace complete IDT or ASIS testing. Currently many other companies are offering allergen-specific serology testing, but based on a recent study test results do not agree well between laboratories [70].

Are IDT and ASIS reliable for the identification of canine adverse food reactions?

Many laboratories offer food allergen-specific IgE panels despite the fact that several studies have suggested that IDT and ASIS are not reliable in diagnosing CAFR[49, 71–73]. IDT for example has a very low sensitivity (10–33 %) and a high variable specificity (50–95 %)[49]. Thus, it is worth to reinforce the concept that IDT and ASIS should not be used to make a diagnosis of CAFR.

Some promising results were obtained by patch testing for food components[74], but at this point the test method is at an experimental stage and will require further evaluation.

Do any drugs interfere with IDT and/or ASIS?

The administration of drugs that can inhibit the release of histamine, and possibly other inflammatory mediators, inducing false negative results needs to be carefully considered when performing an IDT. In fact, antihistamines, glucocorticoids, progestational compounds, β2 adrenergic agonists, bronchodilators, tricyclic antidepressants may interfere with IDT [49]. On the contrary, ketoconazole, essential fatty acids, cyclosporine and oclacitinib seem to interfere less with IDT[75–78]. Similarly, some sedatives should not be used to tranquillize the patient, such as oxymorphone, ketamine/diazepam, acepromazine and morphine [79]. On the contrary, xylazine hydrochloride, medetomidine (dexmedetomidine), tiletamine/zolazepam, thiamylal, halothane, isofluorane, and methoxyfluorane can be safely used [49]. Recommendations on the use of propofol for IDT are still controversial. In one study propofol reduced the histamine reaction, while in a more recent study in atopic dogs the IDT reactions were enhanced [80, 81].

A recent evidence-based review assessed the withdrawal time for IDT and ASIS of commonly used antiinflammatory drugs [46]. Although withdrawal times may vary due to duration of treatment, dosage and type of drugs, the following withdrawal times for common anti-inflammatory medication have been suggested [46]:

IDT: antihistamines (7 days), short-acting oral glucocorticoids (14 days), long-acting injectable glucocorticoids (at least 28 days), topical glucocorticoids (14 days), ciclosporin (probably not needed), pentoxifylline (none)

ASIS: antihistamines (probably not needed), short-acting oral glucocorticoids (none), long-acting injectable glucocorticoids (<28 days), topical glucocorticoids (none), ciclosporin (none)

Abbreviations

AD: Atopic dermatitis; ICADA: International Committee for Allergic Diseases in Animals; IDT: Intradermal Testing; ASIS: Allergen-specific IgE Serology; FAD: Flea allergy dermatitis; CAFR: Cutaneous adverse food reaction; ASIT: Allergen-specific immunotherapy.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors thank the other members of the ICADA for their critical review of this manuscript. These members are, in alphabetical order, Drs. Didier Carlotti, Melissa Eisenschenk, Alexander Koutinas, Thierry Olivry, Jon Plant, Helen Power, Pascal Prélaud, Cherie Pucheu-Haston, Manolis Saridomichelakis, and Regina Wagner. The authors acknowledge the editorial team of BMC Veterinary Research for waiving the publication charges for this article.

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Reprint paper*

Laryngeal paralysis in dogs: An update on recent knowledge

Adriaan M. Kitshoff[1], Bart Van Goethem, Ludo Stegen, Peter Vandekerckhove, Hilde de Rooster

SUMMARY

Laryngeal paralysis is the effect of an inability to abduct the arytenoid cartilages during inspiration, resulting in respiratory signs consistent with partial airway obstruction. The aetiology of the disease can be congenital (hereditary laryngeal paralysis or congenital polyneuropathy), or acquired (trauma, neoplasia, polyneuropathy, endocrinopathy). The most common form of acquired laryngeal paralysis (LP) is typically seen in old, large breed dogs and is a clinical manifestation of a generalised peripheral polyneuropathy recently referred to as geriatric onset laryngeal paralysis polyneuropathy. Diagnosing LP based on clinical signs, breed and history has a very high sensitivity (90%) and can be confirmed by laryngeal inspection. Prognosis after surgical correction depends on the aetiology: traumatic cases have a good prognosis, whereas tumour-induced or polyneuropathy-induced LP has a guarded prognosis. Acquired idiopathic LP is a slow progressive disease, with dogs reaching median survival times of 3–5 years after surgical correction.

* This paper originally appeared in *Journal of the South African Veterinary Association* 84(1), Art. #909, 9 pages.
Eur J Comp An Pract (2015), Winter 25(4); p20-33
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Introduction

It is the authors' opinion that the incidence of laryngeal paralysis (LP) is higher than commonly perceived. This is mainly a result of incorrect diagnosis because of a failure to recognise the typical clinical signs. The authors' experience has shown that many cases that are correctly diagnosed are given an improper grave prognosis. New findings regarding idiopathic LP make the disease progression and response to therapy easier to comprehend (Stanley et al. 2010). Adaptations of the surgical techniques and the use of the unilateral arytenoid lateralisation drastically decreased the associated complications (MacPhail & Monnet 2001; White 1989).

The aim of this article is to sensitise the reader to the clinical signs and treatment options for LP. An update will also be given on the laryngeal anatomy, aetiology and the diagnosis of LP in dogs. The most commonly encountered complications are also discussed.

Anatomy

The larynx is a semi-rigid organ composed mainly of hyaline cartilage and muscles (Evans 1993). During inspiration, contraction of the cricoarytenoideus dorsalis (CAD) muscle results in abduction of the arytenoid cartilages and vocal cords, opening up the glottic lumen and allowing air to pass freely (Evans 1993). Failure of the CAD muscle to contract will result in narrowing of the glottic lumen and respiratory stridor (Monnet & Tobias 2012).

[1] Department of Small Animal Medicine and Clinical Biology, University of Ghent, Belgium
Email: adriaan.kitshoff@ugent.be

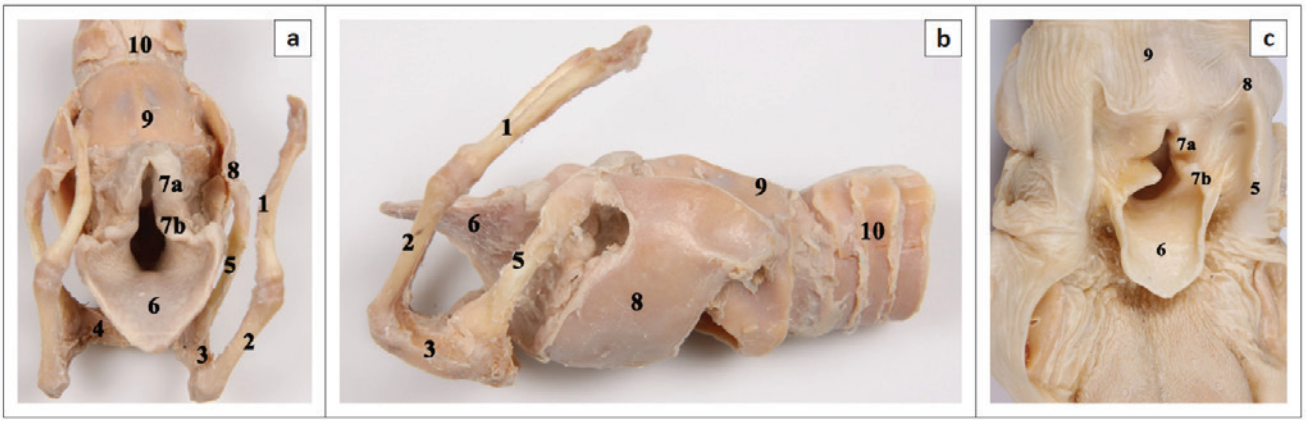

FIGURE 1: Embalmed cadaver specimen of a canine larynx, depicted as, (a) rostrodorsal view with the muscles removed, (b) lateral view after removal of the muscles and (c) rostrodorsal view with the dorsal aspect of the oesophagus removed. Evident in these views are the, (1) stylohyoid, (2) epihyoid, (3) ceratohyoid, (4) basihyoid (5) thyrohyoid, (6) epiglottis, (7a) corniculate process of the arytenoid cartilage, (7b) cuneiform process of the arytenoid cartilage, (8) thyroid cartilage, (9) cricoid cartilage and (10) trachea.

Source: Photographs by M. Doom

The cartilages of the larynx include the epiglottic, arytenoid (paired), sesamoid, inter-arytenoid, thyroid and cricoid cartilages (Figure 1). The arytenoid cartilages have the most complex structure. Their irregular shape is the result of the corniculate, cuneiform, muscular and vocal processes (Evans 1993). The muscular process is situated just lateral to the cricoarytenoid articulation and acts as an insertion site for the CAD muscle (Evans 1993). The corniculate process is the longer of the two dorsal processes and forms the dorsal margin of the laryngeal inlet. The other dorsal process, the cuneiform process, is situated more rostroventrally than the corniculate process (Evans 1993). The ventral part of this process lies in the aryepiglotic fold forming most of the lateral boundary of the laryngeal inlet (Evans 1993). The ring shape of the cricoid cartilage creates a rigid structure that supports the more elastic thyroid and arytenoid cartilages (Monnet & Tobias 2012).

The thyropharyngeus (TP) muscle is situated on the dorsal and lateral aspect of the larynx (Hermanson & Evans 1993). This muscle originates on the lateral aspect of the thyroid cartilage and it extends dorsally to the pharynx to insert on the median plane (Hermanson & Evans 1993). Contraction of this muscle, together with the cricothyroideus muscle, results in constriction of the middle pharyngeal area that assists in swallowing and prevents air from entering the oesophagus (Hermanson & Evans 1993). Opening of the glottis is caused by contraction of the CAD muscle (Hermanson & Evans 1993). This muscle originates from the dorsolateral surface of the cricoid cartilage and inserts on the muscular process of the arytenoid cartilage (Hermanson & Evans 1993). Contraction of the muscle results results in caudodorsal displacement of the arytenoid cartilage (abduction).

All the intrinsic muscles of the larynx, except the cricothyroideus muscle, are innervated by the caudal laryngeal nerve (terminal portion of the recurrent laryngeal nerve) (Hermanson & Evans 1993). The left recurrent laryngeal nerve (RLN) arches around the aorta and ascends on the left side of the trachea, whereas the right RLN arches around the right subclavian artery and ascends on the right side of the trachea (Evans & Kitchell 1993). As the recurrent laryngeal nerves ascend, they give rise to the paralaryngeal recurrent nerves that run parallel to the RLN (Evans & Kitchell 1993). The paralaryngeal recurrent nerves supply sensory innervation to the oesophagus and the trachea (Evans & Kitchell 1993).

Aetiologies and classification

Laryngeal paralysis can be congenital or acquired and, depending on the aetiology, it occurs unilaterally or bilaterally (Monnet & Tobias 2012; Stanley et al. 2010). A hereditary form of LP has been described in Siberian huskies and bouviers des Flandres (O'Brien & Hendriks 1986; Venker-van Haagen 1982). A loss of motor neurons in the nucleus ambiguus as a result of an autosomal dominant trait, with secondary Wallerian degeneration of the recurrent laryngeal nerves, has been identified in the bouvier des Flandres (Parnell 2010).

This disease results in either unilateral or bilateral paralysis and, generally, presents in dogs less than 12 months of age (Burbidge 1995; O'Brien & Hendriks 1986; Ridyard et al. 2000; Venker-van Haagen 1982).

Congenital LP polyneuropathy has been reported in Rottweilers, bouviers des Flandres, bull terriers, Dalmatians, German shepherd dogs, Afghan hounds, cocker spaniels, dachshunds, miniature pinchers and Siberian huskies (Bennnett & Clarke 1997; Braund 1994; Braund et al. 1994; Braund et al. 1989; Eger et al. 1998; Harvey & O'Brien 1982; Mahony et al. 1998; O'Brien & Hendriks 1986; Ridyard et al. 2000; Venker-van Haagen 1982). Clinical signs indicating the presence of a polyneuropathy can also be present. These clinical signs include hyporeflexia (all four limbs), decreased postural reactions, hypotonia and appendicular muscle atrophy (Braund 1994; Braund et al. 1994; Davies & Irwin 2003; Gabriel et al. 2006; Mahony et al. 1998; Ridyard et al. 2000). In young Dalmatians and Rottweilers, axonal degeneration together with loss of myelinated nerve fibres of the RLN and paralaryngeal recurrent nerves are observed (Braund et al. 1994; Braund et al. 1989; Mahony et al. 1998). Phenotypic characteristics, such as white coat, freckles and blue eyes, have been linked to LP in Siberian huskies and German shepherd dogs (O'Brien & Hendriks 1986; Polizopoulou et al. 2003; Ridyard et al. 2000).

Acquired LP can be caused by trauma to the RLN or vagus nerves in the cervical or thoracic region (e.g. bite wounds, surgical trauma, mediastinal tumour) (Monnet & Tobias 2012). Diseases such as neuropathies, caudal brainstem disease, endocrine diseases (hypothyroidism and hypoadrenocorticism), myasthenia gravis, paraneoplastic syndromes, idiopathic myositis, systemic lupus erythematosus and organophosphate toxicity can also result in LP (Burbidge 1995; Dewey et al. 1997; Kvitko-White et al. 2012; MacPhail & Monnet 2001; Michael 2002; Monnet & Tobias 2012; White 1989). The term geriatric onset laryngeal paralysis polyneuropathy (GOLPP) has recently been used to described the commonly encountered syndrome of acquired idiopathic laryngeal paralysis (AILP) (Monnet & Tobias 2012; Parnell 2010; Stanley et al. 2010). Strong evidence exists that this form is a prominent clinical sign of a generalised peripheral polyneuropathy (Jeffery et al. 2006; Stanley et al. 2010). It commonly occurs in breeds such as Labrador retrievers, Rottweilers, Afghan hounds, Irish setters, golden retrievers, Saint Bernards, Irish setters and standard poodles (Gaber, Amis & Le Couteur 1985; Monnet & Tobias 2012).

In contrast to the congenital form, AILP is typically seen in middle-aged to older large breed dogs (Burbidge 1995; Parnell 2010). Male dogs are presented about twice as often as females (Burbidge, Goulden & Jones 1991; MacPhail & Monnet 2001; White 1989).

Clinical signs

Dogs with unilateral LP (mostly left-sided) will only display clinical signs during strenuous activities (i.e. working dogs) (Monnet & Tobias 2012). Failure to abduct the arytenoid cartilages during inspiration results in increased resistance to airflow and turbulence through the rima glottidis leads to the typical inspiratory stridor (Stanley et al. 2010; Venker-van Haagen 1982). Dysphonia is caused by the inability to tense the vocal cords, which results in the dog's voice changing to a weak, hoarse bark (Parnell 2010). Partial obstruction of the upper airways by the paralysed arytenoids leads to exercise intolerance (Burbidge 1995; Parnell 2010).

Respiratory distress (which can lead to cyanosis) can easily be exacerbated by excitement, exercise, elevated environmental temperatures, pulmonary oedema or the presence of bronchopneumonia (Millard & Tobias 2009; Monnet & Tobias 2012; Parnell 2010). The functional airway obstruction can also be worsened by secondary laryngeal oedema and inflammation (Harvey & O'Brien 1982; Millard & Tobias 2009). Overweight dogs with LP present with more severe clinical signs than normally conditioned animals (Broome, Burbidge & Pfeiffer 2000).

Advanced diagnostics can reveal the presence of concurrent bronchopneumonia, megaoesophagus, hiatal hernia or gastro-oesophageal reflux (Burnie, Simpson & Corcoran 1989; Stanley et al. 2010). These can lead to excessive coughing, gagging and regurgitation in affected patients. In one study, oesophageal motility was decreased in all 32 dogs with AILP (Stanley et al. 2010). This was a result of a peripheral neuropathy and was more pronounced if a liquid diet was fed (Stanley et al. 2010). A decrease in oesophageal motility can be clinically silent (Stanley et al. 2010). Dysphagia can be a symptom of peripheral polyneuropathy and can sometimes be seen in patients with LP (Monnet & Tobias 2012). Congenital LP in dogs is usually the result of a polyneuropathy complex and presents in dogs less than 12 months of age (Monnet & Tobias 2012). This form of the disease is characterised by signs of LP together with lenticular cataracts and neurological signs such as tetraparesis (worse in the pelvic limbs), hyporeflexia in all

four limbs, decreased postural reactions, hypotonia and appendicular muscle atrophy (Braund 1994; Braund et al. 1994; Davies & Irwin 2003; Gabriel et al. 2006; Mahony et al. 1998; Ridyard et al. 2000). Concurrent diseases, such as megaoesophagus and aspiration pneumonia, can also be present or can develop during the course of the disease (Braund 1994; Braund et al. 1994; Mahony et al. 1998; Ridyard et al. 2000). In 15 dogs with AILP that underwent a full physical neurological examination in one study, all showed neurological abnormalities in addition to respiratory-related problems (Jeffery et al. 2006). These abnormalities included decreased postural reactions, deficits in spinal reflexes and deficits in cranial nerve function (Jeffery et al. 2006). Clinical signs related to the generalised polyneuropathy can be subtle and care should be taken as they can be overlooked when dealing with a dyspnoeic dog (Jeffery et al. 2006). Neurological dysfunction (ataxia) of the hindlimbs in these older dogs is often misinterpreted as weakness or as an orthopaedic condition (Jeffery et al. 2006). This generalised polyneuropathy is a slowly progressive degenerative condition that affects peripheral nerves (Stanley et al. 2010). Obvious clinical signs of general polyneuropathy and dysphagia can take months to years to develop (Jeffery et al. 2006; Stanley et al. 2010).

Diagnosis

Laryngeal paralysis should be suspected in every patient displaying inspiratory stridor, hoarse voice changes and exercise intolerance. The inspiratory dyspnoea does not resolve with open mouth breathing and will worsen with mild lateral compression over the larynx (Monnet & Tobias 2012).

Clinical signs and signalment are integral parts when diagnosing LP. Bouviers des Flandres and Siberian huskies less than 12 months of age with only respiratory problems are suspected to suffer from hereditary LP (O'Brien & Hendriks 1986; Venker-van Haagen 1982). Middle-aged dogs with respiratory problems consistent with LP combined with neurological dysfunction are suspected of having congenital LP, which is mostly the result of a peripheral polyneuropathy (Monnet & Tobias 2012). Older dogs with exercise intolerance, inspiratory stridor and dysphonia are suspected of AILP. The signalment, together with the history, has a specificity of 91.6% and a sensitivity of 98.5% in all dogs with grade 3 and 4 laryngeal paralysis (Broome et al. 2000).

Laryngeal inspection is essential in order to rule out other causes of laryngeal stridor (e.g. laryngeal tumour) and confirm the suspected diagnosis of LP (Broome et al. 2000). Direct visualisation of the larynx can be achieved via transnasal or peroral laryngoscopy. As the latter has a 95% interobserver agreement, it is considered the gold standard of diagnosis (Broome et al. 2000; Radlinsky et al. 2009; Smith 2000). Transnasal laryngoscopy has the advantage that it can be performed in large breed dogs using only sedation and local anaesthesia (Radlinsky, Mason & Hodgson 2004).

Prior to laryngeal examination, an intravenous catheter is placed and the dog is preoxygenated for at least 3–5 min (Millard & Tobias 2009; Smith 2000). The dog is placed in sternal recumbency and the head is held in a normal anatomic position (Gross et al. 2002; Jackson et al. 2004; Smith 2000). To prevent a false positive diagnosis, only a light plane of anaesthesia is maintained (Gross et al. 2002; Jackson et al. 2004; Monnet & Tobias 2012; Smith 2000). The aim is to achieve relaxation of the jaw muscles without affecting the laryngeal reflexes or depressing respiratory movements (Burbidge 1995). Anaesthetic protocols such as diazepam–ketamine combination are avoided because they result in suboptimal laryngeal exposure during laryngoscopy as a result of poor muscle relaxation (Gross et al. 2002). When drug combinations of acepromazine–propofol, acepromazine–thiopental or diazepam–ketamine were used, half of the normal dogs in one study failed to show arytenoid abduction during inspiration (false positive diagnosis) (Jackson et al. 2004). The same study concluded that intravenous thiopental as a sole drug was best for maintaining laryngeal function (Jackson et al. 2004). Although respiratory depression results when using thiopental as induction agent, a very light plane of anaesthesia results in tachypnea, which is ideal to evaluate the larynx (Turner & Ilkiw 1990). Patients suspected of LP should be examined until they almost reach a plane of consciousness (Burbidge 1995). When laryngeal inspection is not conclusive, doxapram HCl (1.1 mg/kg), which induces deep inspiratory movements, can be useful to differentiate normal dogs from dogs with LP (Tobias, Jackson & Harvey 2004). The increased velocity of airflow, however, will result in an increase in the negative airway pressure, which results in paradoxical arytenoid movement that can lead to complete laryngeal obstruction (Tobias et al. 2004).

Laryngeal inspection involves the evaluation of the arytenoid cartilages for active abduction during inspiration

and passive adduction during expiration (Monnet & Tobias 2012). Immobile arytenoids and vocal cords in an appropriately anesthetised dog indicate bilateral LP, whereas asymmetrical motion of the arytenoids is indicative of unilateral disease (Monnet & Tobias 2012). To avoid false negative diagnoses of LP in patients with paradoxical movement of the arytenoids, it is helpful if an assistant indicates the inspiration phase to the clinician who is performing the laryngeal inspection. Paradoxical movement in LP patients occurs when the increased negative airway pressure during inspiration results in adduction of the arytenoids and, subsequently, the positive pressure during expiration results in passive return of the arytenoids to their resting position (Burbidge 1995).
This is encountered in up to 45% of dogs with LP (Olivieri, Voghera & Fossum 2009). Excessive negative pressure can lead to secondary elongation of the soft palate and eversion of the laryngeal saccules (Millard & Tobias 2009). The constant rubbing of the arytenoid cartilages against each other can result in mucosal ulcerations and oedema at the level of the corniculate processes (Monnet & Tobias 2012).

Other diagnostic methods, such as sound signature identification, tidal breathing flow-volume loops, electromyography, blood gas analysis and plethysomography, can assist in confirming the diagnosis of LP (Amis & Kurpershoek 1986; Bedenice et al. 2006; Burbidge 1995; Yeon et al. 2005). Echolaryngography has been studied but proved less sensitive for diagnosing LP than direct visualisation (Radlinsky et al. 2009; Rudorf, Barr & Lane 2001).

Thoracic radiographs should be taken in all dogs suspected of LP in order to assist in the diagnosis of underlying diseases, such as cervical and cranial mediastinal masses, and to identify other pathologies such as megaoesophagus, aspiration pneumonia and noncardiogenic lung oedema (Monnet & Tobias 2012). In dogs suspected of a megaoesophagus, positive contrast oesophograms could confirm the diagnosis; although, this is not performed routinely because of the increased risk of aspiration (Millard & Tobias 2009). In patients with confirmed laryngeal paralysis, 7% – 14% are subsequently diagnosed with hypothyroidism (Asulp et al. 1997; Dixon, Reid & Mooney 1999; Jaggy et al. 1994; White 1989; Zikes & McCarthy 2012). In dogs showing clinical signs of weakness, megaoesophagus, other peripheral or central neurological signs, exercise intolerance, dermatological abnormalities (hyperpigmentation, alopecia, poor coat quality and pyoderma), lethargy or obesity, free thyroxine and thyroid-stimulating hormone should be tested (Jaggy et al. 1994; Jeffery et al. 2006).

