FECAVA
Federation of European Companion Animal Veterinary Associations

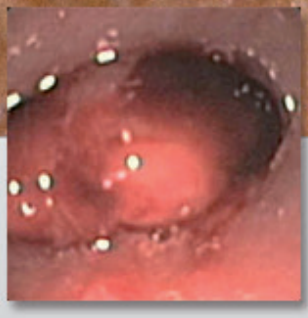
EJCAP ONLINE

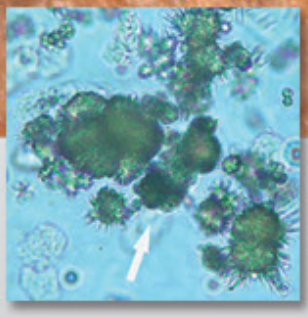
Volume 23(2), Summer 2013

An update on feline Leishmaniasis in Europe

Snuffling and sneezing in cats

Urine sediment evaluation in the dog and cat





Risk factors of cruciate disease, Primary hyperaldosteronism in cats, Modulating fertility in cats and dogs, Haemophilia A and B in dogs, and more...

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COMMISSIONED PAPER (F)

Canine Leishmaniasis[#] - an update

Maurice Roze[1]

SUMMARY

Canine leishmaniasis is a vector-born disease which is caused by a flagellate protozoan (genus *leishmania*) named *Leishmania infantum*. It is endemic in many countries and transmitted by the bite of phlebotomine sand flies. Two successive hosts, a vertebrate and an arthropod, are needed to complete this parasite's life cycle. Many species of mammals can be infected but the dog is the main reservoir for *L. infantum*. The epidemiology of the disease is constantly evolving with the appearance of new foci of infections. Resistance of the dog to infection depends on a cellular immune response (Th1) and its susceptibility is related to a humoral response (Th2).The usual clinical signs are: lymphadenopathy, skin lesions (alopecia, scaling), weight loss, and excessive weakness. In advanced cases, nephropathies and haemorrhages may also occur which can be fatal. The first line of treatment involves a combination of Meglumine antimoniate (100 mg/kg/day) and Allopurinol (20-30 mg/kg/day). Treatment is aimed at achieving a clinical cure; it will not eliminate the parasite. In the past few years progress has been made in the field of diagnosis, case follow-up (Real-time PCR), and prevention (long acting insecticides and vaccination).

Key words: Dog, Leishmaniasis, Canine leishmaniasis

This paper was commissioned by FECAVA for EJCAP

Introduction

In 2005, the JSAP published an article on canine leishmaniasis[1]. In 2013 veterinarians are still concerned by this disease and in the 8 years since this article was published, knowledge about the physio-pathogenic and immunologic mechanisms of leishmaniasis have evolved, epidemiological data have been undated, and new diagnostic and preventive medicines have become available. The purpose of this article is to provide an update on the diagnosis, treatment, and prevention of canine leishmaniasis (CanL).

CanL is a disease caused by a flagellate protozoan (genus *leishmania,* named *infantum*) which is transmitted by sand flies. Thirty species of leishmania infect mammals and around twenty of them infect human beings. Among those twenty, ten also infect dogs. All leishmaniases are vector-borne parasitic diseases transmitted by the bite of a sand fly (a blood sucking *dipteran* fly). Zoonotic leishmaniases is considered as one of the main zoonotic disease risks worldwide. The W.H.O. has classified

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[#] Editor's note: Leishmaniosis or Leishmaniasis? Readers may be a little confused by the use of the words Leishmaniosis and Leishmaniasis in the two papers in this issue of EJCAP. The two spellings appear in both papers in the text and reference list. Each refer to exactly the same condition. Leishmaniasis was the original term universally used. It is the official term used by WHO and in the OIE zoonotic diseases list and is the term still used universally for the human disease. On the other hand readers will find that nowadays Leishmaniosis is the term almost always used for the animal disease in veterinary journals and text books.

classified them as a high priority diseases. It is estimated that 16 million human beings are infected in 88 countries; 2 million new cases are reported annually[2]. Sand flies are blood sucking diptera. They belong to the genus *Phlebotomus* in the Old World and the genus *Lutzomyia* in the New World. Each sand fly is specific to the leishmania that they transmit. The geographical distribution of leishmaniases corresponds to the distribution of the vector and mammal reservoirs.
Two clinical forms of human leishmaniases are recognised: a visceral form and a tegumentary (cutaneous or mucosal) form. When humans become infected with *L. infantum* (the agent responsible for CanL) it results in development of the visceral disease. Unlike the disease in humans, dogs suffering from CanL can present with various forms, some of which are fatal. About 2.5 million dogs are estimated to be infected in Southern Europe. As dogs are the main reservoir for these parasites, leishmaniasis represents not only a very important public health problem but also a serious veterinary problem[3].

The severity of the disease both in dogs and immuno-compromised people justifies the high level of interest the disease has provoked in the medical and veterinary profession. The geographical extension and the outbreak of new foci of infections makes CanL a disease that all veterinarians in Europe should be able to recognise and manage whatever their area of expertise.
It is therefore necessary for clinicians to keep up to date about developments concerning the transmission of the disease, diagnosis, treatment, and prevention[4].

KEY POINTS

- CanL is a vector-born zoonotic disease caused by a flagellate protozoan *Leishmania infantum*.
- The disease is transmitted by the bite of phlebotomine sand flies.
- *L. infantum* is the agent of a visceral form of leishmaniasis in humans.
- *L.infantum* is the agent of multisystemic form of leishmaniasis in dogs.

The Parasite

Identification

Leishmania are protozoa of the flagellated class, *Trypanosomatidae* family, and belong to the genus *leishmania*. A defined enzymatic profile of *leishmania* allows classification in units called zymodemes[5].

Agents of human and canine Leishmaniases

Only about twenty species of leishmania are pathogenic for humans. Half of them (ten) can also infect the dog. They are: *L. infantum, L. donovani, L. tropica, L. major, L. arabica, L. braziliensis, L. mexicana, L. amazonensis, L. combiensis, and L. peruviana*. The most widespread species is *L. infantum* (named *L. chagasi* in South America).This species infects humans and dogs in the Mediterranean Basin, Portugal, West Africa, Southern Asia and South America[2]. Canine infection only has been reported in North America.

Parasite biology

Leishmania require two successive hosts (a sand fly vector, and a vertebrate) to complete their life cycle. In each of the hosts a different morphological life stage occurs[3].

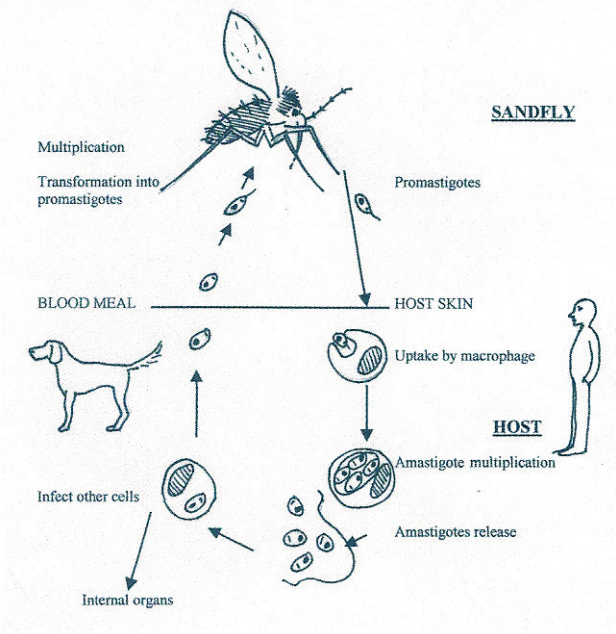

Fig 1 The life cycle of Leishmania infantum.

Parasite biology in the host

After inoculation by an infected sand fly, adhesion between the host's phagocytic cells, Langerhans cells, and the parasite promastigote (the flagellate form) occurs. The adhesion is facilitated by complement receptors. Promastigotes are then included in the host's cells inside a vacuole called the parasitophorous vacuole. In this vacuole, the parasite loses its flagellum and changes into a form called an amastigote [6]. These amastigotes appear as ovoid bodies (size: 1.5 µm to 5 µm) inside the host's macrophages. They contain a nucleus, a kinetoplast (mitochondrial type structure) and an internal remnant of the flagellum. This form, the amastigote, is very easy

to recognise. It can be observed by the practitioner in stained smears obtained from the bone marrow or from lymph node aspirates. Usually, foreign organisms are destroyed by the body; here instead of being destroyed the parasites multiply and develop different mechanisms for their protection (e.g. pH and oxygen metabolite neutralisation).

After phagocytosis, captured antigens are presented to lymphocytes. Then two types of outcome related to the immune response of the host can occur. In resistant dogs (those that do not go on to develop the disease), parasites do not disseminate further than the local lymph node. Their dissemination is prevented, mainly by the production of interferon by the host cells. In susceptible dogs (those which are going to develop the disease) the parasites disseminate to internal organs (lymph nodes, spleen, and bone marrow)[7]. Amastigote multiplication leads to cell rupture and infection of other cells. Inflammatory reactions occur in the organs which are colonised. The incubation time can be from months to several years.

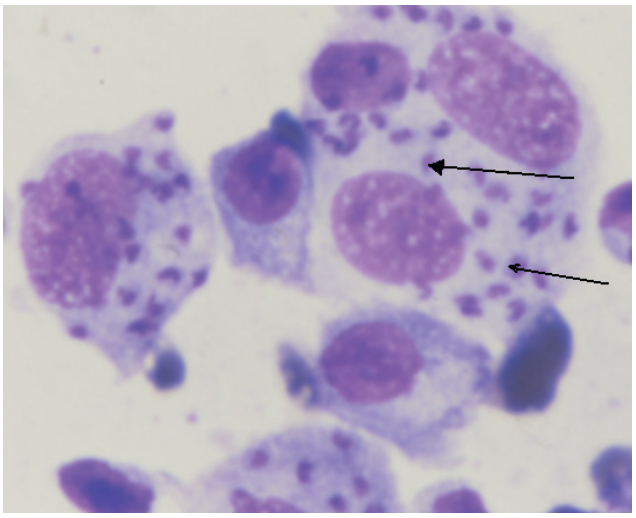

Fig 2 Leishmania amastigotes (arrows) observed in macrophages.