Myasthenia gravis is infrequently associated with LP (Jeffery et al. 2006). In dogs with LP presenting with clinical signs of regurgitation (megaoesophagus), dysphagia, multiple cranial nerve abnormalities, generalised or focal neuromuscular weakness or exercise intolerances, acetylcholine receptor antibody titres need to be measured to rule in or out myasthenia gravis (Shelton 2002). Acquired myasthenia gravis can be associated with hypothyroidism or hypoadrenocorticism, or present as paraneoplastic syndrome associated with thymomas, osteogenic sarcoma, cholangiocellular carcinoma and cutaneous lymphoma (Shelton 2002). An attempt should be made to rule out these primary conditions when a diagnosis of myasthenia gravis has been made.

Medical treatment of respiratory distress

Patients with LP can present in acute respiratory distress, resulting in cyanosis and hyperthermia (Burbidge 1995). Emergency treatment is essential and consists of oxygen supplementation, administration of a sedative and cooling of the patient (Burbidge 1995; Millard & Tobias 2009). The route of oxygen supplementation depends on what is tolerated by the patient and can include an oxygen cage, flow-by oxygen, an oxygen hood, a facemask or a nasal cannula (Mazzaferro 2009). If cyanosis, dyspnoea and hypoxia (SpO_2 < 95%) persist despite oxygen supplementation, a temporary tracheostomy or temporary intubation under light anaesthesia should be considered until laryngeal swelling decreases or surgical correction can be performed (Millard & Tobias 2009). Temporary intubation is selected if the time of intubation is expected to be just a couple of hours, whereas tracheostomy tubes are used for longer-term management (Millard & Tobias 2009). Fluids are administered with caution as pulmonary oedema can develop in animals with severe upper respiratory tract obstruction (Monnet & Tobias 2012). Sedation using acepromazine (0.005 mg/kg – 0.020 mg/kg) and butorphanol (0.200 mg/kg – 0.400 mg/kg) has been recommended (Millard & Tobias 2009). Additionally, short-acting cortico-steroids, such as dexamethasone (0.100mg/kg – 0.500mg/kg) or prednisolone sodium succinate (0.200mg/kg – 0.400mg/kg), can be administered in the case of laryngeal oedema (Millard & Tobias 2009). Hyperthermia should be differentiated from true pyrexia that can occur as a result of aspiration pneumonia. Temperatures lower than 41.0°C are not life

threatening unless prolonged and therapy to cool patients should only be instituted if temperatures are elevated above this level (Mazzaferro 2009; Millard & Tobias 2009). Cooling can be achieved by clipping the fur, by wetting the animal, by applying ice packs over well-vascularised regions (neck, axilla and inguinal region), by fanning the wetted patient or by the rectal administration of cool isotonic fluids (Mazzaferro 2009). Continuous monitoring of the temperature is important and cooling procedures should be discontinued as soon as the body temperature reaches 39.4°C to prevent iatrogenic hypothermia (Mazzaferro 2009).

Conservative management of LP can be considered in older patients with minimal to moderate clinical signs. This involves anti-inflammatory drugs to decrease laryngeal swelling and a weight loss programme for overweight patients (MacPhail & Monnet 2008). The owners should also be educated on the changes in the patient's routine and environment. A cool area should be prepared for the patient, especially in the warmer months of the year. Patients should not be allowed to perform strenuous exercise. Short walks using a harness can be permitted during the cooler periods of the day.

Surgical treatment by cricoarytenoid cartilage lateralisation

Surgical management is advised in all LP patients with severe clinical signs (MacPhail & Monnet 2008; Monnet & Tobias 2012). The aim of surgery is to increase the size of the rima glottidis (LaHue 1989; Millard & Tobias 2009; Monnet & Tobias 2012). As resistance of airflow is inversely proportional to the radius to the power of four, according to Poiseuille's law, even a small increase in size will make a substantial difference (Monnet & Tobias 2012).

Many surgical techniques have been developed and successfully applied. They can be classified as intralaryngeal or extra-laryngeal procedures (Figures 2 and 3).

Cricoarytenoid cartilage lateralisation is currently considered the procedure of choice (Monnet & Tobias 2012). The objective of this procedure is to prevent passive adduction of the arytenoid cartilage during inspiration by fixing it to a neutral to slightly lateralised position (low tension technique) (Bureau & Monnet 2002). This modification still allows adequate epiglottic coverage of the rima glottidis during swallowing and is believed to reduce aspirationrelated complications (Bureau & Monnet 2002).

Unilateral cricoarytenoid lateralisation (UCAL) is performed via a lateral approach (LaHue 1989; Monnet & Tobias 2012). Dogs with unilateral LP are corrected depending on the affected side, whilst dogs with bilateral LP have the lateralisation procedure on the left side if the surgeon is righthanded (MacPhail & Monnet 2001; Monnet & Tobias 2012).

Unilateral correction is sufficient to relieve clinical signs in most bilaterally affected dogs (Monnet & Tobias 2012). Placing a sandbag under the neck elevates the laryngeal region and the skin incision is made over the larynx, just ventral to the jugular vein (Monnet & Tobias 2012). A combination of blunt and sharp dissection through the subcutaneous muscles (platysma and superficial sphincter

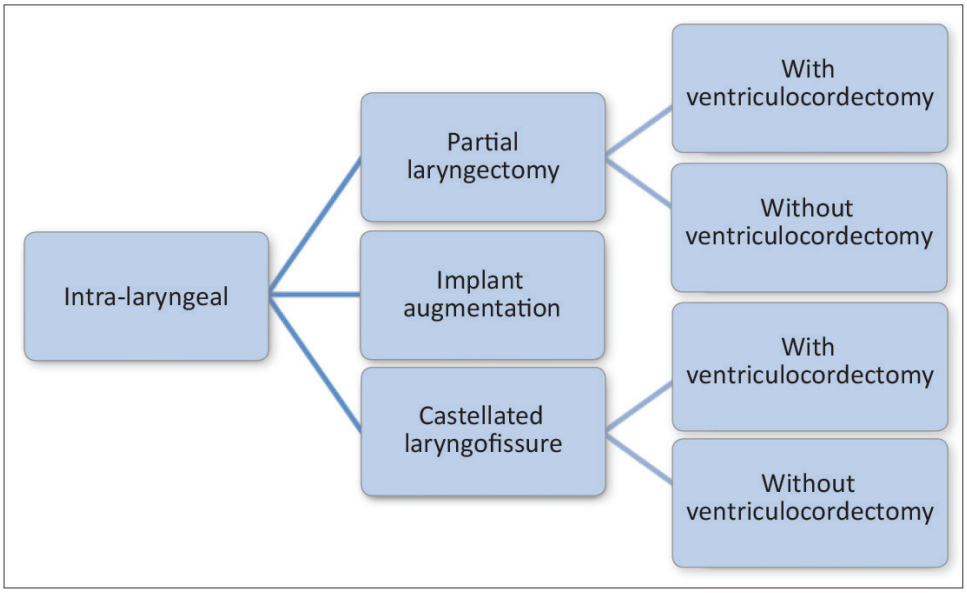

FIGURE 2:
Schematic diagram indicating the different intra-laryngeal surgical procedures in dogs with laryngeal paralysis.

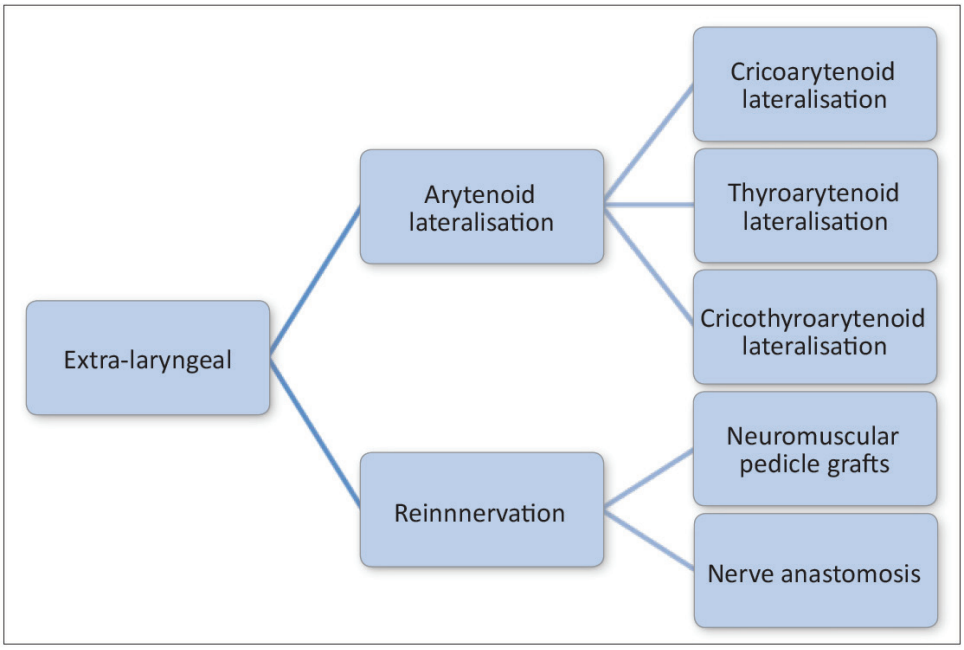

FIGURE 3:
Schematic diagram indicating the different extra-laryngeal surgical procedures in dogs with laryngeal paralysis.

colli muscles) and subcutaneous tissue exposes the TP muscle. This is then incised at the dorsocaudal rim of the lamina of the thyroid cartilage, avoiding penetration of the laryngeal mucosa. Alternatively, the TP muscle can be split along the direction of its muscle fibres (Nelissen & White 2011). Cricothyroid disarticulation may be performed in the adult dog when additional exposure is required. As an alternative, a stay suture can be placed through the lamina of the thyroid cartilage to achieve atraumatic lateral retraction.

The muscular process of the arytenoid cartilage is usually prominent and easily palpable because of the neurogenic atrophy of the CAD muscle (Griffin & Krahwinkel 2005). A transverse incision is made through the CAD muscle and dissection is continued carefully until the cricoarytenoid articulation is visible (Monnet & Tobias 2012). The cranial part of the joint capsule is left intact during dissection of the cricoarytenoid joint (Bureau & Monnet 2002). A nonabsorbable monofilament suture (e.g. polypropylene) on a tapercut needle is recommended for fixing the arytenoid. Depending on the size of the dog, USP 2/0 (< 40 kg) or USP0 (> 40 kg) is used (Demetriou & Kirby 2003). The suture is anchored dorsally on the caudal border of the cricoid cartilage, taking care not to penetrate the laryngeal lumen. It is recommended that extubation be attempted after performing this step as inadvertent suturing of the endotracheal tube can occur (Weinstein & Weisman 2010). The needle is then passed through the muscular process of the arytenoid in a medial-to-lateral direction (Monnet & Tobias 2012). Older dogs can have brittle laryngeal cartilages that can tear during suture placement (Monnet & Tobias 2012). For this reason, needle selection is very important to decrease the risk of tearing or even fracturing of the cartilage once the suture is tightened. Some authors advise pre-drilling a small hole in the arytenoid cartilage using an 18-gauge hypodermic needle before needle placement (Monnet & Tobias 2012).

The suture is carefully tied until resistance from the tensed remaining part of the joint capsule is felt (Bureau & Monnet 2002). Alternatively, the suture can be tied under direct visual endoscopic control after temporary extubation (Weinstein & Weisman 2010). Adequate abduction is defined as any degree of abduction resulting in an increase in the glottic diameter without axial displacement of the dependant (non-surgically treated) side (Weinstein & Weisman 2010) (Figure 4).

Meticulous apposition of the TP muscle, using a continuous suture pattern with monofilament absorbable suture material is essential to decrease the chance for postoperative dysphagia (Nelissen & White 2011). The subcutaneous tissues are closed in two layers and the skin is closed routinely.

Postoperative complications occur in 10% – 58% of dogs (Gaber et al. 1985; Hammel, Hottinger & Novo 2006; MacPhail & Monnet 2001; Snelling & Edwards 2003). These include gagging or coughing, aspiration pneumonia, recurrence of clinical signs (caused by implant failure or cartilage tearing), residual stridor, respiratory distress, gastric dilatation volvulus, seroma or haematoma formation, and death (Millard & Tobias 2009; Monnet & Tobias 2012). It

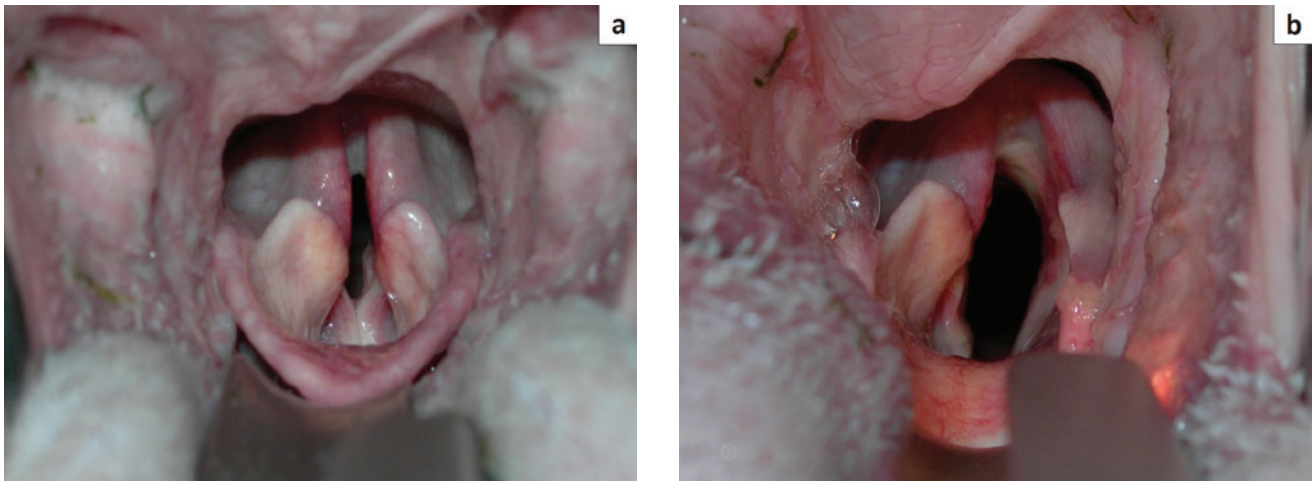

FIGURE 4: Laryngeal inspection in a 10-year-old Maltese dog with laryngeal paralysis, depicting, (a) preoperative appearance and (b) left arytenoid abduction after unilateral arytenoid lateralisation.

Source: Photographs by B. Van Goethem

should be kept in mind that dogs carry a lifelong risk for the development of respiratory tract complications postoperatively (MacPhail & Monnet 2001). Aspiration pneumonia is the most frequently noted complication, occurring in about 8% – 24% of dogs postoperatively (Demetriou & Kirby 2003; Hammel et al. 2006; MacPhail & Monnet 2001; Snelling & Edwards 2003; White 1989). Low-tension techniques are believed to decrease the incidence of postoperative aspiration pneumonia (Bureau & Monnet 2002).

About 5% of patients require a contralateral procedure because of arytenoid fragmentation, avulsion of the lateralisation suture or inadequate lateralisation (White 1989). Recurrence of clinical signs postoperatively is seen more commonly in small breed dogs (Snelling & Edwards 2003). Complications during the postoperative period can be minimised by sound knowledge of the anatomy, meticulous tissue handling and avoidance of laryngeal lumen penetration (Monnet & Tobias 2012). Factors that negatively influence the surgical outcome include age, concurrent respiratory tract abnormalities, oesophageal disease, neurological disease or neoplastic disease and the placement of a temporary tracheostomy tube (MacPhail & Monnet 2001). Unilateral cricoarytenoid lateralisation has a good clinical outcome, with 88% – 90% of dogs showing an improved quality of life in the postoperative period (Hammel et al. 2006; Snelling & Edwards 2003).

Variations of this technique exist in which the arytenoid is also fixed to the thyroid (cricothyroarytenoid lateralisation) or solely to the thyroid (thyroarytenoid lateralisation) (Monnet & Tobias 2012). The latter technique results in a less extensive (but satisfactory) opening of the rima glottidis when compared to cricoarytenoid lateralisation and takes less time to perform (Griffiths, Sullivan & Reid 2001). The clinical outcomes of UCAL and thyroarytenoid lateralisation compare well (Griffiths et al. 2001).

Other surgical techniques

Permanent tracheostomy creates a bypass of the larynx (Monnet & Tobias 2012). It is considered in patients that are at risk for postoperative aspiration pneumonia. This includes patients with generalised myopathy, megaoesophagus, hiatal hernia and gastrointestinal disorders (Monnet & Tobias 2012).

Partial laryngectomy (Figure 5) is an older technique involving removal of the vocal cords and a substantial part of the corniculate and vocal processes (unilateral or bilateral) in order to ensure unobstructed airflow without influencing the protective effect on the airway (Harvey 1983a, 1983b). This procedure can result in significant postoperative swelling that might necessitate placement of a temporary tracheostomy tube. Complications are seen in approximately 50% of the dogs and include persistent upper respiratory stridor, coughing, vomiting, aspiration pneumonia, laryngeal webbing and exercise intolerance (Harvey 1983a; Harvey & O'Brien 1982; MacPhail & Monnet 2001; Ross et al. 1991) (Table 1). This abandoned technique has recently regained popularity since the introduction of diode laser arytenoidectomy via transoral approach. No direct postoperative complications were reported in 20 dogs and only 10% developed aspiration pneumonia in the long term (Olivieri et al. 2009).

A recent retrospective study on ventriculocordectomy via ventral laryngotomy has shown some promising results with

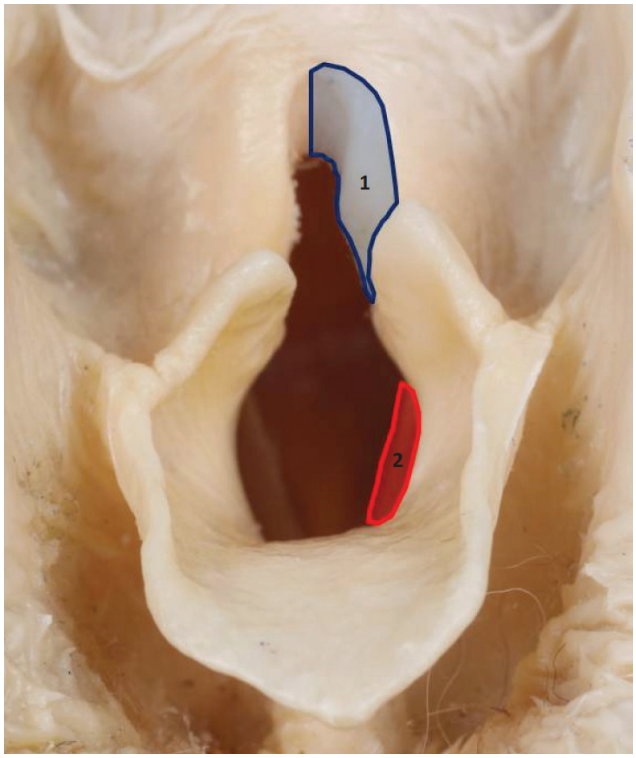

FIGURE 5: Schematic presentation of unilateral partial laryngectomy on an embalmed cadaver specimen of the canine larynx, indicating the area of the arytenoid cartilage to be removed in blue (1) and the location of the vocal fold in red (2).

Source: Photograph by M. Doom

limited short-term and long-term complications. The authors of this article concluded that because of the ease of the procedure, the limited complications and minimal surgical trauma, this technique should be considered for routine use (Zikes & McCarthy 2012).

Castellated laryngofissure is another historical procedure that creates an enlargement of the ventral laryngeal ostium by offset closure of a castellated incision on the ventral aspect of the thyroid cartilage (Figure 6). This procedure is technically difficult, results in severe postoperative laryngeal oedema and requires the placement of a temporary tracheostomy tube for 2–3 days postoperatively (Monnet & Tobias 2012). Variable results have been obtained and the procedure was abandoned (Burbidge et al. 1991).

Reinnervation techniques and neuromuscular pedicle grafts have been used in dogs to restore the abductor function in experimentally denervated patients (Greenfield et al. 1988; Paniello, West & Lee 2001; Rice 1982). These techniques might be of use in patients with acquired LP of traumatic origin. It is likely to be ineffective in patients with polyneuropathy or polymyopathy as a primary cause (Monnet & Tobias 2012).

TABLE 1: Surgical treatment methods with their reported percentages of improvement, aspiration pneumonia, minor complications (persistent stridor, coughing, gagging, panting, seroma formation, exercise intoler ance or vomiting), webbing and mortality rate.

Treatment method	Improvement (%)	Aspiration pneumonia (%)	Minor complications (%)	Webbing (%)	Mortality (%)
Unilateral arytenoid lateralisation[1,2,3,4,5]	90	10–28	9–56		0–14
Bilateral arytenoid lateralisation[6,4]	-	11–89	-	-	67
Bilateral arytenoid lateralisation with ventriculocordectomy[7]	88	15	30	-	0
Castellated laryngofissure with ventriculocordectomy[6]	100	-	40	40	-
Partial laryngectomy, transoral approach with or without ventriculocordectomy [4,8,9]	88–90	6–33	44	8–14	30
Partial laryngectomy, transoral approach – diode laser[10]	100	10	-	0	-
Ventriculocordectomy, transoral approach[11,12]	83	15	40-73	13	-
Ventriculocordectomy, ventral approach[13]	93	3	6	0	-

[1,] Demetriou and Kirby (2003); [2,] Griffiths et al. (2001); [3,] Hammel et al. (2006); [4,] MacPhail and Monnet (2001); [5,] White (1989); [6,] Burbridge et al. (1998); [7,] Schofield et al. (2007); [8,] Ross et al. (1991); [9,] Trout et al. (1994); [10,] Olivieri et al. (2009); [11,] Asulp et al. (1997); [12,] Holt and Harvey (1994); [13,] Zikes and McCarthy (2012).