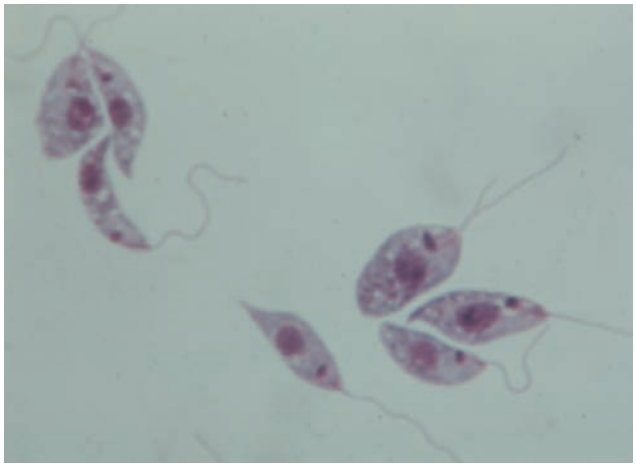

Fig 3 Leishmania promastigotes.

Parasite biology in the vector

Parasite amastigotes, which are localised in the skin of the host at an appropriate depth for being probed by the female insect's mouth parts, are ingested with a blood meal. They quickly change into elongated and flagellated promastigotes (size: 5-20 µm in length, 1-4 µm in width) which contain a nucleus and a kinetoplast. They are mobile, live extracellularly, multiply, and attach to the insect's gut wall. After multiplication, the promastigotes migrate to the thoracic midgut of the insect. Here they change into infectious promastigotes and acquire special properties such as resistance to complement, which will allow them to survive inside the host. Leishmania are specifically adapted to a limited number of vectors (In non-adapted species, Leishmania will be eliminated by the sand fly). This very high specificity probably occurs due to factors relating the parasite (promastigote phospho-lipoglycan) and the insect (gut cell ligands or lectin produced by the female sand fly)[8].

KEY POINTS **The parasite**

- Leishmania require 2 hosts (a sand fly and a vertebrate) to complete their life cycle.
- The parasite exists in two forms: promastigote in the sand fly and intracellular amastigote in the vertebrate.
- Leishmania are specifically adapted to their vectors.

The Vector

The sand fly

Sand flies are the vectors of CanL. They are hematophagous diptera of the *Psychodidae* family, *Phlebotominae* subfamily. Two genera are involved in the transmission of the infection: genus *Phlebotomus* in the Old World and genus *Lutzomyia* in the New World[1, 3]. Only fifteen species of both genera, including six in Europe, are identified as vectors of the parasite. It is only the female sand fly which bites the host and ingests blood meals as the blood meal is needed in the development of the female's eggs. The sand fly vector of CanL can bite a large number of mammals but not reptiles and birds.

In tropical countries, sand flies are active almost all year round. In temperate countries, they are active only during the warm season. In Southern France only two species of

sand flies: *P. ariasi* and *P. perniciosus* are vectors. In the Mediterranean Basin *P. perfiliewi, P. neglectus* and *P. tobbi* are also vectors[8].

Phlebotomine sand flies are very small, silent arthropods active at dusk and during the night. They move within a limited radius (from 400 m for *P. perniciosus* to 2 km for *P. ariasi*).

Each female insect needs several blood meals and each blood meal may last up to several minutes. After landing on its target, the sand fly moves to the head of the animal aiming to bite a hairless zone (inside the pinnae, the muzzle, or eyelids).The bite can be painful. Some months later an inoculation nodule full of amastigotes appears at the bite site from an infected sand fly.

Vector-host interaction

Some dogs are more attractive to sand flies than others. The difference in this tropism could be as a result of a possible previous infection (and the role of factors related to it) or related to odour.

It has been shown that sand fly saliva contains anticoagulants, vasodilatory, and immunomodulatory compounds. Their role as factors in favouring the infection in dogs is not formally established[8].

Transmission

With few exceptions, leishmania are transmitted by the bite of an infected phlebotomine sand fly. A close specificity exists between each species of leishmania and the phlebotomine vector. In the Old World, *L. infantum* is transmitted by sand flies of the genus *Phlebotomus*. In the New World, *L. chagasi* (same as *L. infantum*) is transmitted by sand flies of genus *Lutzomyia*. The sand fly infects the host when a female bites it to ingest a blood meal (which is necessary for the development of the insect's eggs)[8]. Proven or suspected modes of transmission other than sand flies have been described in infected dogs. Proven modes include by blood[9], congenital[10] and venereal[11] transmission; in practice it is recommended to exclude asymptomatic infected dogs when selecting blood donors. Suspected modes of transmission are contact transmission (wounds, bites) or transmission via hematophagous arthropods such as ticks and fleas. This could explain the emergence of autochthonous cases in non-endemic areas with no recognised vectors, as described in foxhound kennels in USA and Canada, and breeding kennels in Europe. Modes of transmission (other than by the sand fly) play only a minor role in the epidemiology of the disease and the role of these modes of transmission still need to be clarified.

KEY POINTS The vector

- Sand flies of the genus *Phlebotomus* are vectors of CanL in the Old World.
- Sand flies of the genus *Lutzomyia* are vectors of CanL in the New World.
- Modes of transmission others than sand flies may exist but with a minor epidemiological role.
- Phlebotomine sand flies are active from dusk to dawn during warm season in temperate countries.

The Host

Animal species

Many species of mammals can be infected by leishmania. Some are accidental hosts whilst others are considered as reservoirs for the parasites. *L. infantum* has been isolated from different species of rodents (such as the black rat and squirrel), equines, bovines, goats, sheep, cats and wild carnivores (foxes, wolves, jackals, fennecs, genets,

Leishmania distribution	Geographical	Proven vectors	Suspected vectors
L.infantum	Mediterranean basin	*Phlebotomus perniciosus P.ariasi*	*P.longicuspis, P.syriacus,etc..*
	Middle East	*P.perfiliewi,P.neglectus P.langeroni,P.tobbi*	
	Southern Asia, Iran, Armenia, Afghanistan	*P.kandelaki*	*P.brevis etc…*
	Central Asia,China	*P.chinensis, P.alexandri*	*P.smirnovi, P.transcaucasius etc..*
L.infantum = L.chagasi	Central-South America	*Lutzomyia longipalpis L.evansi,L.olmeca*	*L.antunesi L.shannoni*

Fig 4. Geographical distribution of L.infantum and its vectors

pine martens)[12]. The prevalence of infections seen in cats is confirmed by several reports (see article on feline leishmaniasis in this issue). The existence of a carnivore reservoir other than dogs (i.e. foxes) has been suspected but not clearly established[13]. In the Mediterranean Basin dogs are the main reservoir for *L. infantum*. Symptomatic dogs are more infective for sand flies than asymptomatic animals, although the latter can still be a source of infection to the vector.

Until now, man was considered a potential host but not a functional of reservoir of infection. However, this may not be true in the case of drug addicts and in cases where people are using the same needles and syringes for injections. Finally, it is worth remembering that blood transfusions pose no danger of man to man transmission as white blood cells are removed before the blood is transfused[2].

Host-parasite interaction

The behaviour of the dogs following exposure to the parasite is very variable. Infection may remain asymptomatic or occur in mild to severe forms[7]. Endemic area studies show that several factors (which include sex, age, genetics, nutritional status, co-infections, and the virulence of the leishmania strain) play a role in the dog's response to infection; however the exact role of these factors has yet to be confirmed. The most important and best known factor is the immune response[14]. Sex does not seem to be a risk factor. Regarding age, there are two peaks of prevalence: before 3 years and after 8 years[4, 15]. All breeds are susceptible although some, such as the Boxer, Cocker Spaniel, Rottweiler, German Shepherd, Foxhound and Neapolitan Mastiff are more susceptible than others. Conversely, breeds such as the Ibizan Hound rarely develop clinical signs of the disease[16, 17]. In fact, it is the orientation of the immune response that affects the resistance or susceptibility to infection.

The key principle which helps us understand the difference in the immune response between infected dogs is the principle of duality between two populations of T helper cell lymphocytes: Th1 and Th2[18]. Th1 cells are involved mainly in cellular immunity. Th2 stimulates B cell differentiation and production of antibodies. The two types of response usually coexist but one is dominant over the other. Resistance to infection is related to the cellular type response which is protective and therefore the infected animal remains asymptomatic. Susceptibility to the infection is related to the humoral response which is not protective and the infected animal goes on to develop the disease[7, 19].

Recent studies have shown that the production of interferon gamma (IFN-γ) by Th1 lymphocytes plays an essential role in the development of a good immune response[20].

Hypergammaglobulinaemia observed in protein electrophoresis performed on a sick dog is the hallmark of an overproduction of antibodies. Not only are the antibodies produced not protective, but they can also result in harmful deposition of immune complexes around the body. In mice and humans the ratio of immunoglobulin sub classes (IgG1:IgG2) can help to determine the type of response produced. Unfortunately, the ratio in the dog does not appear to be correlated with the level of protection[21].

The resistance of the host to infection is not guaranteed. It can be broken if the humoral response is higher than the cellular response. The recent acquisition of these data has helped us to adopt a different attitude towards the leishmaniasis. New diagnostic methods have shown that the majority of infected dogs are not sick[22] therefore, highlighting the presence of the parasite can no longer be considered as proof of the disease.

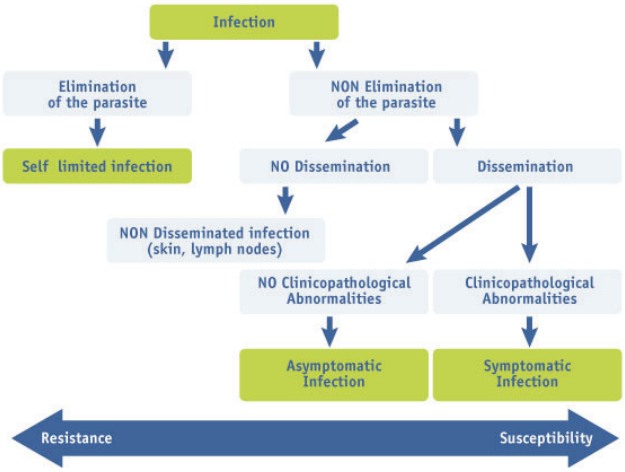

Fig 5 Outcome of the infection of L. infantum in dogs. (From Saridomichelakis M.N. [7])

KEY POINTS The host

- Dogs are the main reservoir for *L. infantum*.
- Man is a potential host for *L. infantum* but is not considered a reservoir of infection.
- The outcome of infection in dogs depends of the type of immune response.
- Resistance to infection depends on a strong Th1 cellular type response.
- Progression to disease is associated with a strong Th2 humoral response.

Public health/human leishmaniasis

Two clinical forms of leishmaniasis are conventionally recognised in humans: the tegumentary (or mucocutaneous) and the visceral form[2].
Tegumentary leishmaniasis most commonly presents with skin lesions in the area where the sand fly has bitten, however, more rarely it can present with lesions of the mucous membranes.
In the Old World (Near and Middle East and Africa) the disease is caused by *L. major* and *L. tropica*. The reservoirs for *L. major* are rodents. The *L. tropica* reservoir is the human. A variant of *L. tropica* called *L. killicki*, has recently been identified in North Africa with rodents as the possible reservoir. In the New World tegumentary leishmaniasis is caused by several species of Leishmania with varied reservoirs: the sloth for *L. guyanensis*, and rodents, dogs and other mammals for other parasites.

Human visceral leishmaniasis (HVL) also known as Kala-azar is a serious disease. *L. donovani* is the agent of HVL in areas of the North East of the Indian Peninsula and Eastern Africa. The reservoir is predominantly human. *L. infantum* (synonymous *L. chagasi*), the agent of CanL, is also the agent of HVL in areas around the Mediterranean Basin and South America (especially Brazil). The reservoir is mainly canine. There are 700 HVL cases per year in South Eastern Europe. The disease is fatal if not treated. *L. infantum* can also rarely cause the cutaneous or mucosal forms of the disease[23]. A large number of individuals living in endemic areas are asymptomatic carriers. These individuals are likely to develop the disease with concurrent immunosuppression (AIDS or immunosuppressive therapy)[24].

HVL Clinical signs:
An irregular fever, pallor, hepatosplenomegaly, and lymphadenopathy combined with pancytopenia were considered as suggestive signs of HVL. Recently, the epidemiology and therefore presenting symptoms have changed. The increased number of immunocompromised patients presenting with HVL (because of HIV infection or immunosuppressive therapy) means atypical signs of the disease are more frequently observed. In Southern Europe, HVL due to *L. infantum* initially was mainly a disease of children; now it is becoming more common in adults[2].

HVL Therapy:
In many countries, the parasites are becoming resistant to pentavalent antimonials. Amphotericin B in a liposomal presentation (Ambisome® (perfusion during 2-6 days)) is now preferred. An alternative medication, Miltefosine is authorised in many countries and has the advantage of being administered orally. The current use of HAART antiretroviral therapy (highly active antiretroviral therapy) gives good results when controlling Leishmania-HIV co-infections. The poor response to anti-leishmanial therapy which is seen in immunocompromised patients is comparable to the poor response seen in susceptible dogs[25].

HVL Epidemiology:
Despite the narrow specificity of the host-vector-parasite relationship, the epidemiology is variable depending on the geographical location [26]. Different factors such as climate (warming) and environmental (urbanization programs) may explain the expansion of the infection in endemic areas[27]. The role of population movements (tourism, wars) can help to explain the emergence of cases of human leishmaniasis outside endemic areas.

HVL Diagnosis:
The diagnosis of HVL is based on serology, direct visualisation of the parasites on smears (from bone marrow or skin) and the use of PCR. The latter is the most common diagnostic tool used in hospitals in developed countries. PCR tests on the kinetoplast DNA provides a result with a good sensitivity. Real-time quantitative PCR is useful in the follow-up of patients with the disease and in determining the prognosis.

HVL Vaccines:
Due to the severity and extent of endemic leishmaniasis, vaccination and the possibility of its application on a large scale, would be the ideal means of prevention[28]. Numerous avenues of research have been undertaken to develop vaccines[29]. The first generation vaccines, using whole parasites, yielded disappointing results. A so called Leish-111f, second generation vaccine consisting of recombinant proteins, provides good protection against cutaneous leishmaniasis however it would not give effective protection against canine infection due to *L. infantum*. Three human vaccines are currently under research. A polyprotein vaccine "LEISH-F3" is being developed in the USA. A DNA vaccine, the "LEISHDNAVAX" is being developed in Germany. Finally there is an

important project called RAPSODI. This project is being undertaken by Virbac in collaboration with IRD and several countries, and is based on the development of a synthetic candidate vaccine based on the immune-dominant properties of the PSA (Promastigote Surface Antigen) protein.
Website: www.fp7-rapsodi.eu

Currently no vaccine against human leishmaniasis is available.

KEY POINTS **Human leishmaniasis**

- Two forms of human leishmaniasis are recognised: the tegumentary form and the visceral form.
- *L. infantum* is the agent of CanL and human visceral lesihmaniasis (HVL).
- Prognosis of HVL is guarded if the disease in not correctly treated and in cases of immune-depression.
- Current treatments are Amphotericin or Miltefosine.
- Currently, no vaccine is available against HVL.

CanL Geographical distribution

Different factors such as climate warming, changes to socio-economic conditions, and population movements may play a role in the evolution of the distribution of the CanL.

CanL is endemic in many countries of the world. In the Old World CanL is endemic in Southern Europe, Africa, The Middle East (Turkey, the Persian Gulf), and Asia. In the New World the disease has been present for a long time in Latin America. In 2000, cases were reported in North America[30, 31].
In France, three successive surveys (1986, 2004, and 2011) highlight a progressive spread of the disease from the Mediterranean to the North and the South West, as well as an increase in cases (autochthonous or imported) in non-endemic areas[32]. At the European level, the disease is also spreading in Portugal, Spain, Italy (ascent of the extension northward), the Balkans, and Greece[33]. The number of clinical cases reported in the United Kingdom, Germany, Netherlands, and Denmark due to importation or travelling continues to grow.

KEY POINTS **CanL Distribution**

- CanL is endemic in more than 70 countries, in Southern Europe, Africa, Asia and Latin America.
- In Europe, CanL is currently spreading from the south to the north.
- There is an increase of new foci and cases (autochthonous and imported) in non-endemic areas (Europe and North America).

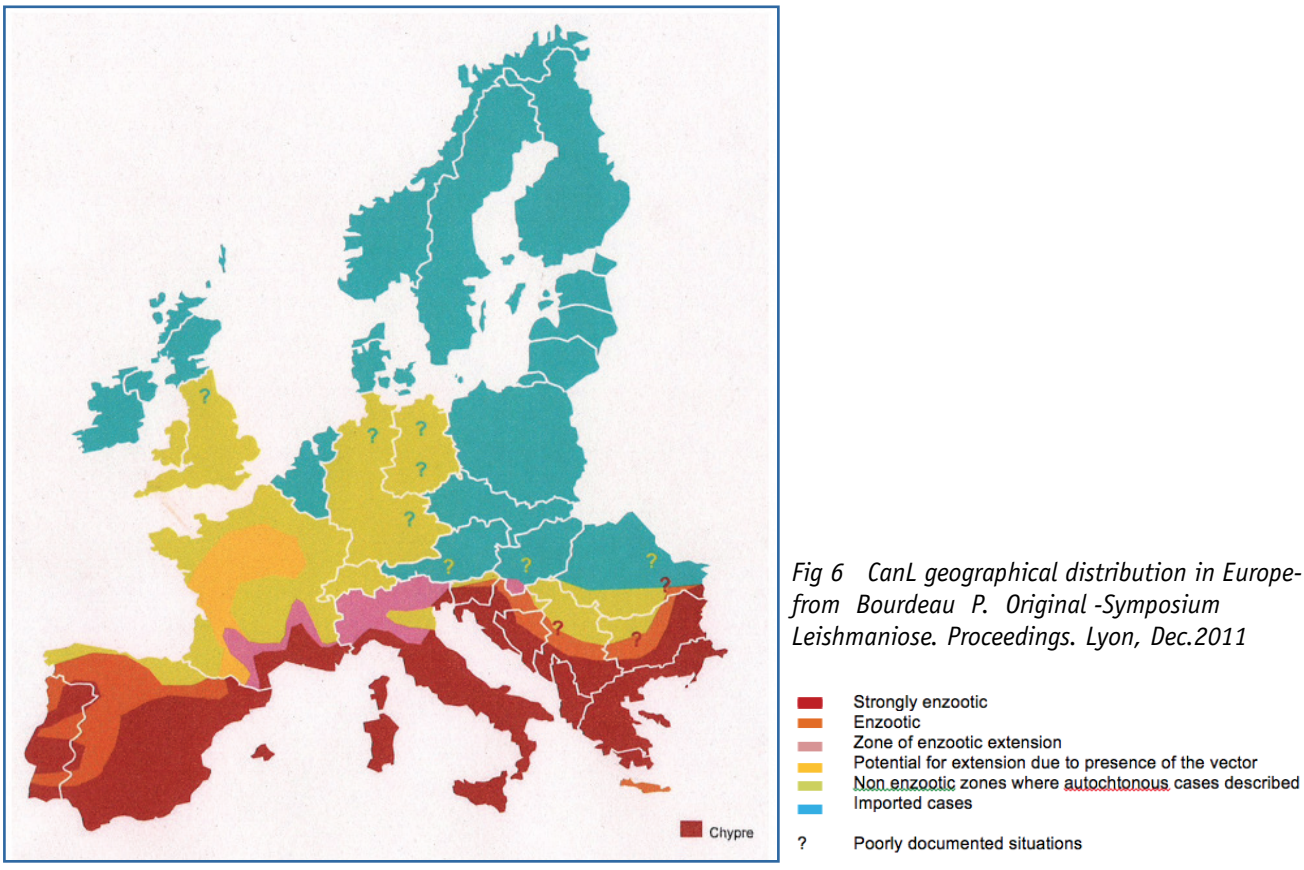

Fig 6 CanL geographical distribution in Europe- from Bourdeau P. Original -Symposium Leishmaniose. Proceedings. Lyon, Dec.2011

CanL Clinical manifestations

Clinical diagnosis of CanL is often difficult because of the variable clinical signs and the long incubation period (from 3 months to 6 years). Clinical signs can appear both in isolation or combination. The most frequently observed signs are: cutaneous lesions (squamosis and alopecia) lymphadenopathy, and an excessive fatigue. In an advanced stage of the disease dogs commonly present emaciated, depressed, with cutaneous scaling, alopecia on ear tips, and lymph node hypertrophy. The most common manifestations (from personal data) are listed in table 1. The others are listed in table 2.