For more information on these sources, please see the full reference list of the article, Kitshoff, A.M., Van Goethem, B., Stegen, L., Vandekerckhove, P. & De Rooster, H., 2013, 'Laryngeal paralysis in dogs: An update on recent knowledge', Journal of the South African Veterinary Association 84(1), Art. #909, 9 pages. http://dx.doi.org/10.4102/jsava.v84i1.909

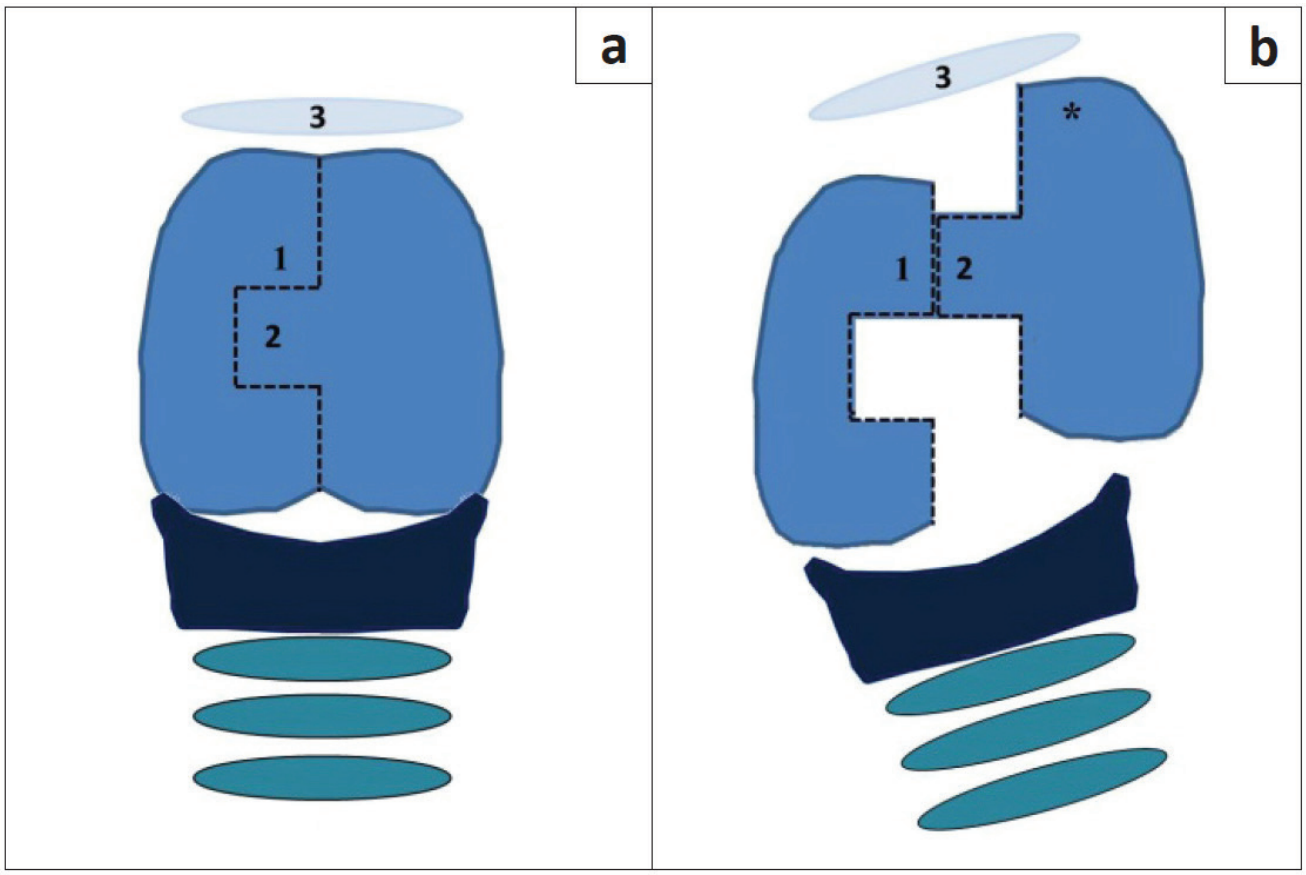

FIGURE 6: Schematic presentation of the castellated laryngofissure technique, depicting, (a) the ventral view of the thyroid cartilage indicating the stepped incision and (b) the advancement of the cartilage flap.
After incision of the cartilage, the flap located at 2 is advanced and positioned lateral to 1, as shown. The cranial border of the cranially advanced thyroid cartilage segment (*) is sutured to the basihyoid (3) with two simple interrupted 2/0 monofilament non-absorbable sutures. The apposed edges in the rostral third (between 1 and 2) are also sutured using the same suture material.

Its routine use is also questioned as it takes a minimum of 5 months for restoration of laryngeal function (Greenfield et al. 1988).

Laryngeal augmentation with implantable devices has been reported ex vivo (Cabano et al. 2011) and in vivo (Kwon et al. 2007) in canine patients. No extensive clinical data exist for the current devices and hence their use can currently not be recommended.

Postoperative care

After surgical treatment, partial obstruction of the larynx will be relieved and the respiratory dyspnoea will resolve immediately. Oxygen therapy should be administered as necessary and perioperative dexamethasone sodium phosphate (0.1 mg/kg – 1.0 mg/kg) can be helpful to decrease laryngeal swelling and oedema (Monnet & Tobias 2012). Food and water is withheld until 12 h after the operation. Heavy sedation in the postoperative period is avoided to preserve the swallowing reflexes (Monnet & Tobias 2012). The patient is first offered canned food rolled into balls (Monnet & Tobias 2012). If no coughing or gagging is observed, small amounts of water can be offered (Monnet & Tobias 2012). The decision to administer postoperative antibiotic is usually case based.

Prognosis and conclusion

A clear distinction needs to be made between the different forms of the disease. Prognosis for hereditary LP is excellent as dogs are cured by surgery. Congenital LP neuropathy has a poor prognosis and most dogs tend to be euthanased within 10 weeks as a result of worsening clinical signs (Davies & Irwin 2003). The prognosis for acquired LP will vary depending on the cause: trauma cases can be cured; neoplasia-induced LP will depend on the tumour type.

Evidence strongly suggests that the most common form of LP in dogs is, in fact, an early stage of GOLPP (Stanley et al. 2010). Even though all complications should be considered when making a prognosis in any dog developing LP as a component of polyneuropathy, this condition progresses slowly, making short-term prognosis more favourable.

Acknowledgements

The authors would like to the Department of Morphology at the Faculty of Veterinary Medicine, Ghent University for supplying the embalmed canine larynxes for the photographs shown in Figures 1 and 5.

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

A.M.K. (University of Ghent) wrote the manuscript. H.d.R. (University of Ghent), B.v.G. (University of Ghent), L.S. (University of Ghent) and P.V. (Veterinary Centre Malpertuus) made conceptual contributions.

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Reprint paper*

Canine recurrent flank alopecia: a synthesis of theory and practice

Sophie Vandenabeele[1], Jan Declercq, Hilde De Cock, Sylvie Daminet

SUMMARY

Canine recurrent flank alopecia is a non-inflammatory, non-scarring alopecia of unknown aetiology and has a visually striking clinical presentation. Although this disease entity is relatively common in the northern hemisphere, there is only scant information in the literature regarding case descriptions. The aim of this article was to review the literature and to describe clinical presentations recognized in practice, which are not always extensively documented in the literature.

* This paper originally appeared in *Vlaams Diergeneeskundig Tijdschrift*, 2014 (83): 275-283. *Eur J Comp An Pract* (2015), Winter 25(4); p34-43 Go to http://www.ejcap.org to see the online presentation of this paper.

Introduction

Canine recurrent flank alopecia (CRFA) is a visually striking disease characterized by cyclic episodes of non-inflammatory hair loss (or coat changes) that can recur annually (Miller et al., 2013a). Several names have been proposed for this unique canine alopecic disease (canine flank alopecia, seasonal flank alopecia, idiopathic cyclic flank alopecia, cyclic follicular dysplasia) but none of the names fit perfectly: complete hair loss is not always seen, alopecia is not always confined to the flank area, and some dogs only experience one episode throughout their entire lives (Paradis, 2009). This intriguing disease was first reported in 1990 by Danny Scott (Scott, 1990). He described a clinically distinct form of waxing and waning non-scarring alopecia in five ovariohysterectomized dogs. Later, it became evident that dogs of either sex and reproductive status could be affected. Although the disease is well recognized in practice, it remains poorly documented in the veterinary literature. The aim of this article is to make a synthesis of the current knowledge from the literature and the different clinical presentations that are recognized in practice, but which are not extensively mentioned in the literature.

Aetiology and pathogenesis

The exact aetiology of CRFA remains unknown. Studies evaluating thyroid function, reproductive hormones and growth hormones in affected dogs have not revealed abnormalities (Curtis et al., 1996; Daminet and Paradis, 2000). However, a localized change in the amount or sensitivity of the hair follicle receptors cannot be excluded (Miller et al., 2013a).

Because the disease is more prevalent in certain breeds, such as Boxers, Airedales, Schnauzers, English bulldogs, Affenpinschers, Griffon Korthals and Bearded collies, a genetic predisposition is suspected (Paradis, 2009; Waldman, 1995; Fontaine et al., 1998). Duration of daylight exposure or changes in light exposure appear to play a role in the development of the lesions. Several observations support the role of light in the pathogenesis of this disease. Firstly, there is the seasonal nature and often annual recurrence of the disease. Interestingly, the onset of CRFA in the northern hemisphere is the reverse of

[1] Department of Medicine and Clinical Biology of Small Animals, Ghent University, 133 Salisbury Avenue, 9820 Merelbeke, Belgium. s.vandenabeele@ugent.be

what is seen in Australia and New Zealand, which means that in both hemispheres, the onset of alopecia coincides with the months with a shorter duration of daylight (Miller et al., 2013a; Paradis, 2012, Basset et al., 2005). Secondly, some cases that have been reported in the literature describe the development of lesions in dogs that were kept in abnormal light conditions; one dog in the northern hemisphere developed lesions in the summer when kept in a dark room (Waldman, 1995; Ando and Nagata, 2000). Light therapy has anecdotally been tried with success as a preventive therapy. In another study, dogs exposed to 100-200 Watt during 15 to 16 hours from September till April did not develop alopecia (Paradis, 1998).

There are two important photo-dependent hormones in the body: melatonin and prolactin. Melatonin is primarily synthesized in the pineal gland and acts at the level of the pars tuberalis of the pituitary. Its production is proportional to the length of the dark period. Decreased retinal daylight exposure results in increased melatonin production. Melatonin is important for reproduction, thermoregulation, coat colour and hair cycling (Paradis, 2000; Stankov et al., 1994).
It is known to be involved in the moulting of several mammalian species. Melatonin implants have been used in foxes and minks to manipulate seasonal coat changes (Valtonen et al., 1995; Rose et al., 1984).

Because of the familial incidence, the association with light exposure and the positive effects of melatonin supplementation, a decreased endogenous melatonin production in genetically predisposed animals is suggested to play a role in the pathogenesis of this disease (Paradis, 1995). Melatonin may act directly on the hair follicle or indirectly through modulation of melatonin stimulating hormone (MSH) and/or prolactin (Paradis, 1995; Fischer et al., 2008).

Prolactin levels are known to inversely correlate with melatonin levels (Messenger, 1993). The increase of melatonin levels and subsequent decrease in prolactin levels induce the formation of a winter coat in sheep (Paradis, 2000; Nixon et al., 2002). Hair follicle cycling is governed by seasonal changes to produce a summer and winter moult, and the other photo-dependent hormone, prolactin, has been implicated as a principal regulator of this process (Messenger, 1993). Prolactin is synthesized in the pineal gland. It has been shown to inhibit growth of anagen follicles in mice and sheep (Nixon et al., 2002; Craven et al., 2006). It is believed to have inhibitory effects at different stages of the hair follicle cycle with the ability to reduce hair length, shorten anagen, induce shedding or lengthen the telogen phase (Nixon et al., 2006; Craven et al., 2006; Thompson et al., 1997). Prolactin may thus very well be an important player in CRFA but no studies have been done to assess its potential role.

Clinical presentation

The age of onset has a wide range: from one year of age to eleven years, with most cases developing clinical signs for the first time between three and six years of age (Miller et al., 2013a; Paradis, 2009; Paradis, 2012). Numerous breeds can be affected, but there seems to be a breed predilection in the Boxer, English bulldog, Airedale, Griffon Korthals, Affenpinscher, Labrador retriever, Golden retriever, Bouvier des Flandres, Dobermann and Schnauzer (Miller et al., 2013a; Paradis, 2009; Waldman, 1995; Fontaine et al., 1998; Cerundolo; 1999). Dogs of either sex and reproductive status can be affected. In practice, the typical clinical presentation of CRFA is a bilateral symmetrical, geographic-shaped, non-scarring and non-inflammatory alopecia in the thoracolumbar area. It is further characterized by a rapid onset of alopecia between the months of November and April in the northern hemisphere. The actual month of onset does not appear to be related to breed, age, sex or reproductive status (Miller et al., 2013a; Paradis, 2012). Hair regrowth can rarely take up to 18 months and permanent alopecia can be seen in chronic recurrent patients. Often, the area of alopecia remains visually recognizable, because regrown hair has a slightly different texture and/or colour (Miller et al., 2013a; Paradis, 2012). Hyperpigmentation in the alopecic area may be striking but is not always present.
The presence or absence of hyperpigmentation in response to light exposure depends on the breed and within certain breeds depends on the individual pigmentation profile of the dog. In some breeds and some individuals, hypermelanization of the skin resulting from endogenous production of factors that stimulate the melanocytes has never been noticed. Breed-related lack of hyperpigmentation is usually seen in the wirehaired pointer, Dalmatian, Dobermann, Vizsla and Weimaraner (Paradis, 2009; Declercq, 2008).

The classical distribution of the lesions is the lateral to dorsolateral thorax and lumbar region. Lesions consist of well-circumscribed patches of alopecia exhibiting a

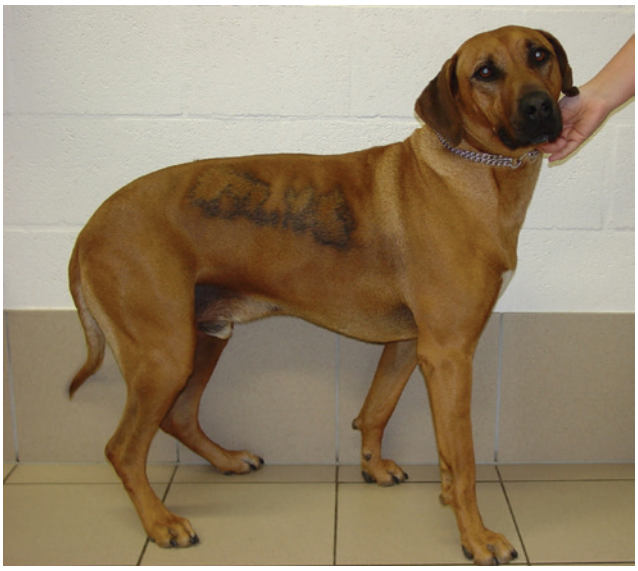

Figure 1. Classical presentation of canine recurrent flank alopecia. A four-year-old Rhodesian ridgeback with bilateral symmetrical, geographic shaped alopecia on the thoracolumbar area and marked lesional hyperpigmentation.

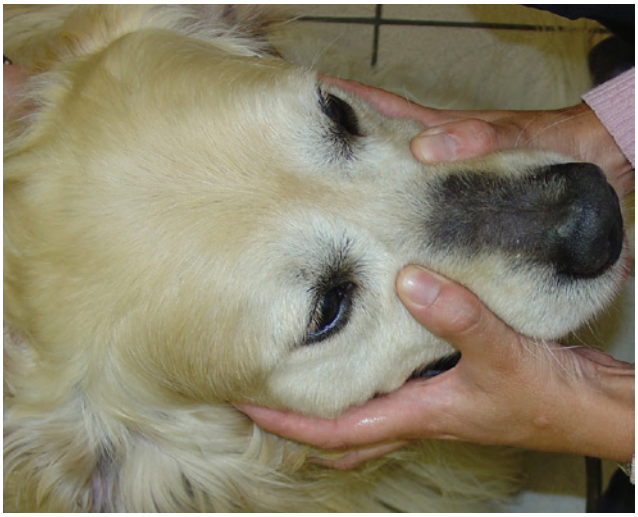

Figure 2. Facial presentation with complete alopecia of the dorsal muzzle in a Golden retriever.

geographic and irregular pattern (Figure 1).
The alopecic area ranges in size from 2 cm to almost the entire thoracolumbar area. Lesions most commonly are bilateral symmetrical, but one side is commonly more affected then the other. Unilateral lesions have been recognized. At the time of onset, there is lesional increased epilation (Miller et al., 2013a).

Several atypical presentations (facial presentation, generalized presentation, flank alopecia without an episode of visual flank alopecia and flank alopecia with interface dermatitis) have been recognized in practice (Declercq, 2008; Vandenabeele, 2007; Vandenabeele, 2014). They are called atypical because the distribution of the alopecia is not confined to or absent in the thoracolumbar area, or because instead of alopecia, there is only a discoloration

Figure 3. Facial presentation with alopecia and hyperpigmentation of the dorsal muzzle and facial folds in a Cane Corso (Picture courtesy of Ilona Schwarzkopf).

and texture change of the coat. The factors that unify all of these cases are the often recurrent nature of the rapid onset of the non-pruritic lesions between November and April (except for the dogs with discoloration of the coat, where coat colour changes and texture changes are seen later in the year and have an onset between April and September) and the spontaneous hair regrowth (Declercq, 2008; Cerundolo and Rest, 2013).

Facial presentation

In these patients, alopecia of the dorsal muzzle and sometimes associated mild alopecia in the periocular region are seen (Figure 2). This is most commonly noticed in the Golden retriever and Labrador retriever (Declercq, 2008; Cerundolo and Rest, 2013; Vandenabeele, 2007).

A visually more striking variation of this presentation is seen in the Bordeaux dog. These dogs present with alopecia and hyperpigmentation of the dorsal muzzle and facial folds. The affected dogs have no alopecia in the thoracolumbar area. The authors of the present study have also seen a Cane corso with this presentation, where the dog had three episodes of alopecia on the facial folds in three consecutive years (Figure 3). The alopecia started in April and hair regrowth was seen in July (Vandenabeele, 2014).

Generalized form

In these dogs, alopecia is present in the thoracolumbar area and other regions, such as the dorsal muzzle, periocular regions, base of the ears, perineum and base of the tail

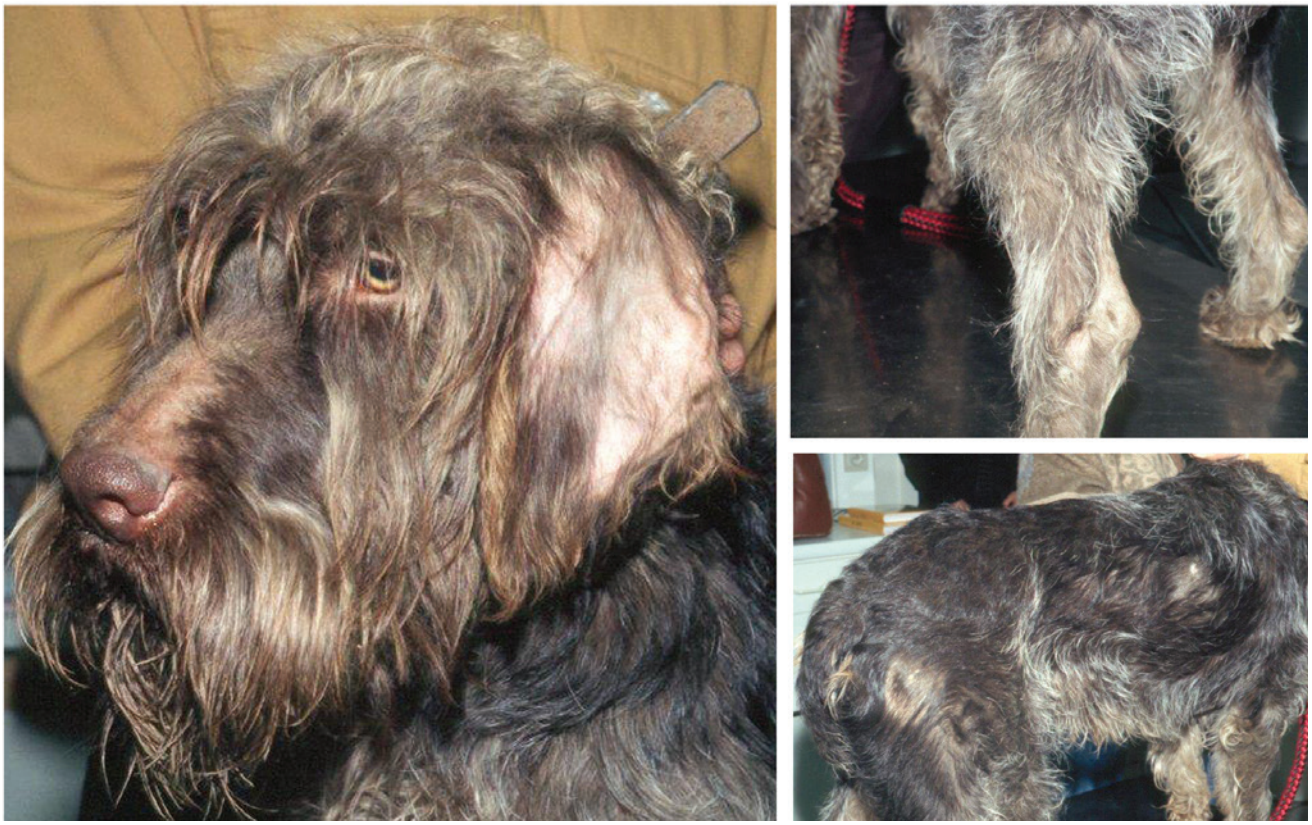

Figure 4. Generalized presentation of canine recurrent flank alopecia in a wirehaired pointer. Note alopecia of the pinnae, flanks and distal extremities.

(Miller et al., 2013a; Declercq, 2008; Cerundolo and Rest, 2013) (Figure 4). This multifocal non-scarring alopecic form has been described in some Airedales, Golden retrievers, Griffon Korthals, Dobermanns, wirehaired pointers and giant Schnauzers (Paradis, 2009). Spontaneous regrowth is seen simultaneously in all of the affected areas.

Flank alopecia without an episode of visual alopecia

Coat colour changes and/or changes in texture of the coat are seen in the flank and thoracolumbar area, without a visual episode of alopecia. These coat colour changes are irregular in distribution and may have a geographic pattern. In the literature, aurotrichia has been described in Schnauzers without preceding alopecia (White et al., 1992). Interestingly, in the study by White et al. (1992), the onset of the discoloration of the coat from silver or black hairs turning into a gold-coloured coat occurred during the months of April till September. This is later than what has been observed in the other forms of flank alopecia. One of the Schnauzers had two consecutive episodes of aurotrichia (White et al., 1992). Idiopathic aurotrichia has also been described in a Bichon frisé (month of onset not reported) (Miller et al., 2013b). The authors of the present study have seen recurrent aurotrichia in a Poodle, Lhasa apso, Cocker spaniel and Yorkshire terrier during the months of April to September (Figure 5).

It is currently unknown why the coat colour change of these dogs occurs at that time of the year. Possibly, the changes in the hair coat represent the recovery phase of the disease and are actually new grown hairs.

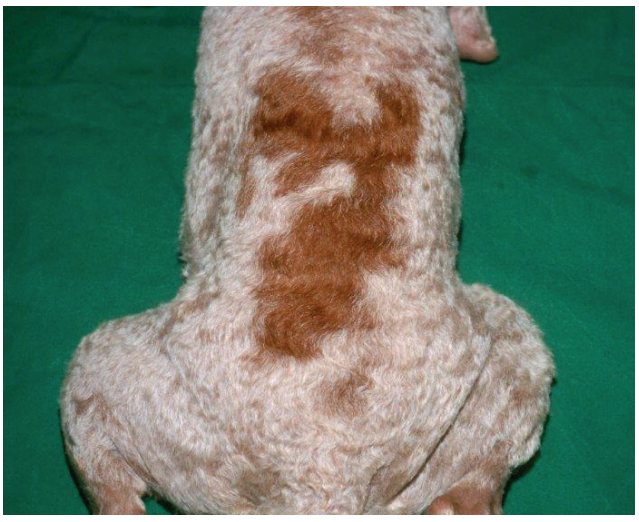

Figure 5. Presentation of canine recurrent flank alopecia without noticeable alopecia. Note the difference in coat colour in an irregular pattern in the thoracolumbar area in this Poodle.