Fig 7 Prevalence of clinical manifestations of CanL.

Table 1: CanL Clinical manifestations with a prevalence over 50% in symptomatic dogs	
Cutaneous lesions:	Non pruritic dermatitis Squamosis, Alopecia, Ulcers
Generalized lymphadenopathy	
Excessive fatigue and/or weight loss	
Polyuria, Polydypsia (signs of renal disease)	

Table 2: CanL Clinical Manifestations with a prevalence under 50% in symptomatic dogs	
Cutaneous lesions:	Hyperkeratosis Depigmentation (nose ,lips and gums) Nodules Inoculation chancres (transient)
Mucosal lesions:	Ulcers Nodules (on tongue, genital mucosa)
Ocular signs:	Keratoconjunctivitis Anterior uveitis Granulomatous blepharitis Granulomatous conjunctivitis
Epistaxis	
Fever	
Onychogryphosis (abnormal claws overgrowth)	
Muscular atrophy (temporal and masticatory muscles)	
Hemorrhagic enteritis	
Lameness (polyarthritis, synovitis, osteomyelitis, phalyngeal osteitis)	
Neurological disorders	

For more details please refer to. Eur J Comp Anim Pract. 2005;15(1): 39-52.[1]

Of all the forms of CanL, chronic renal failure and haemorrhagic syndromes are the most severe. Renal failure is the main cause of death in dogs suffering from leishmaniasis[1, 4, 34, 35].

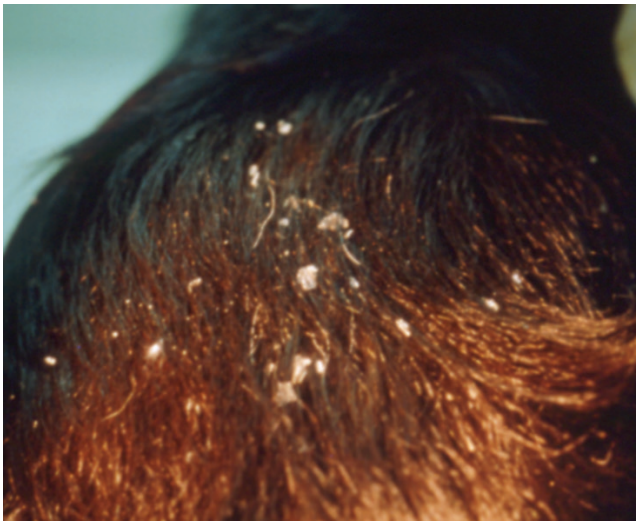

Fig 8 Cutaneous signs of CanL: large typical silvery scales observed on the back of the dog.

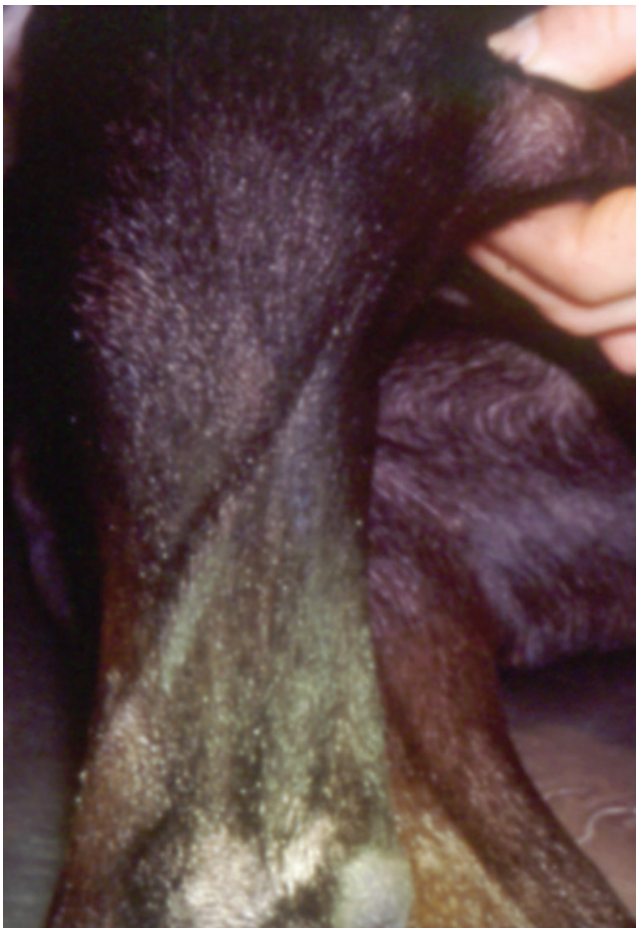

Fig 9 CanL lymphadenopathy. Significant enlargement of the popliteal lymph node. Alopecia and scaling are also observed on the limb.

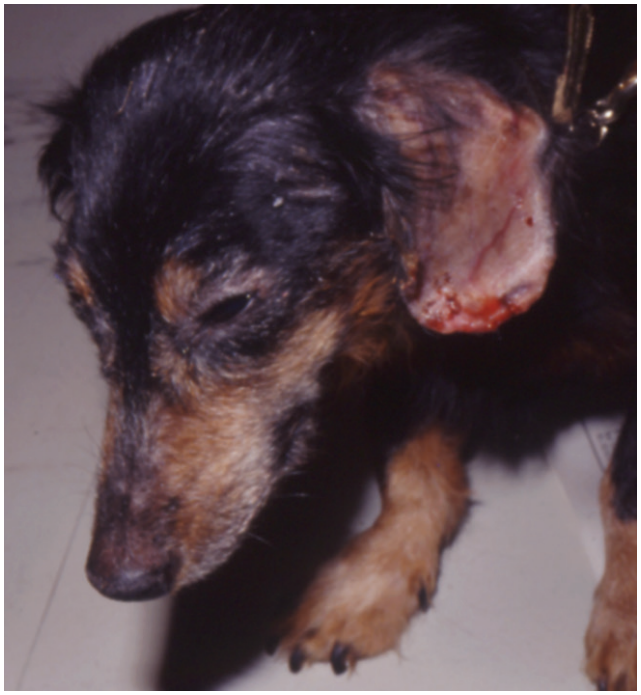

Fig 10 View of the head of a severely affected dog. Alopecia, scaling and bleeding ulcers of the pinnae are observed on this dog.

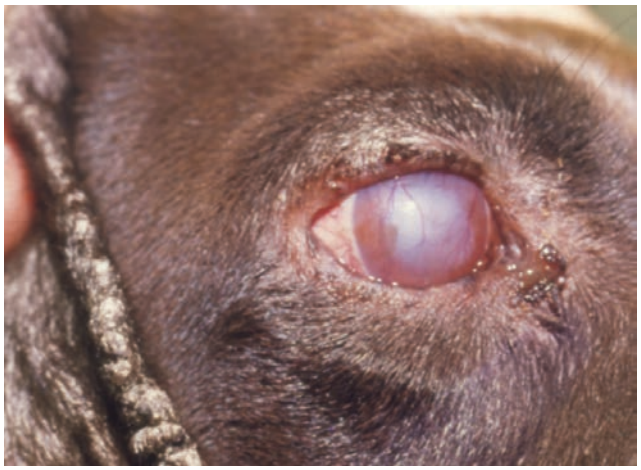

Fig 11 Ocular signs associated with cutaneous signs of CanL. A keratitis marked by an important corneal neovascularistaion, alopecia of the lids and the ears and also hyperkeratosis of the margin of the pinnae are observed.

KEY POINTS **CanL Clinical Manifestations**

- The incubation period may be long (from 3 months to 6 years)
- Clinical manifestations of CanL are very variable.
- Most frequent signs are squamosis, alopecia, lymphadenopathy and excessive fatigue.
- Renal failure is the main cause of death from CanL.
- Many infected dogs are asymptomatic.

Co-existing diseases

Co-existing diseases are common and may complicate the diagnosis for practitioners. The number of co-existing diseases recognised is constantly increasing.
Parasitic diseases (ehrlichiosis, hepatozoonosis, neosporosis, demodicosis, babesiosis, anaplasmosis, and dirofilariosis), immune diseases (pemphigus foliaceus, disseminated lupus erythematosus) and hypothyroidism are well documented co-existing diseases[4, 36]. The large number of co-existing diseases could be due to the depressed immune status of the animal, or for vector-borne diseases deficient anti-vector measures.

CanL Diagnosis

The diagnosis is based on both non-specific and specific methods

Non-specific diagnosis

History

Taking a good history is a very important step in determining the aetiology of the disease. The practitioner must ask very precise questions about the dog's lifestyle and possible movements to and from an endemic area. Additionally, the clinician needs a good knowledge of the geographical distribution of CanL.

Clinical and differential diagnosis

As many infected dogs are asymptomatic, basing the diagnosis solely on clinical signs is not a reliable method for assessing infection[7, 37].
In symptomatic dogs making the diagnosis on clinical signs is often difficult for three reasons[4]:

a) Lesions due to CanL often mimic lesions due to other diseases.
b) Co-existing diseases are frequent.
c) Atypical signs can give a misleading impression.

This is why the list of differential diagnoses is very long. CanL cutaneous lesions look similar to demodicosis, dermatophytosis, pyoderma, ulcerative or nodular dermatitis, and more specifically to immune-mediated dermatitis. In the case of epistaxis, ehrlichiosis, nasal neoplasia and aspergillosis are differential diagnoses. CanL lymphadenopathy may be confused with a lymphoproliferative disease. Many other diseases (polyarthritis and all causes of glomerulonephritis) should be considered as differentials.

Laboratory findings

Non-regenerative anaemia, thrombocytopenia, lymphopenia, hyperproteinaemia with hypergammaglobulinaemia, proteinuria, and hypercreatininaemia can be suggestive of the disease. The laboratory check up is a useful tool for determining the prognosis and follow up of the patient.

Specific diagnosis

Cytology/Histopathology

Observation of leishmania amastigotes in smears from skin lesions, impressions from nodules, lymph nodes or bone marrow is a quick way to confirm the diagnosis. The specificity is excellent but the sensitivity is low[1]. Immunocytochemical techniques are more sensitive. However, these techniques require expertise and they do not give information about the immune status of the dog.

For details of how to collect samples and how to prepare smears please refer to Eur J Comp Anim Pract. 2005;15 (1): 39-52.[1]

Serology

The Indirect immunofluorescent test (IFAT), enzyme like immunosorbent assay (ELISA) and Western blot test determine antibody levels. A positive result is proof of a reaction against an antigen but not proof of the disease. Western blot offers the best sensitivity but needs to be performed in a specialised laboratory. IFAT is currently the more widely used test. High antibody levels are suggestive of a clinical disease; a low titre is suspicious of an infection and requires further evaluation.
Rapid "in clinic" tests:
Different serological tests are currently available for use by the practitioner. They are interesting as they are readily available in the clinic at a low cost. They are very helpful when used in endemic areas to support a clinical suspicion. They give a positive or negative result without a quantitative value. It is often useful to follow up a positive result with quantitative serological laboratory tests[4].

Polymerase chain reaction (PCR)

This biomolecular technique allows detection of leishmanial DNA. It is highly sensitive (kDNA) and specific. It can be applied to any type of sample but solid samples (skin, bone marrow, lymph node, spleen, or conjunctiva) are preferred. A positive result provides proof of the presence of parasitic DNA, but not the proof of a living parasite or the disease. Real-time PCR allows parasitic load quantification and can be used as a tool to follow response to therapy. It is a more sensitive test than conventional PCR. PCR is also very useful in detecting the infection in asymptomatic animals. The practitioner must be aware of possible false positive results in cases of contamination. Information provided by PCR must be viewed in conjunction with information provided by clinical and serological assessments[38].

KEY POINTS **CanL Diagnosis**

- The 1st step in diagnosis is based on history, clinical examination, and quantitative serology.
- The 2nd step in diagnosis includes cytology and/or PCR.

>>> LeishVet is a study group which aims to establish a consensus and formulate recommendations in the management of CanL.
This group proposes useful guidelines for the practical management of CanL[4].
www.parasitesandvectors.com. LeishVet Guidelines

Treatment

Though significant progress has been made in diagnosis and prevention of CanL, to date, no therapeutic protocol is capable of eliminating the infection. The main advance is the use of a polytherapy based on combined administration of antimonial and allopurinol as first line drugs. A clinical cure is nearly always the only achievable result. Discontinuing treatment means, in the majority of patients, relapses occurring within months with a further risk to Public Health[4, 39].

Pentavalent antimonials

N-methylglucamine (Glucantime®) has been and continues to be a widely used treatment. It is the only drug from this group available in Europe. In North America sodium stibogluconate (Pentostam®) is used. It is advised to administer Glucantime® at a dose of 100mg/kg/day, by subcutaneous injections for at least one month of treatment. It has been demonstrated that 80% of the drug is eliminated by the kidney nine hours after administration. Re-occurrence of clinical signs or a rise in antibody titres, often some months after the initial treatment, means the treatment should be repeated as relapses during the first year following treatment are common.

Advantages/Disadvantages

If this treatment is applied as a long term monotherapy it can become expensive for the owner and daily injections can be painful for the dog. Intramuscular injections are not used as there is a risk of pain, fibrosis and abscess formation. The side effects of treatment are usually benign (muscle and joint pain, hyperthermia, and shivering). Severe side effects are uncommon (nephritis, hepatitis, neuritis, and haematological disorders). Nevertheless, if seen the medication should be stopped, and the dose should be reduced in cases of renal insufficiency. The main danger of excessive use of this drug by veterinarians is that it could induce a resistant Leishmania strain[1].

Allopurinol

Allopurinol (Zyloric® or Allopurinol-GNR) is used in human and veterinary medicine to prevent formation of urate calculi. Its use as an antileishmanial drug is more recent than the use of antimonials. Allopurinol is an oral purine analogue which disrupts the synthesis of proteins by leishmania. The daily dose is 20-30mg/kg for an indefinite period of time.

Advantages/Disadvantages

Long term treatment with allopurinol is not expensive. Oral administration makes it easy for the owner to administer. It is a relatively non-toxic drug. The only reported side effect is the occurrence of xanthine uroliths. As the product is not used for treatment of leishmaniasis in human beings, there is no public health risk of inducing parasitic resistance. For these reasons, more and more veterinarians are using allopurinol[40].

Meglumine antimoniate/allopurinol combination

Combination therapy does not increase the chance of eliminating the parasite over monotherapy alone, but dogs treated with polytherapy have longer periods of clinical remission than those treated with monotherapy. The protocol generally applied consists of a subcutaneous injection of Glucantime ® at a dose of 100 mg/kg/day for one to two months with oral administration of allopurinol at a dose of 20 mg/kg/day for several months. The side effects are the same as those of each of the drugs individually[41, 42].

Aminosidine (or paromomycine)

Aminosidine (Gabbriomycin in Italy) is an aminoglycoside antibiotic. It is effective against a wide range of microorganisms. It inhibits protein and mitochondrial synthesis. Administration is by subcutaneous injections at a dose of 5-10 mg/kg twice daily in the dog. Combination therapy: paromomycine (subcutaneous injection -5mg/kg/day) and meglumine antimonial (intramuscular injection-60 mg/kg/twice daily) for one month is more effective than paromomycine alone. Due to its adverse effects (nephro and ototoxicity) the product is contraindicated in cases of renal insufficiency.

Amphotericin B

Amphotericin B is reported to be more effective as an antileishmanial drug than antimonial compounds. The dose is 0.5 to 0.8 mg/kg by rapid IV injections twice weekly. As the product is highly nephrotoxic, it is administered to humans only in hospitals with periodic evaluation of blood creatinine levels. To avoid toxicity, a liposomal formulation (Ambisome®) can be used. This drug is to be avoided in veterinary practice for two reasons. One is the high cost of the product; the other is the danger of inducing drug resistance. Amphotericin B is currently the first line drug used in many countries to treat human leishmaniasis.

Miltefosine

Miltefosine, an alkyl phosphocholine, was developed as an anti-tumor agent. It acts by interfering with the metabolism of lipids in the membrane of the parasites. It is directly toxic to leishmania, and stimulates the host's defenses. Oral miltefosine has been used successfully in India to treat human visceral leishmaniasis. In dogs, the medication is dosed orally at 2 mg/kg (Milteforan®). When used in conjunction with 20 mg/kg/day of allopurinol it is at least as effective as the antimonial/allopurinol combination. The side effects are minor. This medication is a good alternative to the use of the antimonials[39, 43].

Pentamidine (Lomidine®)

This medication is effective in several protozoon diseases. It has been used in humans if the first-line treatment has failed. It has been postulated that the product would induce a good immune cellular response in the dog. Intramuscular injections at a dose of 2 then 4mg/kg on alternate days with a total of fifteen injections are administered. The product has local, renal, pancreatic and cardiac toxicity. The pain and muscular necrosis at the injection site make it an unsatisfactory medication for use in dog.

Spiramycin-Metronidazole combination (Stomorgyl®)

According to a study comparing the results obtained, the association Spiramycin-metronidazole is as effective as the meglumine-antimoniate -allopurinol combinations. The drug is administrated orally to dogs at a dose of 25mg/kg/daily[44].

Quinolones

Quinolones are bactericides and secondarily leishmanicides. They act by blocking DNA replication and transcription. They have minimal toxicity. Two medicaments are proposed as treatment for CanL.

Marbofloxacin (Marbocyl®)

Marbofloxacin administrated orally at a dose of 2 mg/kg/day for 28 days. The absence of toxicity and results obtained would consider this product as a valuable alternative to antileishmanial treatment.

Enrofloxacin (Baytril®)

Enrofloxacin given to dogs at an oral dose of 10 mg/kg led to a rapid clinical improvement but frequent relapses were observed 2 months after the initiation of treatment. In cats it is imperative not to exceed the prescribed dose.

Azoles derivatives

Azoles derivatives are antifungal medications. Ketoconazole® has been administrated orally in dogs at a dose of 10mg/kg for two months. Results are reported as disappointing.

Non-specific immunotherapy

The use of corticosteroid therapy for a parasitic disease is very controversial. It is proposed to be of value in cases where there is a suspected problem with immune complex deposition, for example in acute or sub-acute renal insufficiency or in polyarthritis. Immunosuppressive doses (1mg/kg) of prednisone or prednisolone are used. In nephropathy, corticosteroids are used in combination with allopurinol or with low doses of antimonials, until blood creatinine and urea return to normal[1].

Specific immunotherapy

The goal is to induce a switch from a non-protective immune response (Th2 immunity) to a protective one (Th1 immunity).

Interferon:

Cytokines such as Interferon gamma (IFN-γ) and Interleukins (IL-12, IL-18) have been proposed as potential treatments. Interesting results have been reported with the use of antimony derivatives combined with IFN-γ or an antigenic fraction derived from *L. infantum*. The Interferon dose is 10 iu/day for one or two weeks. Treatment is expensive.

Domperidone:

This molecule is used in humans as an intestinal tract stimulant (Motilium®). It also stimulates lactation. The effects of domperidone, a Dopamine D2 receptor antagonist, have been evaluated in dogs infected by *L. infantum*. The product is administered orally (1mg/kg twice a day for 1 month) and has been proved to be effective in controlling and reducing clinical signs and antibody titers and also increasing the cell mediated immune response. This way of activating the cell mediated immunity means domperidone (Leisguard®) could be considered as a potential preventative medicine in healthy dogs against CanL[39, 45].