Flank alopecia in non-related dogs in the same household has been anecdotally noted. The case description of the flank alopecia in the Affenpinschers by Waldman (1995) mentions that multiple Affenpinschers developed flank alopecia in the winter, when kept in the conservatory, where there was no artificial heating or lighting. The authors of the present study have seen "an outbreak" of flank alopecia in a breeding facility where multiple Bichon frisé dogs developed alopecia at the same time. No more recurrences were seen when the day-night cycle was adjusted in the breeding facility. Another example observed by the authors of the present study is a household with three non-related English Staffordshire terriers where the three dogs develop flank alopecia simultaneously every year between December and February.

Flank alopecia with interface dermatitis

This entity was first described in 2003 by Rachid in Boxers and is characterized by a combination of flank alopecia and interface dermatitis/folliculitis (Rachid et al., 2003; Mauldin, 2005). In Europe, this presentation of flank alopecia has been reported by Van der Luer in an English bulldog (Van der Luer and Bonestroo, 2010). Also, the authors of the present study have seen this presentation in an English bulldog. The distribution of the lesions is very similar to the classical presentation of flank alopecia, with lesions confined to the thoracolumbar area. The difference is the concurrent presence of non-painful and non-pruritic multifocal circular scaly and crusted depigmented plaques within the alopecic area (Figure 6).
The alopecia and the interface dermatitis demonstrate concurrent courses of remission and recurrence in these patients. The relationship between the two types of lesions is not known (Rachid et al., 2003; Mauldin, 2005). The possibility of a superimposed erythema ab igne (chronic radiant heat dermatitis) on CRFA lesions in some of those cases has been suggested (Paradis, 2009). However, the hypopigmentation bordered by the hyperpigmentation is unique to erythema ab igne (Declercq and Vanstapel, 1998). Moreover, histopathological changes typical of erythema ab igne, such as keratinocyte atypia and karyomegaly and a variable number of wavy eosinophilic elastic fibres ("red spaghetti"), are not seen in CRFA with interface dermatitis (Declercq and Vanstapel, 1998; Walder and Hargis, 2002; Rachid et al., 2003; Mauldin, 2005).

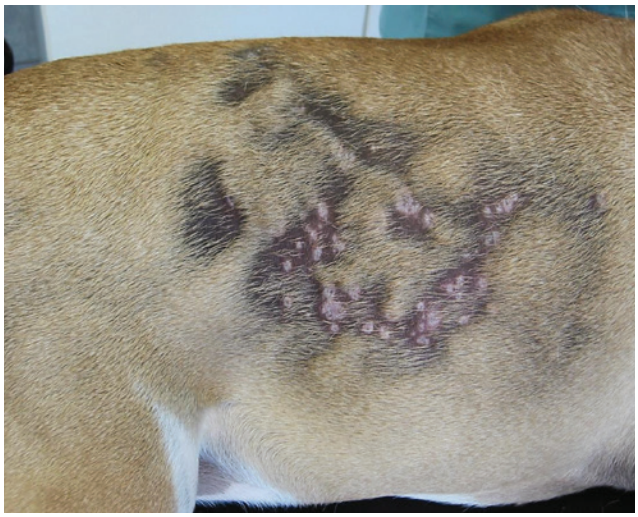

Figure 6. Flank alopecia with interface dermatitis in a 3-year-old English bulldog. Note the thoracolumbar distribution of the lesions with the presence of crusted depigmented plaques within the alopecic area (Picture courtesy of Anja Bonte).

Diagnosis

If a dog is presented with a history of annual recurring alopecia presenting with the typical lesions from November to April and spontaneous regrowth is evident, the diagnosis of CRFA can be made based on the history and striking clinical findings (Miller et al., 2013a; Paradis, 2012).

If a dog is presented for a first episode of CRFA with the typical clinical presentation endocrinopathies, such as hypothyroidism, breed specific hair cycle abnormalities, colour dilution alopecia, post-shaving arrest, erythema ab igne (chronic radiant heat dermatitis), glucocorticoid injection reaction and post-rabies vaccination panniculitis need to be ruled out (Declercq, 2008).

It is of interest that certain breeds that are predisposed for CRFA, such as the Boxer, Airedale and German pointers, are also predisposed for hypothyroidism (Dixon et al., 1999; Paradis, 2009). Hypothyroidism usually presents as a slowly progressive alopecia, as opposed to the rapid onset of alopecia in CRFA. Usually, other coat changes are present in hypothyroid dogs such as a scaly or dull and brittle hair coat. Concurrent pyoderma and otitis are an occasional complaint in hypothyroid dogs (Paradis, 2009). Slow hair regrowth in clipped areas and a rat tail are other clinical findings suggestive of hypothyroidism.

In CRFA, the quality and quantity of the coat in the non-lesional skin are normal. Another difference is that metabolic signs (weight gain, lethargy) are generally seen with hypothyroidism, but not in dogs with CRFA (Paradis, 2009).

Colour dilution alopecia causes an initially dorsally oriented, slowly progressive diffuse, partial alopecia. A variable degree of alopecia can also be noted on the head and rarely, the extremities. Various breeds such as Dobermann, Chihuahua, Italian Greyhound, Yorkshire terrier, Whippet are predisposed for colour dilution alopecia. It is associated with diluted colours of brown and black. These coat colours are referred to as blue, grey, fawn and red (Laukner, 1998). Affected dogs present with clinical signs before the age of 1 year and rarely later in life (Laukner, 1998).

There are a variety of breed-specific alopecic diseases in dogs that have erroneously been classified as follicular dysplasia. Because these forms of alopecia are not developmental abnormalities and do not represent one specific disease, it has been decided that hair cycle abnormalities would be a better denomination for this form of alopecia (Cerundolo et al., 2009).
These hair cycle abnormalities have a true breed predisposition and have been reported in the Portuguese water dogs, Chesapeake Bay retriever, Curly Coated retriever and Irish water spaniels (Cerundolo et al., 2009; Laffort-Dassot et al., 2002; Cerundolo et al., 2005). The alopecia in these breeds can wax and wane, but there is no seasonal influence and the hair regrowth is never complete in these breeds. The caudal thighs and ventral neck are often involved and these are areas that are not affected in dogs with CRFA.
Moreover, these dogs do not respond to melatonin treatment (Cerundolo et al., 2009; Cerundolo et al., 2005).

In post-clipping alopecia, there is a lack of regrowth at the site of previous clipping (Miller et al., 2013c). Especially if the dog was clipped in the thoracolumbar or dorsal area (as might be seen with epidural anaesthesia), clinical resemblance with CRFA is possible. This disease usually effects Nordic breeds such as the Siberian husky, Alaskan malamute, Samoyed, Pomeranians and Chow Chows without age or sex predilection (Miller et al., 2013c; Gross et al., 2005a). It has been proposed that in those breeds, hair regrowth might take a lot longer because of a prolonged telogen phase that possibly developed to save energy in those cold climates. In this case, the prolonged telogen phase is responsible for the post-clipping alopecia (Credille, 2000). These breeds are not predisposed for CRFA, but post-clipping alopecia can be seen in other breeds too. Post-clipping alopecia is diagnosed by the signalment, the history of previous clipping and clinical presentation (Miller et al., 2013c; Gross et al., 2005a). Endocrinopathies, such as hypothyroidism, hyperadrenocorticism and alopecia X should be ruled out.

If a dog presents with unilateral alopecia in the thoracolumbar or dorsolumbar area, erythema ab igne and injection reactions need to be ruled out.

Erythema ab igne is a typically unilateral dermatosis that occurs at the site of repeated exposure to moderate heat. Lesions are commonly seen at the dorsolateral thoracic region and consist of irregular branching areas of alopecia with erythema and hypopigmentation bordered by hyperpigmentation (Miller et al., 2013d; Gross et al., 2005b; Walder and Hargis, 2002; Declercq and Vanstapel, 1998) (Figure 7).

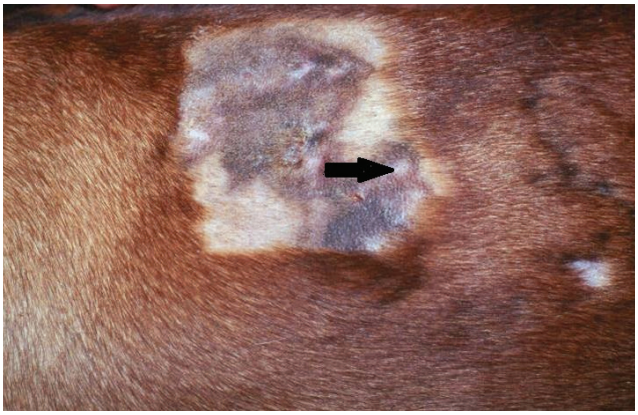

Figure 7. Clinical presentation of erythema ab igne. Note the alopecia with erythema and hypopigmentation bordered by hyperpigmentation (arrow) on the flank of this mixed breed dog subjected to a heat lamp post whelping.

The distribution of the lesions and the irregular alopecia are similar to CRFA, especially flank alopecia with interface dermatitis. The hypopigmentation bordered by the hyperpigmentation is unique to erythema ab igne (Declercq and Vanstapel, 1998). Histopathological changes consist of keratinocyte atypia and karyomegaly, scattered apoptotic or vacuolated basal cells with an interface dermatitis, adnexal atrophy and a variable number of waxy eosinophilic elastic fibres (“red spaghetti”) (Gross et al., 2005b). Erythema ab igne is diagnosed by a history of chronic access to heat sources, such as a heating pad, electric blanket,

burning stove or heat lamp, with typical clinical signs and typical dermatopathological changes (Miller et al., 2013d; Gross et al., 2005b; Walder and Hargis, 2002; Declercq and Vanstapel, 1998). It is of interest to note that glucocorticosteroid injections also can cause either alopecia or changes in coat colour and coat texture (Declercq, 2008; Miller et al., 2013e) (Figure 8).

The alopecia is focal and often there is a concurrent atrophy of the skin with slight scaling. Mineralized injected material in the deep dermis can be seen or palpated; moreover, the underlying musculature can be atrophic

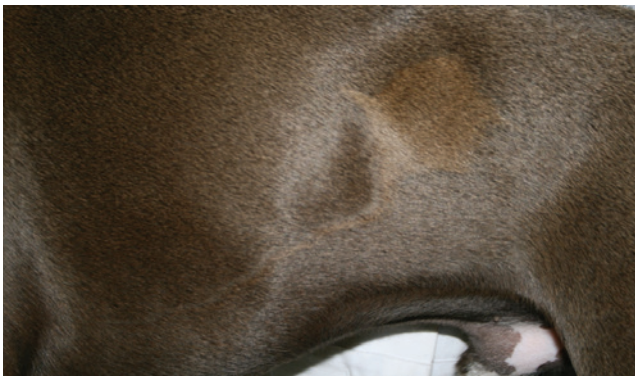

Figure 8. Focal discoloration of the coat with concurrent atrophy of the skin in a Weimaraner caused by a glucocorticosteroid injection.

in severe cases (Miller et al., 2013e). Another type of injection reaction, post-rabies vaccination panniculitis can be seen at the site of rabies vaccine deposition and is considered to be one of the vasculopathic syndromes under the group of ischemic dermatopathy (Gross et al., 2005c). Typically, the lesion is noted two to three months post vaccination. The lesion consists of a focal alopecia with minimal inflammation. The alopecic area may vary in size, and erythema is minimal or absent. Lesional hyperpigmentation can be seen (Miller et al., 2013e). Injection reactions can be diagnosed by history, clinical presentation and histopathology (Miller et al., 2013e; Gross et al., 2005b).

If a client does not want to wait for spontaneous regrowth, a biopsy and histopathological examination are warranted. In active lesions, the fairly typical histopathological changes consist of infundibular hyperkeratosis extending to secondary follicles and sometimes even into the sebaceous gland ducts (Figure 9). The hair follicles demonstrate an atrophic base and may be malformed. These fore-mentioned changes create a specific dysplastic appearance of the hair follicles resembling a malformed foot, hence called "witch's feet" or "octopus-like hair follicles" (Gross et al.,

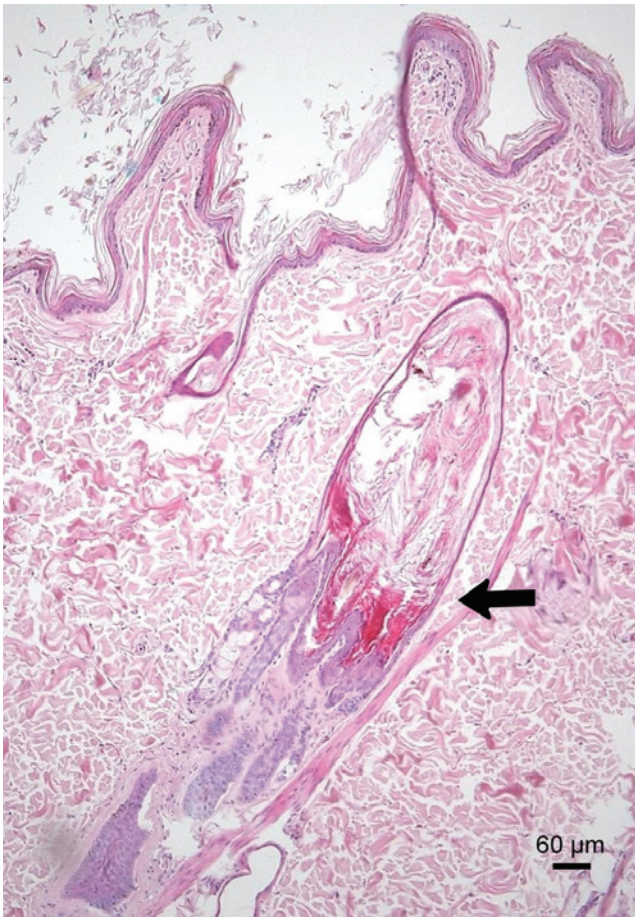

Figure 9. Photomicrograph. Haematoxylin and eosin stain. Note the infundibular hyperkeratosis extending into the secondary follicles, creating the shape of a witches' foot (arrow).

2005d). The size of the adnexae is normal, but sebaceous glands may be melanised. Melanin aggregates may also be present in the follicular lumen (Bagladi et al., 1996; Miller and Dunstan, 1993). The timing of the biopsies greatly influences the histopathological changes (Gross et al., 2005d; Paradis, 2009). When patients are biopsied early in the disease process, most follicles are in the telogen or catagen phase. However, more often, patients are biopsied when the alopecia has been present for several months and is in the regression phase. If biopsied then, the follicles will often already be in anagen phase, and the infundibular orthokeratotic hyperkeratosis might not be prominently present (Fontaine et al., 1998). This is similar to what can be seen when the disease presents with just coat discoloration. In those cases, follicular hyperkeratosis and anagen follicles can be seen. The described follicular changes are suggestive but not pathognomonic for CRFA (Gross et al., 2005d; Paradis, 2009). Dysplastic hair follicles and abnormal melanin aggregation occur in both follicular dysplastic diseases and endocrine skin diseases (Rothstein et al., 1998).

Clinical management

Dogs with this disease are otherwise healthy, and the disease should be considered as a cosmetic disease. As spontaneous hair regrowth does occur (albeit potentially incomplete with recurrent episodes), benign neglect can be a valid treatment option (Miller et al., 2013a; Paradis, 2009).

Because of a variable timing of the spontaneous regrowth and the unpredictable course of the alopecic periods, evaluation of treatment, either curative or as a preventive measure is very difficult to assess objectively.

Melatonin is considered the initial treatment of choice, if treatment is requested (Miller et al., 2013a). The optimal dose, best route of administration and the duration of treatment and best time of initiation of treatment are currently unknown, as placebo-controlled studies have not yet been published. A success rate of 50-75% has been reported based on anecdotal information (Paradis, 2009). Melatonin implants at 12 mg/dog have been successfully used as a preventative treatment in dogs with recurrent episodes of CRFA (Paradis, 2000). Oral melatonin can be administered at a dose of 3 to 6 mg per dog twice to three times daily during 4 to 6 weeks (Paradis, 2000). This duration of treatment is based on a study in mink showing that melatonin induces the anagen hair cycle within a 4 to 6-week period (Paradis, 2000). However, once the hair cycle is restarted, melatonin is no longer necessary for continuous growth and maturation of the pelage (Valtonen et al., 1995). Treatment should be initiated shortly after the onset of the alopecia or 1 to 2 months before the anticipated onset of the alopecia. Melatonin is a safe drug, without side effects, but due to its interaction with reproductive hormones, it should not be used in breeding animals (Paradis, 2000).

In summary, canine recurrent flank alopecia is a fascinating disease unique to dogs with an unknown pathomechanism and with several clinical presentations. The proposed name does not fit perfectly for this disease entity. The course of the disease and its response to melatonin therapy are unpredictable.

Acknowledgement

The authors wish to thank Dr Anja Bonte and Dr Ilona Schwarzkopf for providing the clinical pictures for Figures 3 and 6.

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Reprint paper*

Relationship between ultrasonographic findings and histopathological diagnosis in dogs with inflammatory bowel disease.

Miryam Martinez[1], Francisco José Pallarés, Maria del Mar Sempere, Marta Soler, Agustina Anson, Juana Carrillo, Amalia Agut

SUMMARY

Inflammatory bowel diseases (IBD) are a group of disorders characterized by persistent, recurrent and nonspecific gastrointestinal signs of undetermined cause, associated with histopathological evidence of mucosal inflammation. The diagnosis of these diseases is a challenge, and intestinal histopathology is always required to establish a definitive diagnosis. Abdominal ultrasound is a useful tool in the diagnostic approach to gastrointestinal diseases, because the morphology of the gastrointestinal tract can be evaluated. The aim of this study was to assess the ultrasonographic findings in the abdominal cavity in dogs with IBD, and to determine the relationship between the ultrasonographic findings and the histopathological diagnosis. In this study, 22 dogs were included, which presented with clinical signs associated with IBD. All dogs underwent abdominal ultrasound and a definitive diagnosis of IBD was obtained by histopathologic analysis. The most common ultrasonographic abnormalities were enlargement of the regional lymph nodes, increased duodenal wall thickness, abdominal effusion and poor intestinal wall layer definition. In conclusion, there was no correlation between the histopathological diagnosis and the ultrasonographic findings.

* This paper originally appeared in *Clin. Vet. Peq. Anim.* 2013; 33 (3): 197-204.
Eur J Comp An Pract (2015), Winter 25(4); p20-33
Go to http://www.ejcap.org to see the online presentation of this paper.

Introduction

Inflammatory bowel diseases (IBD) are a group of disorders characterized by persistent, recurrent and nonspecific gastrointestinal signs, of undetermined cause, associated with histological evidence of mucosal inflammation when other causes of enteritis have been excluded.[1,2] These diseases are classified according to the histopathological findings, including the type of inflammatory cells and the area of intestine affected.[1]

Dogs and cats with IBD may present with a variety of non-specific clinical signs. The most common are weight loss, vomiting, diarrhoea, lethargy and variable appetite (anorexia, polyphagia). Other less common signs are tenesmus, haematochezia, regurgitation, abdominal pain and ascites.[3-5] However, the clinical signs relate to the intestinal area affected. Symptoms are chronic (more than three weeks) and recurrent with exacerbation and remission periods.

The diagnosis of these diseases is a challenge and reached by exclusion of other causes of chronic enteropathy (systemic diseases, parasitic infections, food allergy and tumours) which have a similar presentation. Intestinal histopathology is always required to establish a definitive diagnosis.[3,4]

[1]Veterinary Teaching Hospital. University of Murcia. 16, Campus de Espinardo. 30100 Espinardo, Murcia, Spain. Email: mmg10194@um.es

Abdominal ultrasound (US) is the tool of choice in the diagnostic approach to small animals with chronic vomiting and diarrhoea.[6] Ultrasonography is useful to assess the wall thickness, wall layering, layer echogenicity, motility and content of the gastrointestinal tract (GIT).[7] IBD often leads to transmural thickening of the intestinal wall.[6-8] In some instances, the relative thickness of the layers may also change while the total wall thickness remains normal.[7,9] Changes in the echogenicity of the mucosa (hyperechoic mucosal speckles and striations),[6,7,10] poor intestinal wall layer definition and intestinal corrugation have also been described previously.[7,11]
The aim of this study was to assess the ultrasonographic findings in the abdominal cavity in dogs with IBD, and to determine the relationship between the ultrasonographic findings and the histopathological diagnosis.

Material & methods

This is a retrospective study. Twenty-two dogs were included, which presented with a history of chronic GI signs, at the Veterinary Teaching Hospital of Murcia University (Spain), from 2008 to 2011. Inclusion criteria were dogs with a history of GI signs for over 3 weeks, and when non-gastrointestinal diseases or others causes of chronic enteritis had been excluded. All dogs had haematology, biochemistry, radiography, abdominal ultrasound and faecal analysis. None of them were taking any treatment, and there was no response to the dietary therapy in those in which it was trialled. Intestinal biopsies were required to provide a histopathological diagnosis of IBD.
In all cases of this study, clinical signs, duration of the clinical signs and physical exam were also recorded. Abdominal US examination was performed on all dogs, with two different linear array transducers, a 7 MHz (Logiq 500 General Electric Medical Systems, Spain) and 4-13 MHz (MyLab 70, Esaote, Spain). The following parameters were recorded: appearance of intestinal wall layers, wall thickness (compared to the reference values; duodenum ≤ 5.1 mm (< 20 Kg) ≤ 5.3 mm (20-29 Kg), ≤ 6 mm (>30 Kg), jejunum ≤ 4.1 mm (< 20 Kg) ≤ 4.4 mm (20-29 Kg), ≤ 4.7 mm (>30 Kg)[7]), changes in echogenicity of the mucosal layer (classified as normal, with bright speckles or hyperechoic striations), corrugation of the intestine, the relative motility and content of different segments of the GIT; thickness (reference value: < 7 mm)[9], short/long axis ratio (reference value: < 0.5 [12]) and echogenicity of the mesenteric lymph nodes, relative echogenicity of mesenteric fat and presence of peritoneal effusion.
The intestinal biopsies were obtained either by endoscopy or exploratory laparotomy. Formalin-fixed, paraffin-embedded biopsy samples were sliced at a thickness of 4 µm and stained with haematoxylin and eosin stain. Histological examinations were performed to confirm a definitive diagnosis of IBD. The IBD was classified depending on the type of cellular infiltrate (lymphocytic, plasmacytic, granulomatous, eosinophilic or mixed), location (stomach, small intestine or colon) and degree of inflammation (normal, mild, moderate or severe).
A descriptive statistical analysis was performed with Excel (Office: Windows, Version 2010 Microsoft, Redmond, WA, USA).

Results

Twenty-two dogs were included in the study. The details of these animals are shown in Table 1. There were 15 males and 7 females. The average age was 4.8 years

Table 1. Animals with Inflammatory bowel disease.

BREED	AGE (Years)	GENDER
West Highland White Terrier	7.5	M
American Cocker Spaniel	3	M
Brittany Spaniel	12	M
Schnauzer	2	F
Mixed Breed	2.5	M
Brazilian Mastiff	3.5	M
Yorkshire Terrier	2.5	M
Siberian Husky	9	F
Weimaraner	0.5	F
German Shepherd	3	F
Golden Retriever	6	F
English Cocker Spaniel	7	M
Fox Terrier	11	M
Boxer	1.5	M
Bull Terrier	0.75	F
English Cocker Spaniel	7	M
Fox Terrier	5	M
Mixed Breed	2	M
German Shepherd	3.5	M
German Shepherd	6	F
Andalusian Hound	4	M
Mixed Breed	7	M

M: Male; F: Female

(range from 6 months to 12 years) and no breed was over-represented.

Duration of clinical signs

All twenty-two dogs included in our study had gastrointestinal signs with a duration of 3 weeks to 2 years. In 5/22 animals signs lasted <1 month, in 10/22 dogs between 1-3 months, in 4/22 between 3-6 months, and in 3/22 >1 year.

Clinicopathological findings

The most common clinicopathological findings in serum were leukocytosis, increased hepatic enzymes, decreased total proteins with hypoalbuminemia and electrolyte imbalances.

Ultrasonographic abnormalities

There were no ultrasonographic changes in 5/22 animals (22.7%). Ultrasonographic abnormalities (Fig. 1) were observed in 17/22 (77.2%) dogs. Lymphadenopathy was reported in 10/17 dogs (58.8%) (thickness: mean 8.5 mm; range 7-10.8 mm) while the short/long axis ratio remained normal (<0.5)[12]. The duodenal wall was thickened in 5/17 (29.4%), abdominal effusion was visualized in 5/17 (29.4%), poor intestinal wall layer definition in 4/17 (23.5%) and the jejunal wall was thickened in 3/17 (17.6%) dogs. Hypoechoic mesenteric lymph nodes were present in 3/17 (17.6%) dogs. Other findings including: hyperechoic speckles and striations on the mucosa, hyperechoic mesenteric lymph nodes and intestinal corrugation were also observed (2/17, 11.7 %).

Histopathological analysis

In 15 of 17 (88%) animals with ultrasonographic changes, the inflammatory infiltrate was mixed (lymphocytes and plasmatic cells) and the definitive diagnosis was lymphoplasmacytic enteritis in 8/17 (47%), in 3/17 (17.6%) enteritis associated with gastritis, in 2/17 (11.7%) enteritis associated with gastritis and colitis, in 1/17 (6%) enteritis associated with colitis and in 1/17 (6%) gastritis associated with colitis. In 2/17 (12%) dogs, only one type of cell was observed, in 1/17 (6%) lymphocytic enteritis was diagnosed and in 1/17 (6%) the diagnosis was plasmacytic enteritis associated with colitis.

Regarding the degree of inflammation (Fig. 2), six of 17 (35%) animals had gastritis, of which five (83%) were mild and one (17%) was moderate. Enteritis was diagnosed in 15/17 (88%) dogs, of which five were mild (33%), five moderate (33%), two severe (14%) and three undetermined (20%). Finally, when the diagnosis was colitis in 7/17 (41%), two were of a mild degree (29%), one moderate (14%), three severe (43%) and one undetermined (14%).

In 5/22 dogs without ultrasound changes, the final diagnosis was lymphoplasmacytic enteritis, in three of them (60%), one mild, one moderate and one

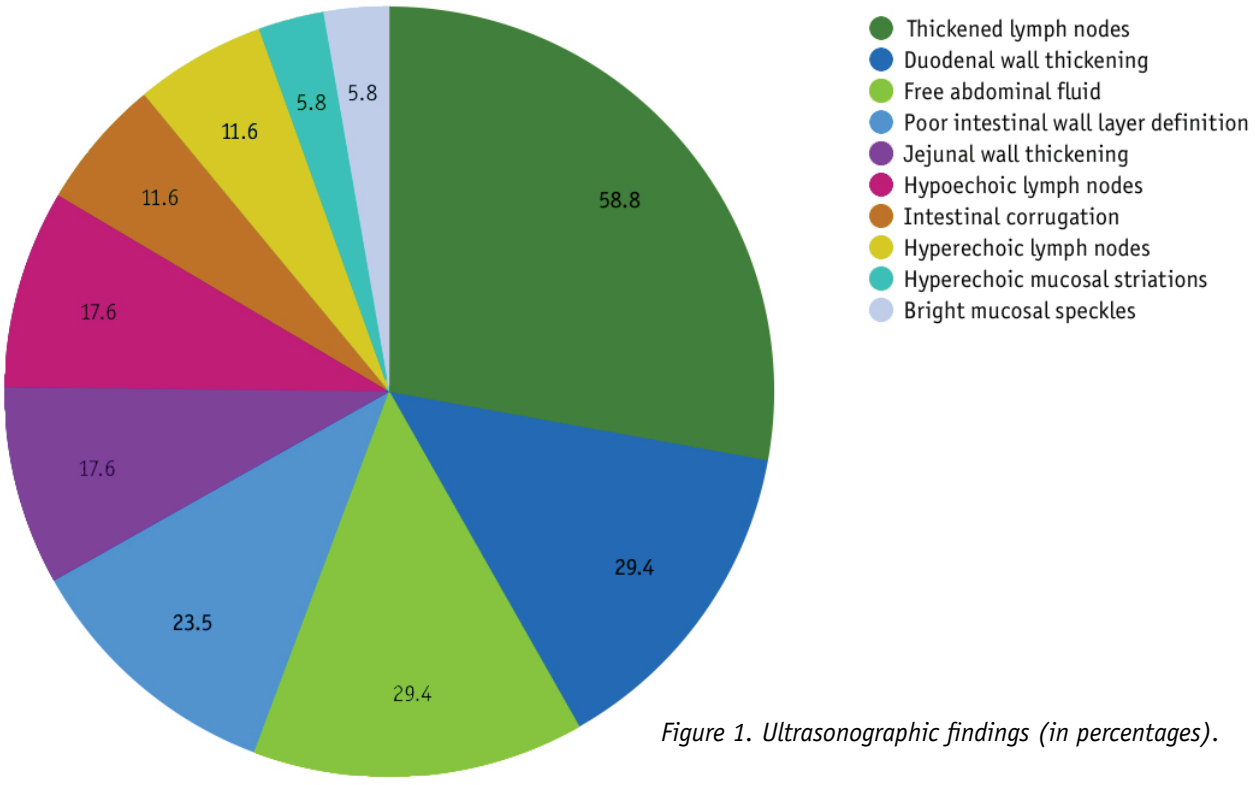

Figure 1. Ultrasonographic findings (in percentages).

Table 2. Relationship between sonographic findings and histopathological diagnosis.

case number	SONOGRAPHIC FINDINGS*										HISTOPATHOLOGICAL DIAGNOSIS
	a	b	c	d	e	f	g	h	i	j	
1.								X	X		lymphoplasmacytic enteritis
2.				X				X			mild lymphoplasmacytic gastritis. moderate lymphoplasmacytic enteritis. severe lymphoplasmacytic colitis.
3.								X			mild lymphoplasmacytic gastritis. mild lymphoplasmacytic enteritis.
4.	X		X				X				severe lymphoplasmacytic enteritis.
5.	X		X		X						lymphoplasmacytic enteritis. lymphangiectasia.
6.	X		X								moderate lymphoplasmacytic enteritis.
7.								X			severe lymphoplasmacytic enteritis.
8.						X		X			moderate lymphoplasmacytic gastritis and colitis.
9.		X								X	mild lymphoplasmacytic enteritis.
10.	X					X					plasmacytic enteritis and colitis.
11.		X						X			mild lymphoplasmacytic gastritis, enteritis and colitis.
12.			X								mild lymphoplasmacytic gastritis and enteritis.
13.						X	X				mild lymphocytic colitis.
14.						X		X		X	moderate lymphoplasmacytic enteritis.
15.	X							X			mild lymphoplasmacytic enteritis.
16.								X			mild lymphoplasmacytic gastritis. moderate lymphoplasmacytic enteritis.
17.						X	X	X			mild lymphoplasmacytic enteritis. severe lymphoplasmacytic colitis. lymphangiectasia.
18.											mild lymphoplasmacytic gastritis and enteritis. lymphangiectasia.
19.											mild lymphoplasmacytic enteritis.
20.											mild lymphoplasmacytic enteritis.
21.											mild lymphoplasmacytic colitis.
22.											moderate lymphoplasmacytic enteritis.

** a. Free abdominal fluid, b. Corrugation, c. Poor layer definition, d. Bright mucosal speckles, e. Hyperechoic mucosal striations, f. Duodenal wall thickening, g. Jejunal wall thickening, h. Thickened lymph nodes, i. Hyperechoic lymph nodes, j. Hypoechoic lymph nodes Five dogs (18-22) had normal ultrasonographic findings.*

undetermined; one dog (20%) had mild lymphoplasmacytic enteritis associated with gastritis and another (20%) had mild lymphoplasmacytic colitis.

Relationship between ultrasonographic findings and histopathological diagnosis

These findings are shown in Table 2.

Five of 17 dogs with ultrasonographic changes had free abdominal fluid and the final diagnosis was lymphoplasmacytic enteritis of differing degrees. Also, in one of them enteritis was associated with colitis and in another with lymphangiectasia.

Intestinal corrugation was observed in 2 dogs with mild lymphoplasmacytic enteritis. One of them was associated with mild gastritis and colitis.

In 4/17 dogs with poor intestinal wall layer definition the histopathologic diagnosis was lymphoplasmacytic enteritis of different degrees. In one of these cases ultrasonography detected free abdominal fluid and jejunal wall thickening, where the final diagnosis was severe lymphoplasmacytic

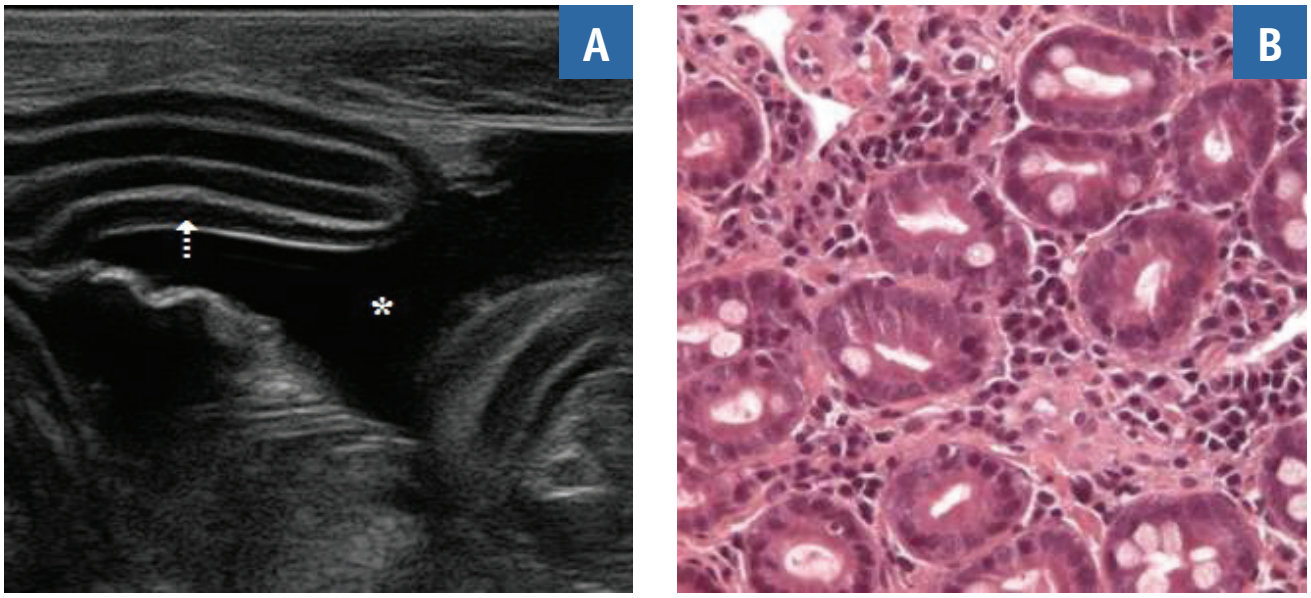

Figure 3. A. Longitudinal image of jejunal segment in a German Shepherd with a history of chronic diarrhoea. The wall of the jejunum is diffusely thickened due to muscularis thickening (white arrow). Free abdominal fluid is also present (asterisk). B. Histological image of the mucosa (x40, HE). Note the lymphoplasmacytic infiltrate within the lamina propria. The definitive diagnosis was moderate lymphoplasmacytic enteritis.

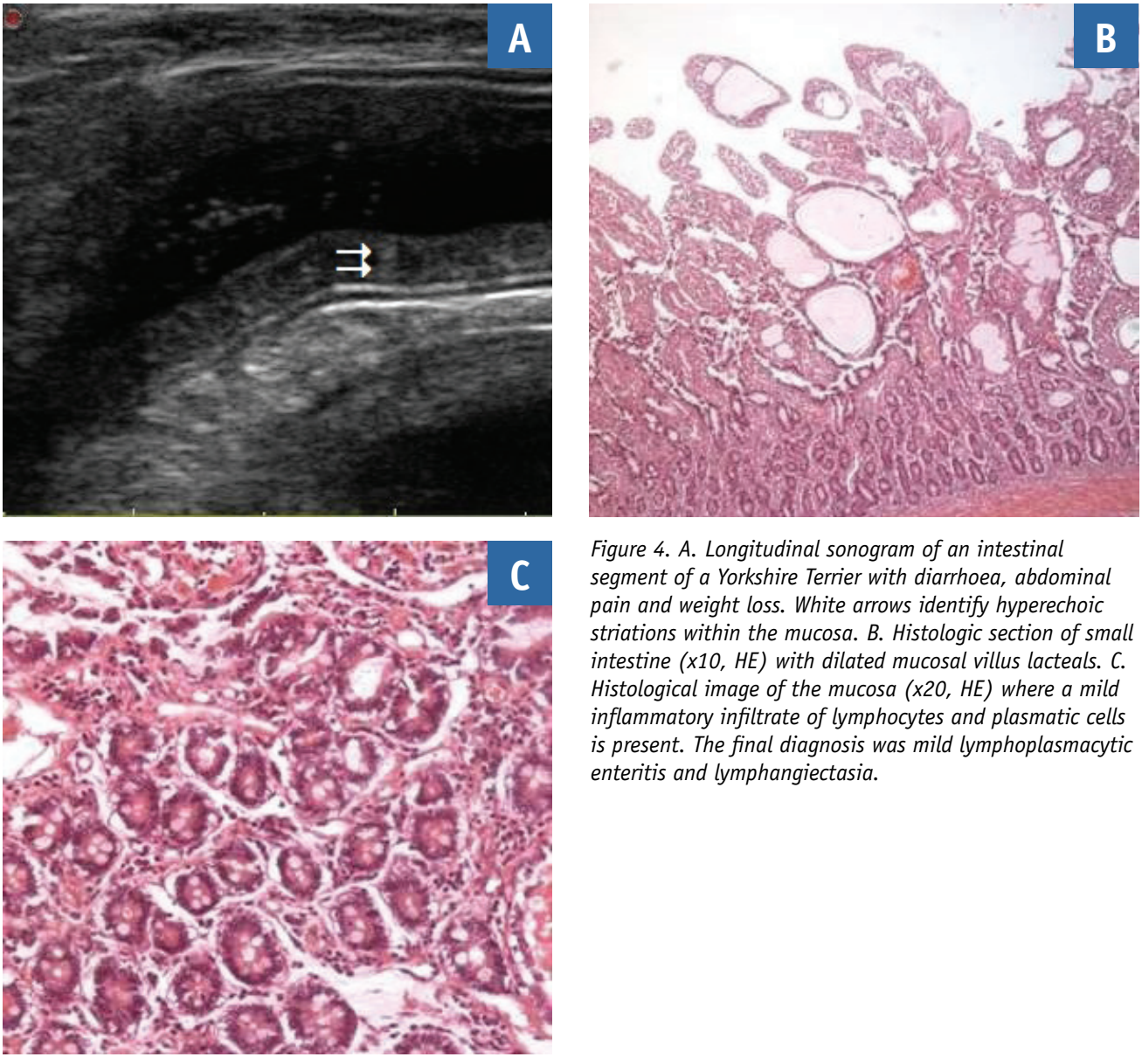

Figure 4. A. Longitudinal sonogram of an intestinal segment of a Yorkshire Terrier with diarrhoea, abdominal pain and weight loss. White arrows identify hyperechoic striations within the mucosa. B. Histologic section of small intestine (x10, HE) with dilated mucosal villus lacteals. C. Histological image of the mucosa (x20, HE) where a mild inflammatory infiltrate of lymphocytes and plasmatic cells is present. The final diagnosis was mild lymphoplasmacytic enteritis and lymphangiectasia.

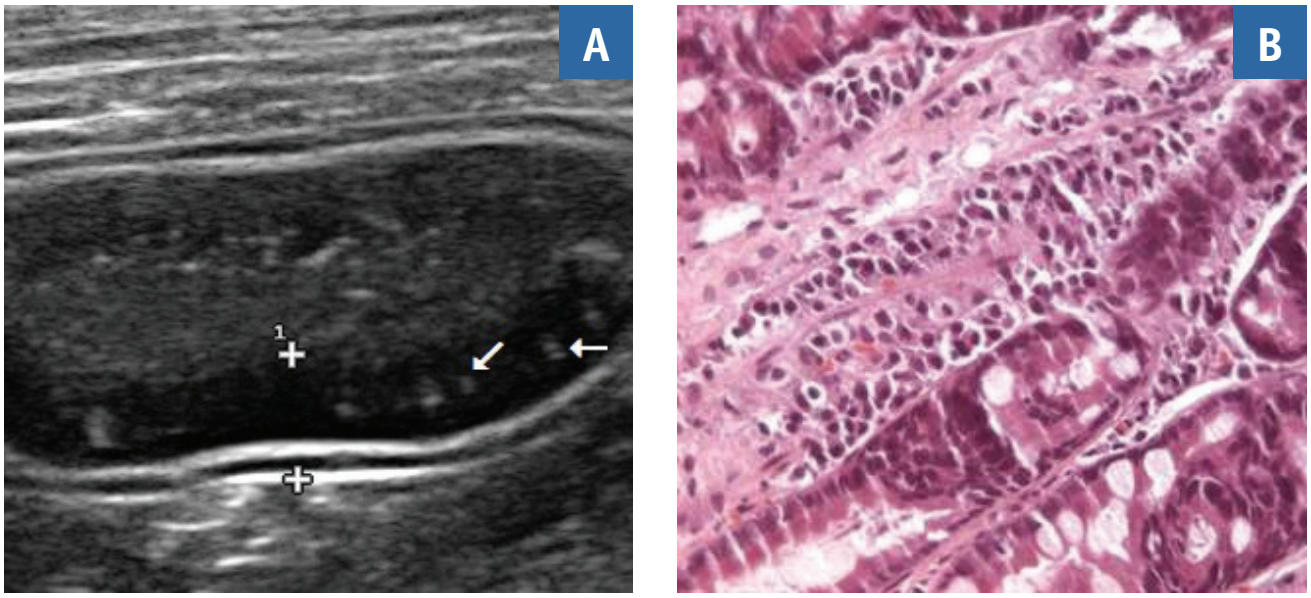

Figure 5. A. Longitudinal sonographic image of the small intestine of a dog with chronic diarrhoea and intermittent vomiting. Note the bright speckles within the mucosa (arrows). B. Histologic section of the villi (x40, HE). An inflammatory lymphoplasmacytic infiltrate and increased connective tissue within the lamina propria (fibrosis) is present. The definitive diagnosis was moderate lymphoplasmacytic enteritis associated with gastritis and colitis.

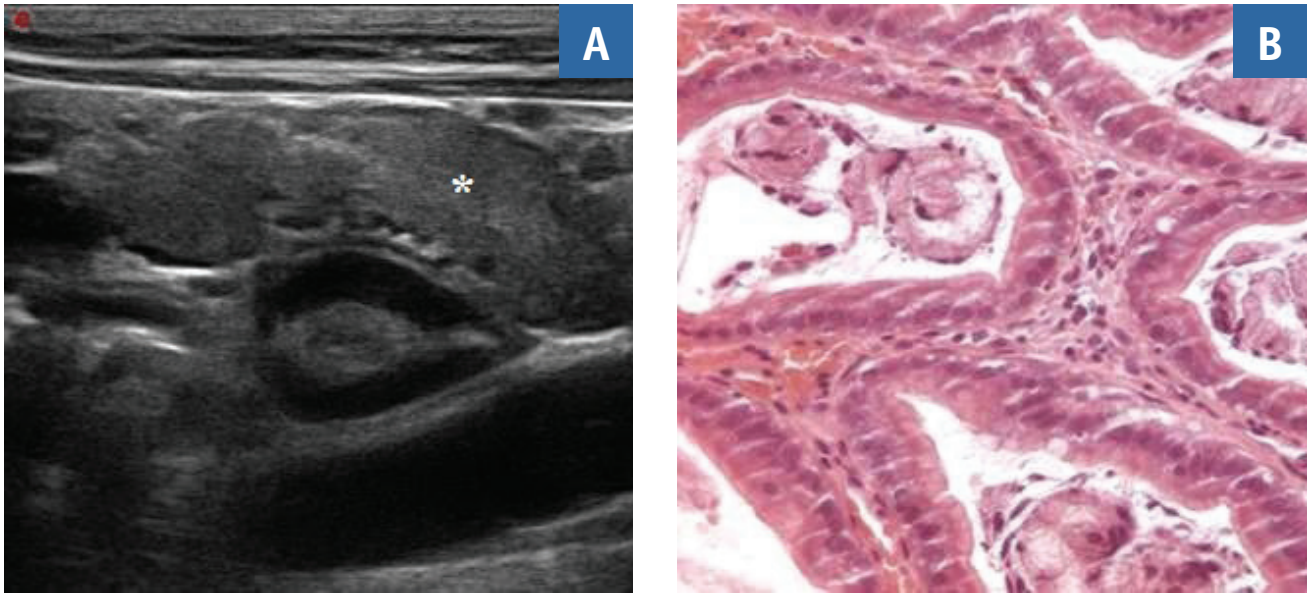

Figure 6. A. Sonogram of a dog with chronic vomiting and weight loss. No abnormalities were present in the GIT. Note the regional lymph node enlargement (asterisk). B. Histological image of the intestinal mucosa (x 40, HE); A mild inflammatory infiltrate of lymphocytes and plasmatic cells are evident within the lamina propria. The final diagnosis was mild lymphoplasmacytic enteritis associated with gastritis.

enteritis (Fig.3). In another case, it was associated with free abdominal fluid and hyperechoic mucosal striations, and the final diagnosis was lymphoplasmacytic enteritis and lymphangiectasia (Fig.4)

The presence of bright mucosal speckles was observed in one dog with lymphoplasmacytic gastritis associated with enteritis and colitis of different degrees (Fig.5).

Duodenal wall thickening was visualized in 5/17 animals, which were diagnosed with enteritis, mild in 1/5 dogs, moderate in 1/5, and of undetermined degree in 1/5 animals. In 2/5 dogs, even though an association with jejunal wall thickening was found, no histological abnormalities were identified within the small intestine, and in these animals the final diagnosis was moderate lymphoplasmacytic gastritis associated with colitis, and mild lymphocytic colitis.