Ocular Manifestations

These manifestations deserve a special mention. There is no correlation between the antibody titre and the severity of clinical signs. Only the techniques with high sensitivity (PCR, Western blot test) are reliable for the diagnosis of ocular leishmaniasis. Specific treatments for the disease are not effective against intraocular inflammation (Uveitis). It is essential to control the inflammation quickly with symptomatic treatments (locally with mydriatic eye drops and/or methylprednisolone sub-conjunctival injections, and systemically with several months of anti-inflammatory treatment)[46, 47, 48].

KEY POINTS **Treatment**

- Therapy is long. It leads to a clinical cure but not to elimination of the parasite.
- First line therapy is: an injection of meglumine antimoniate (100 mg/kg/day -1 or 2 months)/ and oral allopurinol (20mg/kg/day-several months).
- A rigorous follow up is needed.

Diagnosis and treatment

After the history collection and clinical examination, the use of quantitative serological tests is the first step in diagnosis.

- Symptomatic animals with a high antibody levels require treatment (antimonial meglumine + allopurinol combination).
- Asymptomatic animals or symptomatic animals with mild clinical signs with low antibody levels require further investigations (cytology or PCR). If the cytology or PCR is positive, treatment should be initiated (monotherapy: allopurinol).
- Allopurinol therapy maintenance is suggested for preventing relapses. An abnormal renal profile gives a guarded or poor prognosis.

Follow up

- 1 month after treatment: examination, laboratory checks (biochemical profile, CBC, urinalysis).
- Every 6 months: Serology or Real- time PCR.

Prevention advice

- Tell the owner to keep dogs indoors from dusk to dawn during the season of phlebotomine activity.
- Use a combination of the application of a long acting topical insecticide and vaccination.

Prevention/Control

There are theoretically three ways to control CanL: control of vectors, prevention of sand flies bites, and control of the canine reservoir.

Control of vectors

Measures may relate to the reduction of the microhabitat favorable to sand flies near houses or places where dogs live (e.g. avoid stagnant water); insecticide spraying can be used to target the environment. Mass spraying is effective but impossible to achieve in urban or suburban areas. On the other hand, use of indoor insecticide treatment is possible. The pyrethroids are slightly toxic to humans and are very toxic to cats and fish.

Prevention of sand flies bites

The simplest and easiest measures are to avoid trimming a dog's fur and keeping the dog indoors from dusk to dawn during the season of phlebotomine activity (April to November in the Mediterranean Basin). Additionally, all dogs living in or going into an endemic area should be treated with an effective insecticide. Pyrethroids are the most widely used product.

Collars:

Several experimental and in the field studies have shown that deltamethrin impregnated collars (Scalibor®) can significantly reduce sand fly bites and the transmission of *L. infantum*. The collars have to be replaced every 5 months and applied 2 weeks before travelling[49].

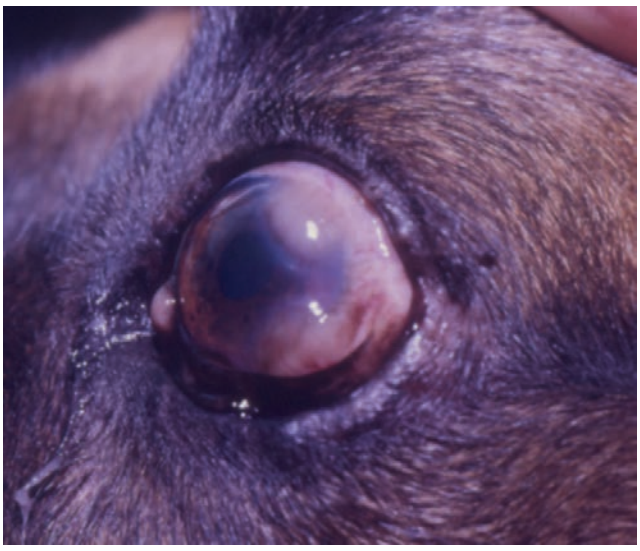

Fig 12 CanL ocular lesions. Two large limbal granulomas are invading the cornea. Another granuloma is seen on the third eyelid free margin. These granulomas are full of parasites.

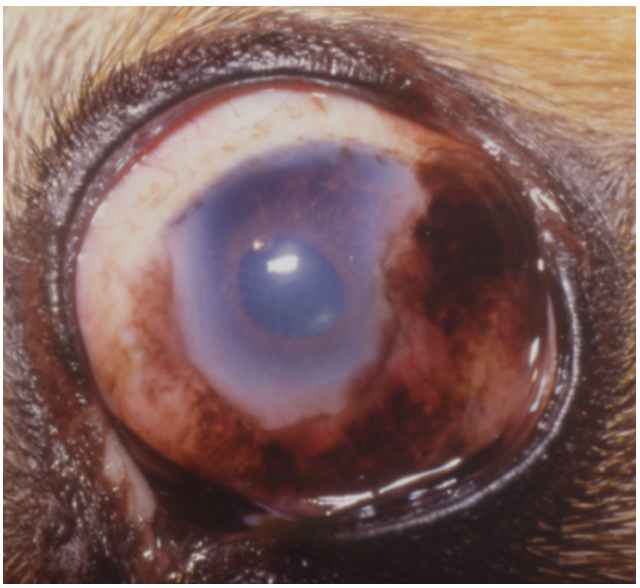

Fig 13 CanL ocular lesions. A dense corneal pigmentation and a whitish corneal infiltrates secondary to a severe keratitis are observed

Spot on:
A combination of permethrin and imidacloprid (Advantix®) provides shorter lasting but efficient protection. It needs to be administered every 3 weeks[50].
Sprays:
A combination of pyriproxyfen and permethrin (Duowin®). One of the advantages of this formulation is that it ensures good protection of the extremities (muzzle). It must be repeated every 3 weeks[51].

Reservoir control

Dogs are the main reservoir of *L. infantum*.
Culling programs
Elimination of symptomatic dogs in endemic areas by culling is not a viable option. This is because of the high rate of asymptomatic infected animals and the fact that this type of measure would be considered ethically unacceptable in many countries as dogs are considered family members.
Chemotherapy
Obviously, early detection and early treatment of infected dogs decreases the transmission of *L. infantum*. In endemic areas, an area-wide and systemic prophylactic therapy before the activity season of phlebotomine sand flies has been proposed. It is difficult to persuade owners of clinically healthy dogs to accept a measure of this type.
Vaccination
For many years, much to the pet owner's disbelief, European veterinarians practicing in endemic areas have been unable to provide an effective vaccine against a so serious and widespread disease as leishmaniasis[1].
From March 2011, a vaccine (CaniLeish®) finally got the approval from the European Medicines Agency (EMA) for Europe. In the New World, two other vaccines are approved for the Brazil. The CaniLeish® vaccine, launched by Virbac laboratories in the countries of Southern Europe, is currently available throughout Europe. It will soon be marketed in North Africa, South America, and then in India.
Unlike most existing vaccines that stimulate humoral immunity, the CaniLeish® vaccine stimulates the cellular immunity responsible for achieving efficient protection against leishmaniasis. A group of proteins, the excreted-secreted proteins (ESP), are used as they are the best stimulators of cellular immunity directed against *L. infantum*[52]. Tests on dogs, in extreme conditions in endemic areas show that the CaniLeish® vaccine reduced the risk of animals developing clinical disease by 4 times. It is recommended that serological screening is performed before vaccination. If the test is negative, the vaccination protocol can start. Primary immunisation consists of 3 subcutaneous injections three weeks apart, from the age of 6 months. Annual re-vaccination is required. Mild transient local (swelling and pain) or general (hyperthermia, listlessness, digestive disorders) reactions were observed with the vaccination. This vaccine is an important step in preventative medicine[53]. The combination of the application of a long acting topical insecticide and vaccination should provide a good protection[4].

KEY POINTS **Prevention/Control**

- Advise the owners to keep the dog indoors during activity periods of sand flies (from dusk to dawn during the warm season in temperate areas)
- Use of pyrethroid insecticides in spot-on applications and collars
- Vaccination. A vaccine (CaniLeish®) is currently available in Europe

Management of CanL infection by the Practitioner

Leishmaniasis due to *L .infantum* is a disease of increasing concern to the population. The veterinarian has an important role to play not only as a therapist but also as informant. Dogs living or having stayed in endemic areas are, of course, brought to the veterinarian because they are sick. However in these areas it must be remembered that symptomatic infected animals, asymptomatic infected animals, and healthy animals coexist[1, 4, 39].

Conclusion

CanL remains a severe disease, the management of which is difficult for the owner and the veterinarian. Although medical treatment is still unable to eliminate the infection, there is currently a consensus on therapeutic protocols. Progress in the development of diagnostic tools available allows more rational management of infected animals. Finally the development of a vaccine has been an important step in the prevention of the disease.

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COMMISSIONED PAPER (I)

An update on feline Leishmaniosis in Europe

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SUMMARY

Zoonotic visceral/cutaneous leishmaniosis is caused in Europe by *L. infantum*. Historically the cat has been considered to be an unusual host for this parasite. However, in recent years serological and molecular investigations have demonstrated that in endemic areas, due to the high prevalence of *Leishmania* infection the disease may have some relevance in cats. Infected cats are a recognised source of infection for sand flies; this has raised the question of the cat's epidemiological role in endemic areas.

In the last 25 years sporadic cases of natural disease have been reported in cats. There are no controlled clinical trials on feline leishmaniosis and the clinical features of the disease in cats can be only inferred from case reports. A review of forty-one published cases of naturally occurring disease in Europe suggests that feline leishmaniosis caused by *L. infantum* is a systemic disease affecting adult cats. Impaired immune competence is a suspected risk factor for disease development. Lymph node enlargement is the most common clinical sign; skin lesions are evident in half the cases. Diagnosis is confirmed by serology. Antibody levels may be low in some affected cats - in these cases parasitological or molecular techniques should be used to confirm the diagnosis. Empirical use of allopurinol or meglumine antimoniate is usually the treatment of choice. Treatment induces clinical recovery but does not eliminate the infection.