The most common finding was mesenteric lymphadenopathy (10/17) and the diagnosis was lymphoplasmacytic enteritis of different degrees. In two cases it was associated with

gastritis and colitis (Fig.6), and colitis and lymphangiectasia.

Discussion

Ultrasound is a non-invasive imaging technique that is included in the diagnostic approach to gastrointestinal disorders in dogs, allowing assessment of wall thickness, wall layering, motility, content of the GIT, regional lymph nodes, and presence of free fluid in the abdominal cavity.[7]

IBD is a common disorder in dogs, characterized by the presence of an inflammatory infiltrate of different cell types within the intestinal mucosa, which has been thought, to be correlated with ultrasonographic changes. However, these diseases do not always induce changes detectable by ultrasound or these changes overlap with findings in intestinal neoplasia, and intestinal biopsies are required to achieve a definitive diagnosis.[7] In this study, 5 animals were diagnosed with IBD without showing sonographic changes. In these dogs, the degree of the disease was mild, except in one of them which was moderate. One possible explanation, is that the degree of the disease was not sufficient to cause sonographic changes but was significant enough to result in clinical signs.[8] However, other cases where we found sonographic changes were diagnosed with mild IBD. Therefore, no correlation was found between ultrasonographic findings and histopathological diagnosis.

In our study, the most common ultrasonographic finding was mesenteric lymphadenopathy with normal short/long axis ratio (10/17 dogs; 58.8%). This lymphadenopathy was associated with intestinal wall abnormalities, thickening of jejunal or duodenal wall and hyperechoic speckles in the mucosal layer. This association suggests the presence of reactive lymph nodes due to an inflammatory process. This regional lymphadenopathy has been previously reported in inflammatory conditions.[14] Thickening of the intestinal wall is a sonographic finding previously reported in humans with IBD.[15] The veterinary literature has also documented the presence of ultrasonographically detectable wall thickening in IBD.[14] Otherwise, more recent assessments indicate that the wall thickness is not able to establish a diagnosis of inflammatory disease.[8] Nevertheless, in this study we found 6 cases with increased intestinal wall thickness, in 3 dogs duodenal wall thickening was present, in one jejunal wall thickening and in 2 of them the wall of the duodenum and jejunum was thickened. By definition, in IBD the intestinal wall is infiltrated with inflammatory cells [16] and has been thought that may cause intestinal wall thickening. On the contrary, other reports suggest that neither infiltration of the intestinal wall nor dilatation of mucosal and submucosal lymphatic vessels necessarily lead to a thickening of the intestinal wall.[17] Nowadays, increased mucosal echogenicity is the most consistent sonographic finding with IBD.[10]

Both hyperechoic mucosal striations and hyperechoic mucosal speckles, have been described.[10] In our case, these findings were observed in two animals. In one of these cases as previously reported, the mucosal striations were associated with lymphangiectasia as definitive diagnosis.[10] In addition, this finding has been associated with dogs with protein losing enteropathy, where hypoalbuminemia was present leading to free abdominal fluid due to reduced colloid osmotic pressure. [10] In this study, one dog that exhibited hyperechoic mucosal striations also had free abdominal fluid and hypoalbuminemia. In another case, bright mucosal speckles were visualized, which has been speculated to represent a partial section through the dilated lacteal.[10] It has also been thought that it may represent focal accumulations of mucus, cellular debris, protein or gas in the mucosal crypts. However, other authors have found this finding to be a sensitive parameter for inflammatory disease, but being non-specific for differentiating disease category.[6] In our study, this finding was observed in a mild lymphoplasmacytic gastritis associated with moderate enteritis and severe colitis.

Loss of layering is an ultrasonographic finding reported in intestinal neoplasia, although it may appear in cases of IBD.[7] In this study, this finding was observed in cases of mild, moderate and severe enteritis. Therefore, when this finding is made, the most probable diagnosis is neoplasia but the differential diagnosis should include IBD, and a biopsy is recommended to reach the definitive diagnosis.[7] One of the limitations of our study was that 7 patients had not undergone a hypoallergenic diet trial prior to the start of IBD treatment. Therefore, we were not able to rule out food allergy in these animals. However, it has been previously reported that animals that have food allergy do not have ultrasonographic changes within the mucosa [6] and in our case, two of these dogs had sonographic findings consistent with protein-losing enteropathy. Nevertheless, we cannot rule out food

allergy in the other five dogs, since they had a normal mucosa layer.

In conclusion, ultrasonography is a useful technique to assess the gastrointestinal tract and to detect sonographic changes consistent with IBD. However, no correlation was found between ultrasonographic findings and histopathological diagnosis.

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Reprint paper*

A retrospective survey of ocular abnormalities in pugs: 130 cases

Marion Krecny[1], Alexander Tichy, James Rushton and Barbara Nell

SUMMARY

OBJECTIVES: To determine the types and frequency of ophthalmic findings in pugs.

MATERIALS AND METHODS: Retrospective analysis of case records of pugs presented to an ophthalmology unit between 2001 and 2012. Ophthalmological findings were correlated with age, gender, presenting signs and time of onset of disease.

RESULTS: In total, 130 pugs (258 eyes) with a mean (±sd) age of 2.8 (±2.87) years were examined. Ocular abnormalities identified included keratoconjunctivitis sicca (n=39 eyes), macroblepharon (n=258 eyes), entropion (n=258 eyes), distichiasis (n=56 eyes), ectopic cilia (n=8 eyes), conjunctivitis (n=88 eyes), corneal pigmentation (n=101 eyes), opacity (n=63 eyes), ulceration (n=46 eyes), vascularisation (n=35 eyes), iris-to-iris persistent pupillary membranes (n=21 eyes) and cataract (n=18). Keratoconjunctivitis sicca was significantly associated with the presence of corneal pigmentation (P=0.007 for left eyes; P=0.043 for right eyes). However corneal pigmentation was also identified in pugs (n=61) without keratoco njunctivitis sicca. There was a significant influence of ectopic cilia on corneal ulceration (P<0.001). Younger dogs (mean age, 1.28 (±0.45) years) were significantly more affected by distichiasis.

CLINICAL SIGNIFICANCE: The high number of cases of corneal pigmentation without keratoconjunctivitis

sicca suggests that there may be additional yet undetermined factors involved in the development of corneal pigmentation in pugs.

* This paper originally appeared in the *Journal of Small Animal Practice* (2015) 56, 96–102 DOI: 10.1111/jsap.12291, and was submitted by the British Small Animal Veterinary Association. *Eur J Comp An Pract* (2015), Winter 25(4); p20-33 Go to http://www.ejcap.org to see the online presentation of this paper.

Introduction

The pug appears to be increasing in popularity and with its small, stocky and square body shape, round face and large prominent eyes, a high prevalence of disease has been reported. A number of ocular disorders have been described in the pug, some of which are related to the unique anatomical properties of this breed. Common ocular pathologies include entropion, distichiasis, keratocon-junctivitis sicca (KCS), corneal pigmentation (CP), corneal erosion, corneal ulceration, corneal perforation and corneal vascularisation (CV).[10,22]

In recent years an increase in the number of pugs presented for ophthalmic disorders has been noted. However there are very few large retrospective studies on the most common ophthalmic pathologies in the pug.[10] Hence the purpose of this study was to report the types and frequencies of ophthalmic findings in the pug and attempt to identify potential influencing factors for the development of these abnormalities.

[1] Department of Companion Animals and Horses, University of Veterinary Medicine, Vienna 1210, Austria (Author's current address: Tierklinik Parndorf, Heidehofweg 4, 7111 Parndorf, Austria. Email: marion.krecny1@chello.at)

Materials and methods

Study population and ophthalmologic examination

Case records of all pugs presented to the ophthalmology unit of the Department of Companion Animals and Horses of the Veterinary University in Vienna between 2001 and 2012 were reviewed. All dogs were examined by or under the supervision of an ophthalmology diplomate using slit lamp biomicroscopy (Kowa portable slit-lamp SL-14) and direct and indirect ophthalmoscopy (Heine Omega 2C). Mydriasis was induced using 1% tropicamide (Mydriaticum "AGEPHA" Pharmaceuticals) to examine the posterior ocular segments. Schirmer tear test (STT) (Intervet Deutschland GmbH), fluorescein stain (Ophthalmic Strips U.S.P.) and tear film break-up time (TFBUT) were performed. A TFBUT of 20 seconds was considered normal.[15]

Data acquisition

Ocular abnormalities from the case records were categorised according to anatomical region and suspected aetiology and listed on an Excel 2010 spreadsheet (Microsoft Office 2010, Excel 2010) for subsequent statistical evaluation. Additional data, such as gender, age and time of onset of ophthalmic abnormalities according to the history from the owner were also noted.

Statistical analysis

The data were analysed using SPSS v19 (IBM Software). Frequency distribution of gender, age and ocular pathologies were calculated. Influences of age, gender and interrelation between ophthalmic abnormalities were determined using cross tables and tested for significance using Chi squared test. Descriptive predictability models were applied to determine the predicted chance of development of ocular abnormalities based on influencing factors such as age, gender or other predisposing ophthalmic findings. A P value <0.05 was considered significant for all statistical analysis.

Results

Study population

A total of 258 eyes (130 pugs) were examined (Table 1). Sixty-six (50.8%) entire males, 10 (7.7%) neutered males, 39 (30.0%) entire females and 15 (11.5%) neutered females were included.

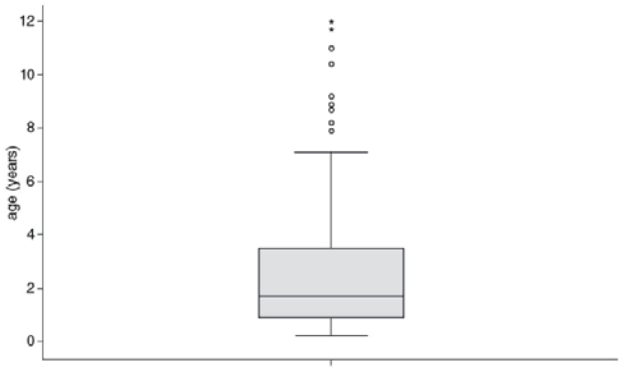

FIG 1. Age distribution of pugs presented

They ranged in age from 0.2 to 12.0 years, with a mean (+sd) of 2.8 (+2.87) years (Fig 1). Presenting signs reported by the owner or referring veterinarian included decreased vision (n=8), blindness (n=4), entropion (n=3), distichiasis (n=6), blepharospasm (n=30), ocular discharge (n=31), conjunctivitis (n=24), corneal opacity including corneal oedema, fibrosis, pigmentation (n=42), corneal injury (n=9) and corneal ulceration (n=6). Less frequent presenting signs included neoplasia of the eyelid (n=2), corneal foreign body (n=1), facial swelling (n=1), reexamination of a repositioned prolapsed eye (n=1), unresponsive pupil (n=1), bulbus prolaps (n=1), hydrophthalmus (n=1), cataract (n=1) and dry eye (n=1). Seven pugs were presented as an emergency, with severe ocular symptoms, which had arisen on the day of presentation.

The duration of symptoms otherwise ranged from 0 to 2880 days, with a mean of 90.25 (±346.15) days.

Ophthalmological findings

All pugs included in the study were identified with bilateral macroblepharon and nasal entropion. Further findings associated with the ocular adnexa included distichiasis, ectopic cilia and conjunctivitis.

Distichiasis was found in 28 left (OS) and 28 right eyes (OD) of 35 pugs. Fourteen pugs were affected unilaterally and 21 pugs bilaterally. Nineteen were entire males, 2 neutered males, 11 entire females and 3 neutered females. Of the 56 eyes affected by distichiasis, CP was found OS and OD in 19 and 16, corneal ulcers in 6 and 7, corneal opacities in 7 and 9 and CV in 6 and 5 cases, respectively. Ectopic cilia were identified OS and OD in six and two cases, respectively (six dogs; four unilaterally and two bilaterally). Five of them were entire males and one was an entire female. CP was found OS and OD in four and one, corneal ulceration in five and two, corneal opacity in two

Table 1. Pugs (presented between 2001 and 2012) with gender, age, symptom duration, STT values, distichiasis, ectopic cilia, conjunctival and corneal pathologies

Case number	Sex	Age (y)	Duration (d)	STT		Distichiasis		Ectopic cilia		Conjunctivitis		Cornea							
												Pigmentation		Opacity		Ulcer		Vascularisation	
				OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
1	f	0·7	nk	14	16	–	–	–	–	–	–	+	+	–	–	–	–	–	–
2	m	2·3	nk	nd	nd	–	–	–	–	–	–	–	–	+	+	–	–	–	–
3	nf	5·1	nk	15	15	–	–	–	–	+	–	+	+	+	–	–	–	–	–
4	m	1	240	17	17	–	–	–	–	–	–	+	+	–	–	–	–	–	–
5	nm	0·7	nk	nd	nd	–	–	–	–	–	–	+	+	–	–	–	–	–	–
6	nm	0·7	nk	15	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	sf	12	nk	7	15	–	–	–	–	–	–	+	+	+	+	–	+	–	+
8	f	0·9	2	15	15	–	–	–	–	–	–	+	+	+	–	–	+	–	–
9	m	0·9	2	nd	nd	–	–	–	–	–	–	+	+	–	–	–	+	–	–
10	m	1	21	15	15	+	+	+	–	–	–	–	–	–	–	+	–	–	–
11	m	3·3	180	20	15	–	–	–	–	–	–	+	–	+	–	–	–	+	–
12	m	1·7	5	15	17	–	+	–	–	–	–	+	+	–	+	–	+	–	–
13	f	1·6	240	15	15	–	+	–	–	–	–	–	–	–	–	–	–	–	–
14	f	1·9	3	15	15	+	+	–	–	–	–	–	–	–	–	–	+	–	+
15	m	3	nk	15	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	m	1·4	nk	15	15	+	–	–	–	–	–	–	–	–	–	–	–	–	–
17	m	8·9	nk	15	15	–	–	–	–	+	+	–	–	+	–	–	–	+	–
18	m	3·1	180	15	18	–	–	–	–	–	+	–	+	–	–	–	–	–	–
19	m	0·3	3	18	12	–	–	–	–	–	–	+	+	+	–	–	–	–	–
20	m	5·5	2880	15	15	–	–	–	–	–	–	+	+	–	–	-	–	–	–
21	m	11	7	nd	nd	–	–	–	–	–	–	+	+	–	–	–	–	–	–
22	nf	1·3	7	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
23	f	1·3	240	18	15	+	–	–	–	+	–	–	+	+	+	+	–	+	–
24	nf	0·3	7	15	15	–	–	–	–	+	+	+	+	+	+	+	+	+	+
25	m	8·7	0	15	e	–	e	–	e	–	e	+	e	+	e	+	e	–	e
26	m	1·9	nk	nd	nd	+	+	+	–	–	–	–	–	–	–	+	–	–	–
27	f	1·3	nk	nd	nd	–	–	–	–	+	+	–	–	–	–	–	–	–	–
28	nm	11·7	1	nd	nd	–	–	–	–	–	+	–	–	–	–	–	–	–	–
29	f	2·5	2	e	18	e	–	e	–	e	–	e	–	e	–	e	–	e	–
30	f	0·5	2	22	21	–	–	–	–	–	+	–	–	–	–	–	+	–	–
31	f	0·2	2	11	14	–	–	–	–	+	+	+	+	–	–	–	–	–	–
32	m	2·6	3	18	17	–	–	–	–	–	+	+	+	–	–	–	+	–	–
33	m	5·5	6	15	15	–	–	–	–	–	–	–	+	–	–	–	–	+	–
34	m	0·9	14	15	20	+	+	–	–	–	–	+	–	+	–	–	–	+	–
35	m	1·2	112	18	15	–	–	–	–	+	+	–	+	–	+	–	–	–	–
36	m	1·3	6	20	13	+	+	+	–	–	–	+	+	+	+	+	–	–	–
37	m	3·5	56	15	15	+	–	–	–	–	–	+	+	+	–	–	–	–	–
38	m	4·2	0	nd	nd	–	–	–	–	–	–	+	–	–	–	–	–	–	–
39	m	4	21	17	10	+	+	–	–	–	–	+	+	–	–	–	–	+	+
40	m	11·7	2	15	9	–	–	–	–	–	–	+	+	+	–	+	+	–	–
41	f	0·3	14	16	15	–	–	–	–	–	–	–	–	+	+	+	+	–	–
42	m	4·9	nk	18	7	–	–	–	–	+	+	+	+	–	–	–	–	–	–
43	f	1·2	nk	15	15	–	–	–	–	+	+	+	+	–	+	–	–	–	–
44	f	0·4	nk	15	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–
45	m	1·7	98	16	16	–	–	–	–	+	+	–	+	+	–	+	–	–	–
46	m	0·3	30	13	13	–	–	–	–	+	+	+	+	–	–	–	–	–	–
47	m	8·2	nk	5	4	–	–	–	–	–	–	+	+	–	–	–	–	–	–
48	m	0·7	nk	13	20	–	–	–	–	–	+	+	+	–	+	–	+	+	+
49	m	0·7	7	15	18	–	–	+	–	–	+	+	–	+	–	+	–	–	–
50	nf	12	nk	10	13	–	–	–	–	+	+	+	+	–	–	–	–	+	+
51	m	0·5	5	20	17	–	–	–	–	+	+	+	–	–	–	–	–	–	–
52	f	2·1	17	15	15	–	–	–	–	+	+	+	+	–	–	–	–	–	–
53	m	1·1	0	15	15	–	–	–	–	+	+	–	–	+	–	–	–	–	–
54	f	0·6	nk	15	15	–	–	–	–	+	+	+	–	–	–	–	–	–	–
55	nm	3·5	nk	15	15	–	–	–	–	+	–	+	+	+	–	–	–	–	–
56	m	0·4	2	14	9	+	–	–	–	+	+	+	+	+	–	+	–	+	–
57	f	0·3	2	0	15	–	+	–	–	–	+	+	–	–	–	–	+	+	–
58	nf	2·2	3	20	15	–	–	–	–	+	+	–	–	+	–	+	–	–	–
59	m	0·3	7	12	14	–	–	–	–	–	–	+	+	–	+	–	–	–	+
60	f	1·5	168	18	18	–	–	–	–	–	–	+	+	–	–	–	–	+	–
61	nm	1·8	2	17	15	+	+	–	–	+	+	+	–	–	+	–	+	–	+
62	m	1·8	nk	15	15	+	+	–	–	–	–	+	+	+	+	–	–	–	–
63	f	0·3	0	15	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–
64	f	0·7	nk	14	18	+	+	–	–	+	+	+	+	–	–	–	–	–	–
65	nf	1·4	nk	12	20	–	+	–	–	+	+	+	+	–	–	–	–	–	–
66	nf	1·3	nk	15	15	+	+	–	–	–	–	–	–	+	+	–	+	–	+
67	nf	7·9	168	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	+

Table 1. (continued)

Case number	Sex	Age (y)	Duration (d)	STT		Cornea													
						Distichiasis		Ectopic cilia		Conjunctivitis		Pigmentation		Opacity		Ulcer		Vascularisation	
				OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
68	f	1·3	1	20	20	–	–	–	–	+	–	–	–	+	–	–	–	–	–
69	nm	4·1	21	15	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70	m	5·5	0	6	15	–	–	–	–	–	–	+	–	–	–	–	+	–	–
71	m	0·5	35	10	18	–	–	–	–	–	–	+	+	–	–	–	–	+	–
72	nm	1·5	nk	15	18	–	–	–	–	+	+	+	+	+	–	–	–	+	–
73	m	2	168	15	15	+	–	–	–	–	+	+	–	–	–	–	+	–	–
74	m	0·3	5	16	17	+	+	–	–	–	–	–	–	–	–	+	–	+	–
75	m	3	5	20	12	–	–	–	–	–	–	–	–	+	–	+	–	–	–
76	m	5	7	nd	nd	–	–	–	–	+	–	–	–	–	+	–	+	–	–
77	f	0·6	3	15	15	–	–	–	–	–	+	–	–	–	–	+	–	–	–
78	f	1·8	336	15	16	+	+	–	–	+	+	+	+	–	+	–	–	–	–
79	f	9·2	7	15	10	–	–	–	–	–	+	+	–	–	–	–	–	–	–
80	f	0·4	4	15	nd	–	–	–	–	–	–	–	–	–	+	–	–	–	–
81	m	1·2	3	nd	nd	–	–	–	–	–	+	–	–	–	+	–	–	–	–
82	m	1·4	nk	15	15	–	–	–	–	+	+	+	+	–	–	–	–	–	–
83	m	nk	nk	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
84	m	1·6	nk	nd	nd	+	+	–	–	–	–	–	–	–	–	+	–	–	–
85	m	0·8	196	15	15	+	+	–	–	–	–	+	+	–	–	–	–	–	–
86	f	0·4	nk	10	15	–	–	–	–	+	–	–	–	–	–	+	–	–	–
87	nm	1	nk	15	15	–	–	–	–	+	+	+	+	+	–	–	–	–	–
88	nm	2	3	10	12	+	–	–	–	–	–	+	–	–	+	–	+	–	–
89	f	5·2	56	20	20	–	–	–	–	–	–	+	+	–	–	–	–	–	–
90	f	1·2	nk	15	15	+	+	–	–	–	–	+	+	–	–	–	–	–	–
91	f	4·8	1344	20	20	–	–	–	–	–	–	+	+	–	–	–	–	–	–
92	f	0·5	nk	15	15	+	+	–	–	+	+	+	+	–	–	–	–	–	–
93	m	1·1	nk	20	20	–	–	–	–	+	+	+	–	–	–	–	–	–	–
94	m	4·2	21	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
95	m	1·2	1	14	13	–	–	–	–	–	–	–	–	–	–	+	–	–	–
96	m	1	2	15	15	–	–	–	–	+	+	+	+	–	–	+	–	–	–
97	m	2·9	10	15	15	–	+	–	–	–	–	+	+	–	+	–	–	–	–
98	m	0·5	1	20	20	–	+	–	–	–	+	–	–	–	+	–	+	–	–
99	m	0·4	3	15	15	–	–	–	–	–	–	–	–	+	+	–	–	–	–
100	nf	2·8	nk	15	15	+	+	–	–	+	+	+	+	–	–	–	–	–	–
101	m	1·7	nk	nd	nd	–	+	–	–	+	+	+	+	–	–	–	–	–	–
102	m	3·2	0	15	15	–	–	–	–	–	–	–	–	–	–	+	–	–	–
103	m	5	nk	15	15	+	+	–	–	+	+	+	+	–	–	–	–	–	–
104	f	410	nk	20	20	+	+	+	+	–	–	+	+	–	+	–	+	–	–
105	f	1·1	nk	15	17	+	–	–	–	–	–	+	+	–	–	–	–	–	–
106	m	7·9	84	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
107	f	6·1	nk	10	6	–	–	–	–	–	–	+	+	–	–	–	–	–	–
108	f	1·9	nk	16	20	+	+	–	–	+	+	+	+	–	–	–	–	+	+
109	f	10·4	1	15	11	–	–	–	–	+	–	–	–	+	–	+	–	–	–
110	m	2·5	2	12	15	–	–	–	–	–	+	–	–	+	+	–	+	–	+
111	nf	5	3	15	15	–	–	–	–	–	+	–	+	–	–	+	–	–	–
112	m	1·6	14	15	20	–	–	–	–	–	–	+	+	–	+	–	–	–	–
113	f	5·3	nk	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
114	nf	2·4	21	15	15	–	–	–	–	–	–	+	+	+	–	–	–	–	–
115	m	2·4	nk	16	14	–	–	–	–	–	+	+	+	–	+	–	–	–	+
116	f	1·7	7	15	15	–	–	–	–	–	–	–	–	+	+	–	–	–	–
117	nf	3·2	168	15	15	–	–	–	–	–	–	+	+	–	–	–	–	–	–
118	f	2·2	14	nd	nd	–	–	–	–	–	–	+	+	–	–	–	–	–	–
119	f	7·1	336	22	21	–	–	–	–	–	–	+	+	–	–	–	–	+	+
120	f	0·2	1	14	5	–	–	–	–	–	–	+	+	+	–	+	–	–	–
121	nm	2·6	nk	24	23	–	–	–	–	–	–	+	+	–	–	–	–	+	+
122	m	5·9	0	25	18	–	–	–	–	–	–	–	–	–	–	+	–	–	–
123	f	1·1	3	15	15	–	–	–	–	+	–	+	–	–	–	+	–	–	–
124	m	1·6	2	15	15	+	+	+	+	+	+	+	–	–	–	+	+	–	–
125	m	3·2	7	15	27	–	–	–	–	–	–	–	–	–	+	+	–	–	–
126	m	0·5	nk	15	15	–	–	–	–	+	+	–	–	+	+	–	–	–	–
127	m	6	2	20	20	–	–	–	–	–	–	+	+	+	–	–	–	+	–
128	nf	8·2	7	15	19	–	–	–	–	–	–	+	+	–	–	–	–	–	–
129	nf	1·8	nk	15	15	–	–	–	–	–	+	–	+	+	–	–	+	–	+
130	m	2·9	nk	25	22	–	–	–	–	–	–	+	+	–	–	–	–	–	–

f Entire female, nf Neutered female, m Entire male, nm Neutered male, nd Not done, nk Not known, e Enucleated.