KEY WORDS: feline leishmaniosis, cat, *Leishmania infantum*

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Introduction

The cat is considered an unusual host for *Leishmania* spp. infection as only sporadic cases are reported from countries where the organism and different species of sand fly vectors are endemic[1]. In Europe leishmaniosis is caused by *L. infantum*. This is the worldwide agent of zoonotic visceral or cutaneous leishmaniosis and causes a potentially fatal disease in humans and dogs. The only *Leishmania* species that has been isolated from cats in Europe is *Leishmania infantum*[2-6]. *L. infantum* infection has also been reported in cats in Iran[7] and in Brazil[8-9]. By the end of the 1990s many studies had been published that reported the use of serological and/or biomolecular

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Editor's note: Leishmaniosis or Leishmaniasis? Readers may be a little confused by the use of the words Leishmaniosis and Leishmaniasis in the two papers in this issue of EJCAP. The two spellings appear in both papers in the text and reference list. Each refer to exactly the same condition. Leishmaniasis was the original term universally used. It is the official term used by WHO and in the OIE zoonotic diseases list and is the term still used universally for the human disease. On the other hand readers will find that nowadays Leishmaniosis is the term almost always used for the animal disease in veterinary journals and text books.

techniques which confirmed that feline infection may be frequent in endemic areas. Additionally, new case reports were published; now detailed information is available on the clinical features, the diagnosis, and treatment of the disease.

Historical reports of *Leishmania infantum* infection in cats in the old world

Leishmania infection was first detected in cats in Algeria in 1912 when amastigotes were found in the bone marrow of a kitten living in the same house as a child who was diagnosed with visceral leishmaniosis[10]. In 1931 Machattie et al. described two cats in Iraq with ulcerative dermatitis and poor body condition. *Leishmania* infection was confirmed by both cytology and isolation[11]. In 1932 leishmaniosis was described in a cat in Spain[12]. In Algeria in 1948 amastigotes were found in an emaciated cat with lymphadenopathy and ulcerative lesions on the lips and the ears[13]. In Tampon (Isle of Reunion) in 1976 a cytological diagnosis of leishmaniosis was obtained from lymph nodes in a cat with chronic pyrexia, lymph node enlargement, anaemia and leucopenia; for the first time the disease was treated in a cat[14]. Following twelve intramuscular injections of Lomidine the authors reported a clinical cure (dose not reported). In Switzerland in 1977 leishmaniosis was considered the causative agent of an exfoliative dermatitis affecting a cat which had travelled in Spain[15]. Following the 1980s clinical cases of leishmaniosis in the cat have been more accurately diagnosed. Additionally, at this time more epidemiological studies were undertaken. The first study was performed on a feline population in Sicily in 1933 where a physician investigated the spleen, liver, and bone marrow of 120 necropsied cats living in the city of Catania[16]. He performed the study in areas where human visceral leishmaniosis was common but cases of feline infection were not confirmed[16].

The disease in cats

Detailed studies on the pathogenesis of feline leishmaniosis are lacking at present. One experimental infection with two different strains (one obtained from a human patient in Brazil and the other from a dog in France) of *L. chagasi/infantum* was carried out in the 1980s and involved the intravenous or subcutaneous inoculation of 21 cats. They were followed for 8-24 weeks[17]. According to physical examinations, cell blood counts, and serum protein evaluations performed during the study none of these cats developed clinical signs of the disease. However, antibody titres significantly rose from week two. The parasite was found on direct visualisation of impression smears taken from the liver, spleen, bone marrow, and to a lesser extent in the blood of cats intravenously inoculated with the parasite. Gross or microscopic lesions were never found at necropsy. These data supported the hypothesis that cats are less susceptible than dogs or hamsters to *Leishmania* infection but this was not verified by performing additional studies with more sensitive methods for demonstrating the persistence of the infection (isolation and PCR), or monitoring (more appropriately) for longer follow up periods. In fact leishmaniosis is usually a chronic disease and even with dogs the incubation period may last many months to years. Most dogs develop a subclinical infection and may go on to develop overt disease at a future point due to the imbalance between the host protective immune response and the pathogen. This balance can be broken by immune suppression

Table 1: Case reports of feline leishmaniosis in Europe in the years 1989-2012.

COUNTRY	N° of cats	Reference
FRANCE	1	Dunan et al., 1989
PORTUGAL	1	Costa Durao et al., 1994
FRANCE	1	Laurelle-Magalon & Toga, 1996
FRANCE	1	Ozon et al., 1998
ITALY	24	Pennisi et al., 1999; Pennisi et al., 2004; Monteverde et al., 2006; Maroli et al., 2007; Caracappa et al., 2008; Ennas et al., 2012; Pennisi et al., 2013
SPAIN	2	Hervas et al., 1999
SPAIN	1	Hervas et al., 2001
ITALY	1	Poli et al., 2002
FRANCE	1	Grevot et al., 2005
ITALY	1	Britti et al., 2005
SPAIN	1	Leiva et al., 2005
SWITZERLAND*	2	Rüfenacht et al., 2005
FRANCE	1	Verneuil, 2006
PORTUGAL	1	Marcos et al., 2009
ITALY	1	Ibba F., 2009
FRANCE	1	Pocholle et al., 2012

*: cats travelled in Spain

or concomitant diseases[18]. Unlike the large number of studies which exist describing the immunology of leishmaniosis in humans, dogs, and other animals, the pattern of the immune response to *Leishmania* infection in cats has never been studied.

The only information that can be found on feline leishmaniosis caused by *L. infantum* in Europe is in a single case report or case series published about 41 cats where a diagnosis had been confirmed by serological, parasitological and/or biomolecular methods (table 1). Thirty-eight of these cats were domestic shorthaired cats, one was a persian, one a crossed Siamese, and in one case the breed was not reported; twenty-three were females and eighteen males, they were aged between two and fifteen years (mean age 8.1 years). Thirty-six were tested for retroviral infections and sixteen cats were positive for antibodies against Feline Immunodeficiency Virus (FIV). Amongst these FIV and *Leishmania* positive cats three were also positive for Feline Leukemia Virus (FeLV) and three were concurrently affected with squamous cell carcinoma (fig.1).

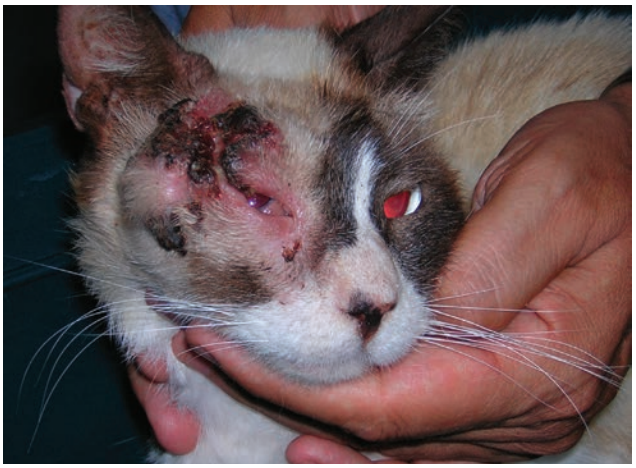

Figure 1: FIV infected cat concurrently affected by squamous cell carcinoma and leishmaniosis (Leishmania amastigotes were found in the site of neoplastic tissue)

Three cats had been treated with immune suppressive drugs and two were affected by diabetes mellitus. This means that more than half of the cats developing the disease could have had impaired immune competence. In humans, co-infection with *Leishmania* and Human Immunodeficiency Virus is a well-known occurrence[37]. A common clinical finding on physical examination at diagnosis was the presence of skin lesions which were evident in about half of cases (21/41); in very few cases (4/41) skin lesions were the only observed sign. Ulcerative dermatitis was the most frequent skin lesion seen (15/41): one or more ulcers mainly on the head,

Figure 2: Small ulcer associated with L. infantum on the face of a FIV positive cat

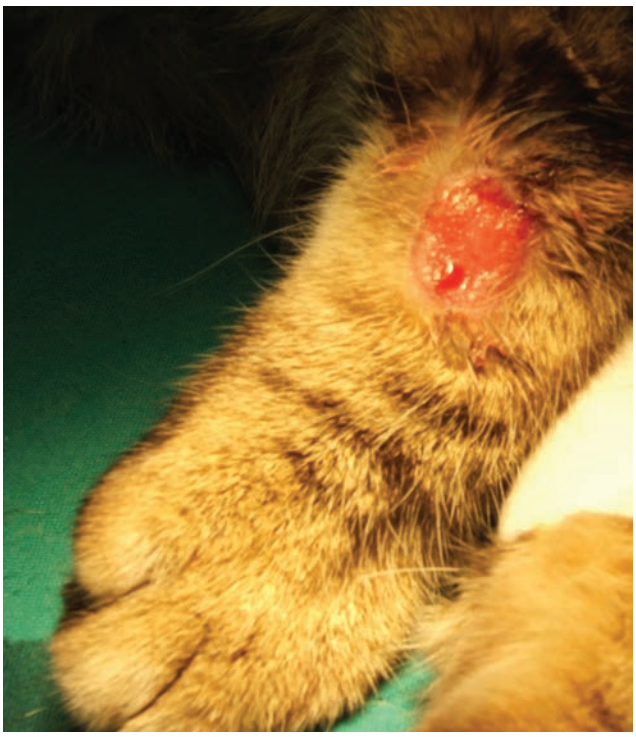

Figure 3: Ulcerative dermatitis associated with Leishmania infection in a cat: large ulcers developed symmetrically on both dorsal carpi

face, ears, eyelids, or symmetrically on carpal or tarsal regions, or the ischial tuberosity were reported (figs. 2-3). Less commonly crusty-ulcerative dermatitis (6 cats) or nodular dermatitis distributed on the face, neck or trunk (4 cats) was observed. Alopecia was also described (6 cats) which was usually associated with dermatitis. In three cases rare haemorrhagic blisters or haemorrhagic nodules were reported (fig 4). Other less common findings were seborrhoea, miliary dermatitis and telogen *effluvium*; each one was reported in one case. More than one type of skin lesion may occur in the same cat (e.g. ulcers and nodules). In all cases, the head was the most involved body region. Itching was reported in 8 cats. *Leishmania amastigotes* were found by cytological or