and one case with ectopic cilia, respectively.
Conjunctivitis was found OS and OD in 40 and 48 cases (56 pugs), respectively. Thirty affected animals were male and 26 were female.
STT was documented OS and OD in 116 and 115 cases, respectively (231 eyes; 117 pugs) and ranged from 0 to 27 mm/min. KCS was diagnosed OS and OD in 20 (15.4%) and 19 (14.6%) cases, respectively – in a total of 39 eyes from 29 pugs (STT<15 mm/min). Nineteen were affected unilaterally and 10 pugs bilaterally. Sixteen pugs were entire males, nine entire females, three neutered females and one neutered male.
Each of the affected pugs had corneal changes. Thirty-one eyes were diagnosed with CP (17 OS and 14 OD), six eyes had a corneal ulcer (4 OS and 2 OD), eight eyes had corneal opacities not related to pigmentation (4 OS and 4 OD) and nine eyes had CV (5 OS and 4 OD).
One hundred and seven pugs had at least one eye with normal physiological STT (≥15 mm) reference values (192 eyes; 96 OS and 96 OD). Twenty-two pugs had normal STT values unilaterally and 85 pugs bilaterally. One hundred and seventeen of the 192 eyes with normal STT values were affected with CP (61 OS and 56 OD), 50 eyes had corneal opacities (29 OS and 21 OD), 36 eyes were diagnosed with corneal ulceration (18 OS and 18 OD; with 2 eyes having ectopic cilia) and 26 eyes had CV (14 OS and 12 OD).
Regarding qualitative changes of the tear film, four pugs had a TFBUT OU<20 seconds of a total of five pugs, in which TFBUT was determined. One pug had a TFBUT of 19 seconds in one and 21 seconds in the other eye. Three pugs with decreased TFBUT were entire males and two were neutered females. All of them (including the pug with high TFBUT values) had corneal changes, i.e. CP, corneal opacity, corneal ulceration and vascularisation.
The most common corneal findings included CP, corneal opacity and ulcerative keratitis. Twenty-three pugs were affected in one eye whereas 68 pugs had bilateral CP. Forty-six entire male pugs (unilateral=14, bilateral=32) were diagnosed with CP, followed by 25 entire females (unilateral=5, bilateral=20), 13 neutered females (unilateral=2, bilateral=11) and seven neutered pugs (unilateral=2, bilateral=5). Corneal opacity not related to pigmentation was found OS and OD in 36 and 27 cases (53 pugs), respectively. Forty-three pugs were identified with unilateral opacities whereas 10 pugs had bilateral opacities. Thirty entire male, 11 entire female, 7 neutered female and 5 neutered male pugs were affected. Corneal ulceration (Fig 2) was noted OS and OD in 27 and 23 cases (46 pugs), respectively.

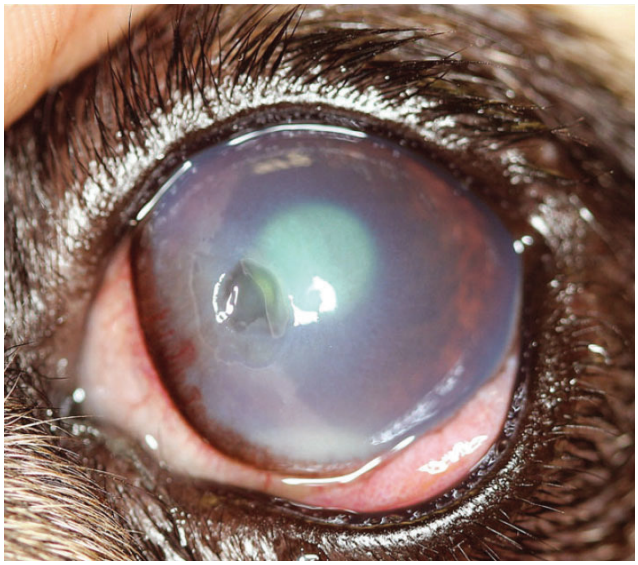

FIG 2. One-year-old pug with a corneal ulcer

Forty pugs had a unilateral ulcer and in three pugs both eyes were affected. Twenty-six were entire male, followed by 12 entire female, 6 neutered female and 2 neutered male pugs. Corneal ulceration in the absence of KCS and ectopic cilia were found OS and OD in 17 cases each (32 pugs; 2 bilateral, 30 unilateral).
Concurrent corneal opacity was identified OS and OD in 11 cases each. Ulcerative keratitis was associated with CV OS and OD in four and eight cases, respectively. The remaining corneal disorders included CV (19 OS and 16 OD; 21 unilateral and 7 bilateral of 28 pugs; of which 4 had corneal ulceration OS and 8 OD), corneal perforation (4 eyes of 4 pugs), descemetocoele (3 eyes of 3 pugs), microcornea (2 eyes of 1 pug), corneal abscess (1 eye), corneal degeneration (uncertain if lipid or calcium; one eye) and adherent leukoma (1 eye).
Further ocular abnormalities of the anterior segment included bilateral iris–iris persistent pupillary membranes (PPMs) in 10 pugs. One pug had iris–iris PPMs, however no information regarding laterality could be determined from the case records. In one case the lens was affected by PPMs unilaterally. The remaining abnormalities of the iris included iris coloboma in one eye and bilateral iris atrophy in one pug. Abnormalities of the lens included cataract formation in 18 eyes of 11 pugs. In 14 eyes (8 pugs) the cataract was located in the nucleus, four eyes (3 pugs) had cortical cataract and one eye complete cataract. One pug was referred because of a laceration of the anterior lens capsule due to a perforating corneal foreign body, requiring phacoemulsification.
One pug was identified with a persistent hyperplastic tunica vasculosa lentis. Vitreous degeneration was found in three pugs.
Fundus abnormalities were identified in two pugs, one of

which had bilateral atrophy of the non-tapetal fundus and the other retinal folds suggestive of retinal dysplasia. Further statistical analysis of posterior segment changes were not performed because of the small number of diseases.

Association with influencing factors

A statistically significant influence of young age on distichiasis was determined. A marked association of ectopic cilia at an early age was noted, however this difference was not significant. There was no statistically significant influence of age or gender on the development of any other ocular abnormality.
There was a significant association of KCS and CP (P=0.007 for left eyes; P=0.043 for right eyes), although a considerable number of pugs with CP in the absence of KCS were also identified.
Furthermore a significant influence of ectopic cilia on the development of corneal ulceration (P<0.001) was revealed.

Discussion

This study clearly demonstrates a high prevalence of pugs with ocular abnormalities at an early age (mean 2.8 years). This finding is in clear contrast to a recent study with a population of 295 pugs, which were presented at a median age of 4.1 years.[10] A recent retrospective analysis of ocular abnormalities in other brachycephalic breeds determined an onset of ocular disorders in a later stage of life (between 3 and 10 years of age).[16] The exact reasons for this age difference at the time of presentation remain speculative, however a bias of the results due to the currently increasing popularity of this breed is possible.
Regarding disease of the ocular adnexa, all pugs were identified with bilateral macroblepharon and nasal entropion. Both findings represent a congenital entity of the pug.[17] However, a statistically significant influence of nasal entropion on the development of ensuing corneal disorders in pugs has not been established to date.[10] Distichiasis and ectopic cilia are inherited developmental eyelid conditions which are common in dogs.[8] This study corroborates the results of the recent study by Labelle et al. (2013), in which no significant association between distichiasis or ectopic cilia and CP was established.
The fact that neither the chronic irritation of nasal entropion, distichiasis or ectopic cilia had any statistical influence on the development of CP is surprising. This result suggests additional yet undetermined factors, in the development of CP. Labelle et al. (2013) confirmed that CP in pugs is not associated with diseases such as tear film deficiencies or ocular abnormalities. In that study a genetic basis for CP in pugs was suggested.[10]

The lack of the aqueous component of the tear film (quantitative KCS) may lead to various ocular disorders: corneal ulceration, CV, corneal scarring and corneal perforation. The most frequently affected breeds are the Cavalier King Charles spaniel, English bulldog, Lhasa apso, Shi tzu, West Highland white terrier and the pug.[22] Giuliano (2013) lists the pug as the sixth most frequently affected dog breed to develop KCS. In the study by Westermeyer et al. (2009), the pug was ranked as the 25th most likely breed to develop KCS with 1491 pugs less than 1 year of age identified as having KCS. The results of this study clearly show a high prevalence of young pugs diagnosed with KCS, however the influence of age on the presence of disease was not statistically significant.
KCS may also manifest as a lack of adequate tear film quality because of an imbalance in the tear film composition (mucin or lipid components), resulting in ocular surface diseases.[9] Qualitative KCS may be diagnosed by determining the TFBUT3 and measuring the level of lipids on the eyelid margin with a Meibometer (Courage-Khazaka).[4,6,14] Normal TFBUT ranges from 19.7 ±5 to 21.53 ±7.42 seconds.[7] Unfortunately the TFBUT was only recorded in five pugs. Four of these five pugs had a deficiency in tear film quality. The high prevalence of low TFBUT values in pugs is corroborated by several other studies.[1,10] There are only a few studies on canine meibometry with mean meibomium lipid concentrations of 179 ±60 MU (meibometer units) measured with the older version of the Meibometer MB550.[14] In contrast 211 ±48 MU and 205 ±41 MU were measured, respectively, in OD and OS using the Meibometer MB550.4 Ewert (2011) used the Meibometer MB550 to examine the concentration of lipids on the eyelid margin of 98 dogs, including four pugs. In that study a mean of 299.47 ±170.[4] MU was reported. To the authors' knowledge no further studies on meibometry in pugs exist. Hence an explanation for the poor tear film quality related to this breed is still lacking.
All pugs with KCS also had concurrent corneal disorders. This result is corroborated by several studies, reporting corneal pathologies (i.e. vascularisation, pigmentation, ulceration) as a consequence of underlying KCS.[21,22] However, it was interesting that a marked number of pugs showed CP and corneal ulceration in the absence of KCS. This result suggests an influence of yet undetermined

factors in the development of CP and corneal ulceration.
In this study, less than half of the population was diagnosed with conjunctivitis, most likely as a consequence of chronic irritation of nasal entropion, distichiasis or ectopic cilia.[17,19]
CP is a melanin deposition in the corneal epithelium and stroma as a result of chronic irritation or inflammation of the cornea.[13] Potential causes include distichiasis, ectopic cilia, trichiasis or insufficient tear production.[2]
The prevalence of CP is well described with several brachycephalic breeds (i.e. pug, Shi tzu, Lhasa apso, Pekingese) being overrepresented.[12] The breed related palpebral fissure of these breeds has been associated with the development of CP.[19]
In one case report, a 12-year-old pug was presented with KCS, pigmentary keratitis OU and a corneal mass, caused by *Toxoplasma gondii*.[18] A recently published study reported on the presence of CP in pugs.[10]
Similar to that study, CP was the most frequently detected corneal disorder in this study (101 eyes, 90 pugs). In the previous study, CP could be found in at least 1 eye of 243 of the 295 pugs.[10] However, CP was clearly the most frequently identified corneal disorder in this study population, hence further investigations are warranted in order to determine additional influencing factors on the development of this disease.
On the basis of the results of this study, the pug is also at high risk for the development of corneal ulceration.[17] Corneal ulcers are a common problem in dogs in general due to various reasons: malposition of the eyelids, eyelash disorders, quantitative and qualitative KCS, foreign bodies and trauma.[11,19,22] A superficial ulcer may rapidly progress to a deep or melting ulcer.
Furthermore, one study determined the pug as having the highest percentages of positive bacterial cultures in cases of corneal ulceration.[20] In the present study, 43 pugs were presented with corneal ulcers and three with unilateral descemetoceles.
Results of bacterial cultures and treatment modalities were not evaluated as a part of this study. However, the results clearly show that the pug is at high risk of developing corneal ulcers.
The presence of iris-to-iris PPMs was observed in the study by Labelle et al. (2013), among which 228 pugs had iris-to-iris PPMs in the left and 232 in the right eye. In contrast to that study, iris-to-iris PPMs were only found in 11/130 pugs in this study, however in some cases presence of PPMs may not have been recorded.
In Austria, pugs are not required to have an ophthalmic examination performed prior to breeding. Therefore there is little information about hereditary ocular disorders such as cataracts.
In this study, cataracts were diagnosed in 18 eyes (11 pugs). In USA, pugs are suspected to have hereditary cataracts.[5] There are no studies regarding the incidence of cataracts in pugs in Austria to date.
The present results suggest that fundus abnormalities are rare in pugs with only 2 of 130 pugs having such changes. A possible explanation may be that some fundus disorders go unnoticed because of underlying corneal changes, such as CP. As with cataracts, data of fundus abnormalities in pugs are lacking.
As in most retrospective studies, there were some limitations, which may have negatively influenced the results. The greatest limitation was an inconsistency in the management of medical records throughout the period in review. Some values, for instance TFBUT were not recorded in most cases, which made the statistical analysis challenging. Furthermore as in any other referral hospital, the results may have been biased because of the study population under investigation. Several dogs are referred at a later stage of the disease, thereby shifting the age distribution of the population. Furthermore the number of pugs in Austria with qualitative or quantitative KCS and CP, which remains undetected because of a lack of ocular discomfort may be higher than anticipated.
However based on the results of this study, it can be concluded that pugs are over-represented with quantitative and qualitative tear film abnormalities. Furthermore an influence of KCS on the development of corneal pathologies was clearly identified. Due to the marked number of pugs with corneal pathologies without underlying KCS an influence of additional contributing factors (e.g. abnormal tear film composition) is assumed. Further studies are warranted to investigate the aetiology of early onset ocular abnormalities in pugs.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Reprint paper*

Heartworm infection caused by *Dirofilaria immitis* in a dog imported to Norway

Liva Ihle Vatne[1]

SUMMARY

Dirofilaria immitis infection was diagnosed in a three-year-old male entire crossbreed dog that had recently been imported to Norway from Romania in accordance with the European pet travel scheme. The dog underwent a number of serological tests on arrival, as advised by the Norwegian Food Safety Authority, and was seropositive for *D immitis*. Further diagnostic tests were performed in order to establish the severity of heartworm disease and thereby make an appropriate treatment plan. The dog had clinical signs that could be attributable to heartworm disease. Echocardiographic examination revealed dilation of the pulmonary artery and linear foreign bodies in the pulmonary artery and the right pulmonary artery branch. Treatment with melarsomine dihydrochloride by intramuscular injection and ivermectin per os was administered in a split regime as recommended by the American Heartworm Society. The clinical signs resolved after completion of treatment. The article includes a general overview of heartworm infection in dogs and discusses the prospect of the disease becoming endemic in Norway. Heartworm infection caused by *D immitis* is at present rarely encountered in Norway; however, disease profiles are likely to change in Scandinavian countries with changes in climate and international pet travel regulations.

KEYWORDS: *Dirofilaria immitis*, Norway, dog, case report, heartworm

* This paper originally appeared in *Norsk Veterinærtidsskrift* 2014;(7) 126:615-620
Eur J Comp An Pract (2015), Winter 25(4); p60-67
Go to http://www.ejcap.org to see the online presentation of this paper.

Dirofilariasis

Aetiology and epidemiology

Dirofilariasis is a general term for an infection caused by nematodes of the genus *Dirofilaria*, where the parasite throughout its life cycle uses mosquitoes as intermediate hosts and vectors, and vertebrates as primary hosts[1]. Species *Dirofilaria immitis* and *Dirofilaria repens* are considered to have the greatest clinical importance, since both are relatively widespread in large parts of the world and have a zoonotic potential[2]. *D repens* establishes itself in the subcutaneous tissues of the primary host, while *D immitis* establishes itself in the cardiovascular system of the primary host (Figure 1).

One case of *D repens* in a dog imported to Norway from Hungary has recently been reported[3]. Dirofilariasis caused by *D repens* is not lethal to the primary host, but it is the most common form of Dirofilariasis found in humans in Europe, and is therefore an important zoonotic disease which may be under-diagnosed in Norway[1,2,4,5].

Infection caused by *D immitis* can be lethal to the primary host as opposed to infection caused by *D repens*[4]. Microfilariae, larvae in the first larval stage (L1), are produced by the female adult worm and released into the bloodstream of the primary host (Figure 1).

[1] Liva Ihle Vatne, BVM&S GPCertFelP CertVC, Groruddalen Animal Clinic, Stovner Senter 3, N-0985 OSLO, Norway. vatnevet@gmail.com

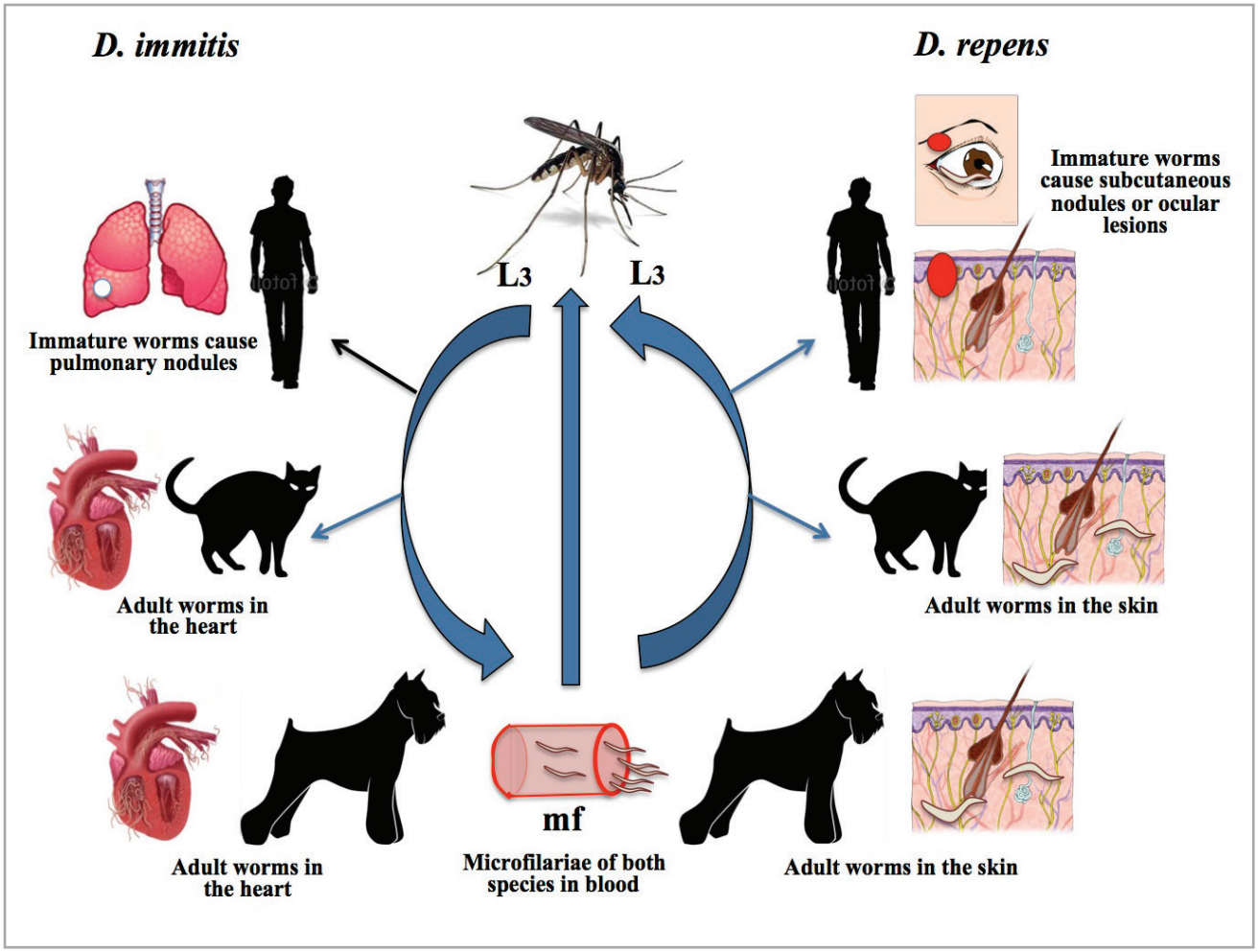

Figure 1: Biological life cycle for D repens and D immitis (diagram designed by Fernando Simón)

Microfilariae cannot continue the life cycle there; further development must take place in the vector. Microfilariae are transmitted to the mosquito when it draws blood from an infected dog. The development from larval stage L1 to L3 can occur in a number of different mosquito species, but requires an average temperature of about 18 °C and a minimum temperature of about 14°C over a period of 30 to 60 days[6,7]. During the infectious larval stage L3, larvae are transmitted through inoculation into the skin of the primary host by the mosquito. After the larvae have been inoculated, they undergo development from stage L3 to L4 in the subcutaneous tissue over a period of about 10 to 12 days. Development from L4 to L5 takes place in the muscle tissue 50 to 70 days after injection[7]. Juvenile worms (L5) penetrate systemic veins and are transported to the pulmonary arteries where they continue to develop into adult worms. In severe cases, worms may also enter the right heart chamber and caudal vena cava, but in most cases the blood flow will retain the worm in the pulmonary artery and its branches[8]. The prepatent period is about six months, where the female worms grow to be 13.5 to 30.5 cm long and can start producing microfilariae[6]. The adult worm can survive for up to 7 years in the primary host, while microfilaria have a lifespan of up to 2 years[1,7].

Heartworms are considered endemic in large parts of the world, including North and South America, Asia, Australia, southern regions of Europe, and in parts of Eastern Europe[1,5]. Factors that determine if heartworms will become endemic to an area include climate; mosquito species/ subspecies and the density of the primary host population. Risk factors for infection include animal species (the dog is the natural primary host); the sex of the primary host (male dogs are more vulnerable than female dogs); the habitat of the primary host (dogs who live outdoors are more exposed to infection); and the size of the primary host (large dogs are more likely to become infected than small dogs)[7]. Dogs who are younger than six months cannot be carriers of adult worms, as it takes about six months from the time the dog becomes infected until the heartworms develop into adults. It is therefore unlikely to see clinical signs in dogs younger than a year old. Heartworms primarily affect dogs, but have also been identified in a number of other animal species including cats, foxes, ferrets, sea lions, and

wild dogs[6,7]. The heartworm *D immitis* in its immature form has sporadically been found in humans, also in Europe[1,2,4,5]. Immature heartworms will generally not migrate to the pulmonary arteries, but will form nodules in lung tissue and subcutaneous tissue[4]. *D immitis* has recently been found in areas which have traditionally been considered heartworm-free[5]. Significant changes in geographic distribution may occur due to spreading from an exposed area as a front, but can also appear locally within a limited area[6]. In Norway, *D immitis* has currently only been found in dogs imported from endemic areas, although there is a fear that the infection may spread via mosquitoes in parts of the country with sufficiently elevated average temperatures[9,10,11].

Pathophysiology

When adult heartworms establish in the pulmonary arteries of the primary host, a number of processes may contribute to the ensuing pathology. As soon as the female worms have matured, antigens are released, causing an inflammatory reaction in the pulmonary microvasculature and larger arteries in the host[7]. The pulmonary artery expands in diameter, and both the endothelium and tunica media thicken. Blood vessels may become obstructed by formation of thrombi and as a result, the arteries dilate even further. The extent of these changes depends on the primary host's response to the infection, as well as the number of worms existing in the animal. Pulmonary hypertension may occur partly due to the thickening of the pulmonary arteries, and partly due to the development of thromboembolism[7,8]. Increased lung tissue density and right-sided cardiomegaly are common radiographic findings in dogs with moderate to severe heartworm infection[8]. Pulmonary oedema and -inflammation occur as a result of increased permeability in the smaller blood vessels, and may be visible on radiographs as interstitial and alveolar infiltrates in the caudal lung tissue[6]. Chronic and severe disease may cause irreversible changes in the lung tissue due to fibrosis. In certain cases, hypersensitivity pneumonitis develops, and this may resemble pulmonary oedema on radiographs[7]. In rare cases eosinophilic pulmonary granulomatosis occurs, which may be mistaken for a pulmonary neoplasm on radiographs4. In addition to invading the lungs and heart of the primary host, other organs may also be affected by heartworm; glomerulonephritis may develop due to the activation of immune complexes, with resulting proteinuria and in some cases, azotaemia[7].

Clinical findings

Heartworm infection caused by *D immitis* will in most cases cause few clinical signs in the affected dog. As the disease progresses, the clinical picture will depend on the duration of the infection, the number of worms in the host, and the dog's response to the infection[6]. Clinical signs of heartworm disease are attributed to the damage caused to the pulmonary arteries and to the right side of the heart, however larvae can also spread to other organs such as the eyes, kidneys, the central nervous system, and subcutaneous tissue[4]. Dogs with a significant number of adult worms often have clinical signs such as coughing and reduced exercise tolerance. Individuals with severe heartworm disease may also exhibit dyspnoea, weight loss, syncope, and signs of right-sided heart failure[4,8]. Dyspnoea is due to hypoxia from vascular and interstitial changes in the lungs, with resulting pulmonary hypertension. Infection may potentially become life threatening in these individuals, and treatment of seriously ill dogs can be fraught with complications. "Caval Syndrome" can be seen in severely affected dogs, where worms invade the right ventricle and caudal vena cava to cause an obstruction of the bloodstream. These dogs may present with clinical signs of right-sided heart failure, intravascular haemolysis, haemoglobinuria, and acute general malaise[8].

Diagnosis

Heartworm infection is directly diagnosed by the visualisation of worms during surgery, or post mortem, or by identification of microfilariae by microscopy. Indirectly, infection can be diagnosed by the use of a number of blood tests to determine the presence of antigens and/or microfilariae in the host. It is important to bear in mind that dogs that have been given heartworm prophylaxis will be microfilaria negative even if they carry adult worms. Antigen and microfilaria tests should therefore be carried out at different stages during the diagnostic process[12]. Serological identification of *D immitis* antigens in serum can be achieved by the use of in-house tests or by tests performed in a number of commercial laboratories. These tests will identify antigens released by the female *D immitis* worm. The specificity of the test is about 98 %, meaning that seropositive dogs are in most cases carriers of the adult worm[7]. Seropositive results are found as early as six months after infection with heartworm larvae by mosquitoes. Individuals with a small number of worms may produce negative tests; the test sensitivity is about 80%[6,7]. An even less sensitive test is one that searches for microfilariae in the circulation. Microfilaraemia may be discovered as early as five months after the individual is infected with heartworm larvae. The most common test for microfilaraemia

is a concentration technique, or a modified Knott's test, where 1 ml EDTA blood is added to 9 ml of formalin, followed by a centrifuge of the haemolysed solution. The sediment is dyed with methylene blue and studied under a microscope with 100x to 400x magnification[6]. Microfilariae may also be found in fresh blood by studying a blood smear under a microscope, or their mobility may be observed inside a haematocrit tube. The latter method is unreliable in individuals with a small number of microfilariae, and would not distinguish between the various types of microfilaria. Molecular testing, such as the polymerase chain reaction test (PCR) and tissue histochemistry, are able to distinguish between different types of microfilaria, but it is important to keep in mind that these tests would be negative in dogs that are carriers of the adult worm but are microfilaria negative[12]. Other diagnostic procedures such as general blood tests, radiography, echocardiography and electrocardiography should be performed in order to determine the degree of severity and guide the treatment strategy (Table 1).

Treatment of adult heartworm infection

The goals of heartworm treatment are to improve the clinical condition of the patient, as well as to attempt elimination of all stages of *D immitis* in order to prevent transmission to other individuals. The most recent recommendation made by the American Heartworm Society (AHS) is that all seropositive dogs are treated for heartworm infection regardless of whether they have clinical signs or not[12]. However in cases where heartworm is suspected, but where the dog tests negative on the antigen test, treatment should be postponed, since treatment in itself may lead to serious consequences for the individual. Treatment of adult heartworms involves the use of an arsenic-based compound that is costly to the owner and may cause side effects. Owners should be informed of costs, risks and potential side effects and written consent should be requested.

Melarsomine dihydrochloride is the most commonly used medication and is administered by deep injection in the lumbar muscles. The dosage is 2.5 mg/kg and the injection is given twice, with a 24-hour interval[7]. An alternative to the traditional standard treatment is to give an initial injection, followed by two more injections given 24 hours apart 4 to 6 weeks later on. This type of treatment is called the "split-dose regimen" and is considered by the AHS to be the optimal treatment method. It is recommended since it appears to limit side effects and eliminates a higher number of worms than the standard injection technique (98% compared to 90%)[12]. Nevertheless potential side effects are associated with this treatment. Mild swelling and pain at the injection site is frequently seen, but this gradually decreases after a few days. Pulmonary thromboembolism will always occur to a certain extent after treatment, as dead worms are flushed through the pulmonary circulation. Embolism can occur between 7 to 28 days after administration of melarsomine dihydrochloride and it may be fatal to the dog in some cases[6,12]. The risk of serious consequences of embolism can be minimised by ensuring that the dog remains physically inactive for several weeks after treatment. Clinical signs of embolism include fever, coughing, bleeding from the nose and mouth, and signs of heart failure. The higher the number of worms in the dog, the greater the risk of developing serious side effects. If the dog shows signs of embolism, it should be hospitalised for intensive treatment and observation. It may be necessary to administer oxygen, heparin, corticosteroids and antibiotics[6]. Prophylactic treatment with corticosteroids to reduce side effects is recommended by some[12]. Thought this has been debated due to the potential pro-coagulation effects, thereby increasing the risk of thromboembolism[7]. In serious cases, including caval syndrome, the dog should undergo surgery to extract as many worms as possible: specially designed alligator forceps are passed into the right heart via the jugular vein under fluoroscopic guidance[8].

Wolbachia pipientis is an intracellular bacterium that lives in symbiosis with *D immitis*. Studies have shown that the inflammatory reaction occurring during heartworm treatment is partly due to the release of *W pipientis*[13]. Doxycycline will reduce the burden of *W pipientis* in all stages of *D immitis* and treatment with antibiotics should be considered for individuals with a large number of heartworms[13]. AHS has recently advised that dogs should be

Table 1: Clinical signs of heartworm disease[12]

mild	No clinical signs or cough
moderate	Cough, exercise intolerance, abnormal lung sounds
severe	Cough, exercise intolerance, dyspnoea, abnormal heart and lung sounds, hepatomegaly, syncope, ascites, death
Caval syndrome	Sudden onset of severe lethargy and weakness, haemoglobinaemia and haemoglubinuria

treated with a recommended antibiotic, such as doxycycline, before initiating the split-dose regimen[12].

The dog should be re-tested for *D immitis* antigen a few months after treatment for adult heartworms. If the dog tests positive, a new test should be carried out six months after treatment, as it may take some time before all of the adult worms have been eliminated. If the dog still tests positive six months after the first treatment, the treatment should be repeated[12].

Treatment of microfilaria infection

All dogs with microfilariaemia should be treated with endectocides. Ivermectin has traditionally been used as a microfilaricidal drug, and is given in a single dose 4 to 6 weeks after treatment with melarsomine dihydrochloride[6,7]. The ivermectin dosage is 50 mg/kg per os, diluted in 1:9 mixture of water or propylene glycol. The diluted solution is equivalent to 1 ml per 20 kg[6]. This dose is also considered to be safe even for more vulnerable breeds, such as Collies, but it is recommended that the dog being kept under observation for 24 hours after administration. With this type of treatment most of the circulating microfilariae are eliminated within a few hours[6]. Side effects such as nausea and vomiting are seen in less than ten per cent of treated dogs, and serious side effects are rare. Ivermectin is not registered for use in dogs, and dog owners should therefore provide written consent, stating that they accept this type of treatment and are informed of its potential side effects. As an alternative, other macrocyclic lactones may be given monthly with the same dose as used for prophylaxis (see section below). Microfilariae will then gradually be eliminated over a period of 6 to 9 months. This is the treatment method now recommended by the AHS[12].

Prophylaxis

Prophylactic treatment of all dogs living in areas exposed to infection is recommended in order to reduce the number of microfilariae in the bloodstream, and thereby prevent transmission to other individuals[12]. Treatment consists of a monthly dose of macrocyclic lactones such as a perorally administrered milbemycin, topically applied ('spot-on') selamectin and moxidectin, or topically applied ivermectin.

Case study

A three-year-old male mixed-breed dog was imported to Norway from Romania in 2012. The dog had been a stray, and was imported from a country where *D immitis* was considered endemic[11,14]. The new owners observed that the dog was coughing daily and had periods of shortness of breath. Prior to import the dog had been vaccinated and treated for parasites, including prophylactic treatment for microfilariaemia, according to current EU regulations for importing pets to Norway[14]. Once in Norway, the consulting veterinarian collected blood samples for serological testing as recommended by the Norwegian Food Safety Authority. A serological antigen test (immunoassay) was carried out and analysed (Laboklin Laboratories, Germany). The blood test was seropositive for *D immitis* and the dog was referred to the author for further diagnostic work up and treatment.

Upon clinical examination, the dog had pink mucus membranes and a capillary refill time of one second. Palpable lymph nodes were considered to be normal and the jugular vein appeared normal without visible pulsations. The femoral pulse was of good quality, was regular, full and synchronous with the heart rate. On cardiac auscultation, the heart rate was normal, no murmurs were detected, and the rhythm was regular, at a rate of 120 beats per minute. The dog was panting and coughing to some extent in the examination room, but no abnormal sounds were heard upon auscultation of the lungs. The Knott's test for microfilaria, carried out at the Laboklin Laboratories in Germany, was negative. Thoracic radiography was performed; the cardiac silhouette was normal, with a Vertebral Heart Score of 10 (normal <10.5)[15]. The lung pattern was unremarkable however a mild dilation of pulmonary arteries was noted[15]. The electrocardiogram was unremarkable. Echocardiography was performed including B-mode, M-mode, colour flow Doppler and spectral Doppler with simultaneous electrocardiography. Morphology and measurements of the heart chambers and walls were normal[16]. Mild dilation of the main pulmonary artery and both the right and left pulmonary arterial branches was noted. Foreign bodies were seen in the main and right pulmonary arteries. The foreign bodies were linear with a circular cross section depending upon the angle of insonation. Based on the appearance of the foreign bodies and the medical history, it was assumed that these were heartworms (Figure 2). Colour and spectral Doppler findings were normal[16]. Echocardiographic evidence of pulmonary hypertension, which is often seen in dogs infected by heartworm, was not detected in this case. The dog was classified as "moderately affected" according to the AHS classification system (Table 1).

Based on clinical and diagnostic results, it was determined that treatment recommended by AHS should be initiated[12].

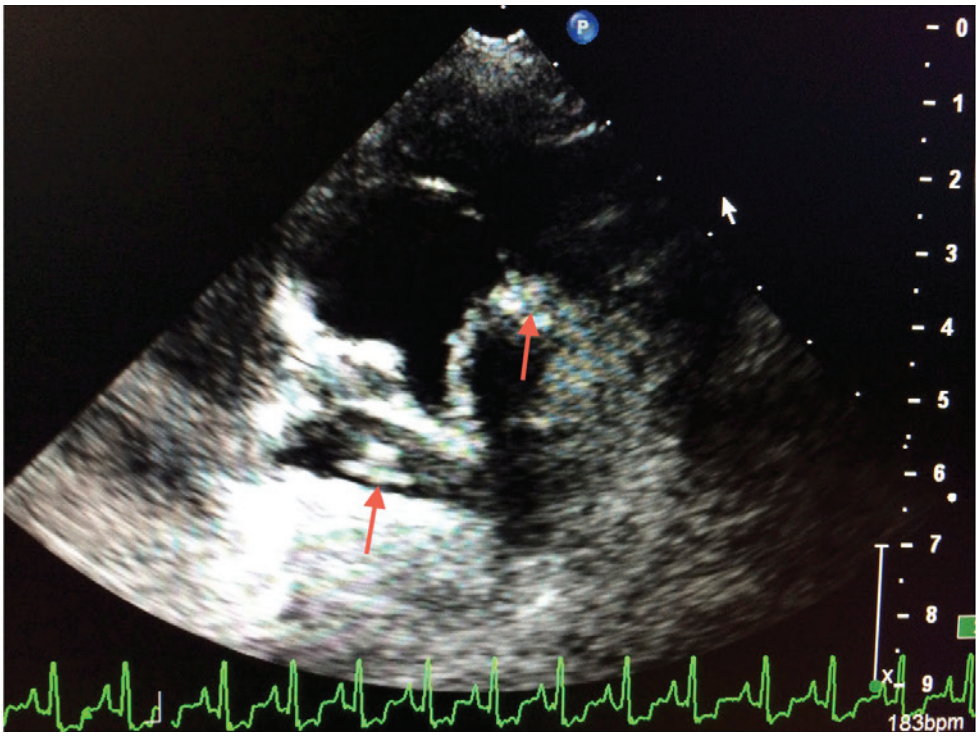

Figure 2: Echocardiography: foreign bodies in the main pulmonary artery and the right pulmonary artery (arrows)

The "split-dose regimen" was chosen as the preferred treatment regimen in an attempt to reduce side effects and to eliminate as many worms as possible. The dog owners signed a consent form stating that they were informed about the costs and potential risks associated with this treatment. The dog received 2.5 mg/kg melarsomine dihydrochloride (Immiticide®, Merial) by deep interlumbar injections on day 1, day 50 and day 51 (Figure 3). Following each treatment the dog was admitted to the veterinary clinic for observation. The dog was released from the clinic in the evenings, for observation by the owners; exercise was limited the first few weeks after each treatment. No side effects were observed following treatments.

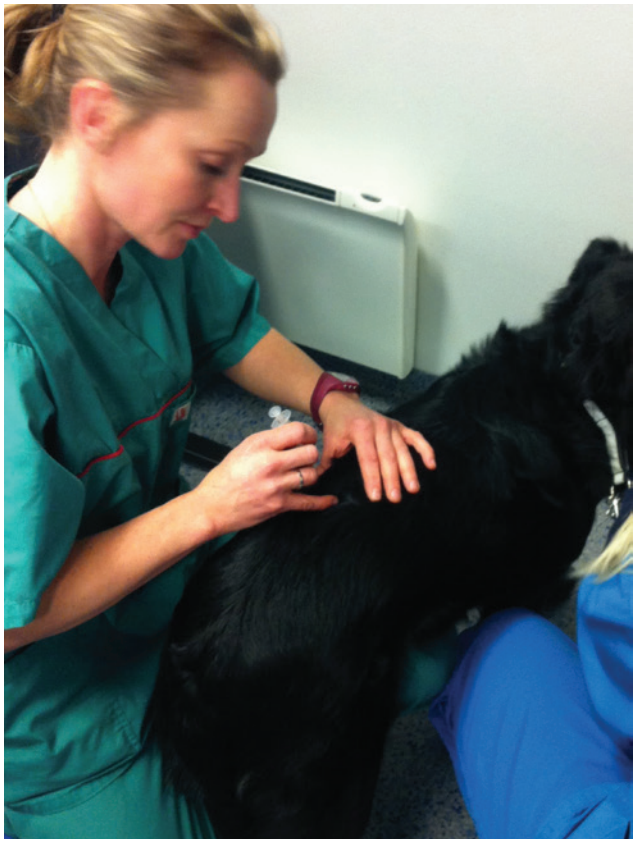

Figure 3: Intralumbar injection of melarsomine dihydrochloride (Immiticide©, Merial)

The dog was given 50 mg/kg ivermectin (Ivomec vet®, Merial) per os diluted with a 1:9 mixture of propylene glycol four weeks after the last injection. Follow-up examinations took place later the same day. The ultrasound examination was now unremarkable; this time, no linear foreign bodies could be visualised echocardiographically in the pulmonary arteries. The dog's clinical signs had improved, but the dog continued to have a mild chronic cough at this stage, and treatment with doxycycline (Ronaxan vet®, Merial, 10 mg/kg once daily) was therefore initiated in order to treat the potential concurrent infection with *Wolbachia pipientis*. Three months later a serological antigen test was carried out (immunoassay, Laboklin Laboratories, Germany); the test was now negative. The owners reported that the dog was in very good physical condition and had stopped coughing. The breathing pattern and respiratory rate were normal.

Discussion

Based on clinical and diagnostic findings it was concluded that the dog in the case study suffered from heartworm disease. The definitive test for heartworm infestation is to directly visualise the worms in the bloodstream. In this case such procedures were not performed. However, the dog was seropositive for *D immitis* and linear foreign bodies were detected by echocardiography. Treatment was initiated based on these findings and because the dog had clinical signs that could be attributable to heartworm disease.

When treatment was completed, the dog was seronegative, and foreign bodies could no longer be detected by echocardiography. A discernible clinical improvement was noted following treatment. In many cases, changes in lung tissue are irreversible, even if treatment often leads to a certain improvement of clinical signs. Irreversibility is correlated with the degree of severity, and in this case the dog was classified as moderately affected. It was therefore not surprising that the cough persisted for a time after treatment had been completed. Another explanation may be that the cough was caused by something other than heartworm disease. Whether or not the clinical signs were caused by *D immitis* alone, or due to a combination if factors, was not determined in this case. A concurrent infection by other pathogens such as *Wolbachia* spp could have contributed to the clinical picture. Further diagnostic investigations such as bronchoscopy and broncho-alveolar lavage were not performed in this case. No side effects from treatment were observed, and the dog owners were very pleased with the clinical result. The dog's general physical condition was good during the entire treatment period. The dog continued to be microfilaria-free and antigen-negative for several months following the treatment regimen, and the cough disappeared.

The Norwegian Veterinary Institute has carried out a study on the health condition of a group of stray dogs imported to Norway from Eastern Europe[10]. Blood and faecal samples from 80 dogs were sent to the Veterinary Institute and examined for a number of pathogens including *D immitis* and *D repens*. Among the dogs that were tested, 7.5% were antigen-positive for *D immitis*[10]. Several of the diseases in question, including heartworm disease, have high morbidity and a significant mortality. The Norwegian Food Safety Authority advises against the importation of dogs from Eastern and Southern Europe in the interest of Norwegian animal and human health. However with the current travel regulations the government cannot prevent such importation from occurring. Norwegian veterinarians are advised to perform tests to determine the incidence of a number of potential diseases among stray dogs imported from Eastern Europe[14]. Tests on imported dogs cannot be imposed on the pet owners, since Norway, as an EEA country has to adhere to international regulations; it is therefore up to each individual pet owner to find out whether or not the imported dog may carry a potentially dangerous disease.

Pathogens and diseases that were previously not diagnosed in Norway have been diagnosed in Norway now that imported dogs are being tested[10,14]. The Norwegian Veterinary Institute has carried out a risk analysis, regarding the importation of stray animals from Eastern Europe to Norway, in order to establish the current situation and provide recommendations[17]. The probability of import of pathogens, not currently found in Norway, was considered high; certain diseases have a zoonotic potential, and therefore present a risk of infection for humans[17]. This emphasises our responsibility as veterinarians to convey the importance of following recommendations by the Norwegian Food Safety Authority to pet owners who choose to import dogs from countries where such infections are endemic. In 2013 the Norwegian authority banned commercial import of dogs from Romania, however individual animals are still legally imported in smaller groups[18]. Illegal commercial importation of dogs from Romania to Norway has also been reported since the new regulation took effect[19].

The Climate Division of the Norwegian Meteorological Institute report that in certain areas of the country, the average temperature could be sufficiently high to enable development of the *D immitis* larvae to a contagious larva stage L3 in Norwegian mosquito species. This was considered more likely to take place along the coast of southern Norway. The minimum temperature, as well as the average temperature, varies from year to year, and the risk will most likely be higher during especially warm seasons (personal communication, Stian Kristiansen, Climate Division, Norwegian Meteorological Institute, Oslo). Larval maturity in the mosquito requires a minimum temperature of about 14°C; and the higher the average temperature, the faster the rate of maturity[7,11]. The risk that Dirofilariasis caused by *D immitis* may become endemic is higher when there are a large number of infected individuals in the area, and when the climate remains stable[1,9,11]. Due to variable weather conditions and a relatively low number of seropositive dogs in the country, this risk is considered to be low in Norway[17].

The risk of significant propagation of *D immitis* is also considered to be low in the neighbouring country of Sweden[20]. However, heartworm disease, once viewed as an exotic disease, could now potentially become a threat to a part of the Norwegian animal population unless certain measures are taken to prevent the proliferation of this parasite.

Acknowledgements

I would like to thank Fernando Simón for kindly designing the diagram in Figure 1. I would also like to thank Professor Bjørn Gjerde and Dr. Stein Istre Thoresen for proofreading the original (Norwegian) version of this paper.

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