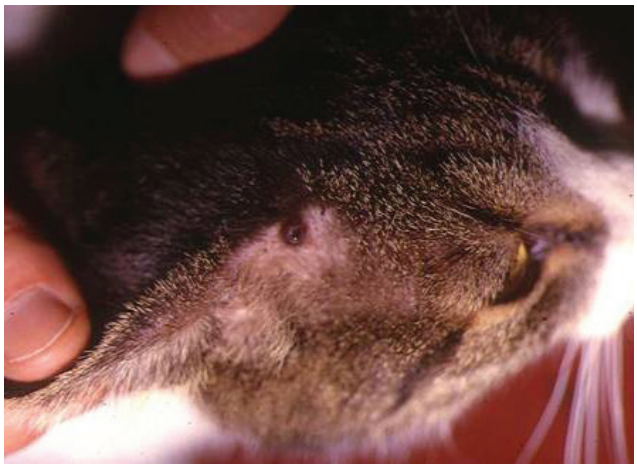

Figure 4: Haemorrhagic nodule (hair clipped)

histological examination of ulcers, nodules, or blisters. Solitary or systemic lymph node enlargement was the most common sign observed (22/41). Cytological evaluation of lymph node smears showed lymphoid hyperplasia with the occasional occurrence of *Leishmania* amastigotes (fig.5). Weight loss (13 cases), ocular lesions (12 cases), reduced appetite (11 cases), dehydration (8 cases), and lethargy (8 cases) were also observed. Uveitis (fig. 6) was the most commonly reported ocular lesion. In some cases the uveitis evolved to panopthalmitis that required ocular enucleation[29, 32, 34]. Amastigotes were found in conjunctival nodules, the aqueous humour, or at histology after enucleation of the ocular bulb. Chronic gingivostomatitis affected about one fourth of the cats (10/41) and a few infected macrophages were found in oral biopsies[38].

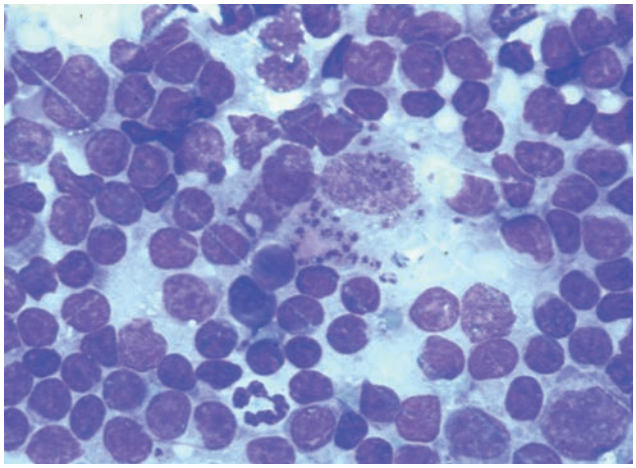

Figure 5: Leishmania amastigotes in a lymph node smear (May Grünwald – Giemsa stain, 63x)

Figure 6: Acute uveitis in a FIV positive cat concurrently infected by L. infantum

A list of other sporadic clinical signs reported in 13 cases included: pale mucous membranes, hepatomegaly, jaundice, cachexia, fever, vomiting, diarrhoea, chronic nasal discharge, splenomegaly, polyuria/polydipsia, dyspnoea, wheezing, and abortion. *Leishmania* amastigotes were isolated from many tissues and organs of some of these cats suggesting a causative role of the parasite in the reported signs (see below).

The most common histological lesion observed on biopsy or at necropsy was diffuse granulomatous inflammation with infected macrophages, CD3+ lymphocytes and plasma cells; reported in the skin, eye, liver, spleen, and kidney[38-40].

Clinical pathological abnormalities in CBC, biochemistry, and urinalysis were reported in some of these cats. CBC data were reported in 34 cases, 8 cases had results within in the reference range. Normocytic normochromic anaemia was found in ten cases; monocytosis in six cases, neutrophilia in five cases, pancytopenia in four cases and lymphopenia in four cases. A biochemical profile was available in 34 cases and the most common abnormalities were increased BUN (6 cats), increased creatinine (5 cats), increased phosphate (3 cats), and increased glucose (2 cats). Serum protein electrophoresis was performed in 26 cases and half of them had an increased level of gamma-globulins, sometimes with a monoclonal pattern (fig.7). Urinalysis was only available in 11 cats. Abnormalities found included proteinuria (4 cats), isosthenuria (3 cats), and glucosuria (2 cats). It must be remembered that FIV infection or other concurrent diseases may contribute to the hematological abnormalities and other clinical findings found. Pancytopenia was found in three FIV positive cats but also in a FIV negative cat[35].

Diagnosis was usually confirmed with more than one technique. The most commonly used diagnostic technique was serology which confirmed *Leishmania* infection in 34 samples: 29 were tested by indirect immunofluorescence antibody test (IFAT), 4 by enzyme-linked immunosorbent assay (ELISA) and western blot (WB), and one by direct

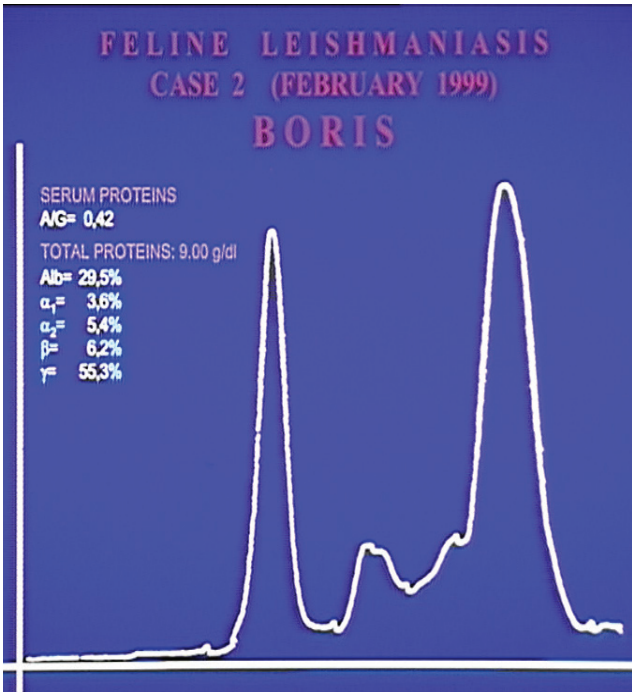

Figure 7: Monoclonal hypergammaglobulinaemia in a cat infected by L. infantum

agglutination (DAT) or indirect haemagglutination tests (IHT). IFAT titre ranged between 40 and 5120 (median 640). In five cases the disease was associated with an IFAT titre which if found in dogs would usually not be considered as diagnostic (≤ 80). A low antibody titre is also known to occur in some affected dogs with clinical signs compatible with leishmaniosis. This confirms that seronegative animals have to be tested with parasitological or molecular tests in order to exclude Leishmaniosis as the diagnosis[18]. PCR was performed in 29 cases and tested positive in samples from a wide range of tissues and organs obtained *intra vitam* or *post mortem*; the most commonly used tissue samples in live animals were the lymph node (18 cats), blood (14 cats), skin (10 cats), tissue from oral swabs (5 cats) and from conjunctival swabs (4 cats). The parasite was also isolated in 14 live cats from tissue mainly obtained from lymph nodes (6 cats), skin (5 cats), bone marrow (4 cats) or blood (3 cats). Cytological evaluation was diagnostic in a total of 16 cases and was performed on tissue from lymph nodes, skin, bone marrow, blood, or smears from nasal discharge.

Treatment followed an empirical approach with drugs conventionally used for canine leishmaniosis: mainly allopurinol (12 cats) or meglumine antimoniate (4 cats). However, there is no information on the pharmacokinetics and safety of these drugs in cats and as such the drug regimen varied. Allopurinol was given at a dose ranging from 10-15 mg/kg BID to 20-25 mg/kg SID or BID. The drug was generally well tolerated but one cat developed an elevation of hepatic enzymes and a second cat developed acute kidney injury of unknown origin two weeks following diagnosis[33, 41]. Meglumine antimoniate was administered subcutaneously at a dose ranging from 5 mg/kg SID (for three periods of 4 weeks with ketoconazole at 10 mg/kg SID per os) to 50 mg/kg SID for 4 weeks[28, 36]. Clinical efficacy was reported for both drugs and in some cases a long term follow up was also available which confirmed that, as usually happens in dogs, treatment resulted in a clinical cure but not the elimination of the parasite[23, 6].

Epidemiological investigations and the role of the reservoir of cats

Many serological and/or molecular studies have been published over the last two decades assessing the prevalence of *Leishmania* infection in feline populations. These studies are not comparable because of different methodologies used. Serological studies were mainly based on IFAT or ELISA techniques and reported a prevalence rate ranging between 3 and 59%. Molecular studies were mostly performed on PCR from blood samples - prevalence rates between 0 and 61% were reported[41]. As the case in dogs, lymph node samples and conjunctival swabs were found to be more sensitive than blood samples for diagnosis[27]. When serological and molecular techniques were used concurrently it was evident that PCR positive individuals may be negative for antibodies and vice versa so therefore, as seen in dogs, animals that test negative at serology may actually be infected.

Retroviral infections were investigated as risk factor for *Leishmania*. Only one study found a positive association between FeLV and *Leishmania*[42], but three recent studies have found a positive association between FIV infection and Leishmania[27, 43, 44].

As demonstrated by both epidemiological studies and experimental infections, cats can be subclinically infected by *L. infantum*. It is unknown if subclinically infected cats can be infectious to *Phlebotomus perniciosus*, the recognised vector of L. infantum in Europe, as there is very limited experience of transmission from a naturally infected cat to the vector[5].

Conclusion

Serological and molecular investigations confirm that in endemic areas *L. infantum* infection is a common occurrence in cats. This provides two important questions: the clinical relevance of the infection in cats and the epidemiological role of infected cats. To determine the clinical relevance of the disease the main issues that need to be addressed in the future by means of prospective controlled trials include: factors influencing the outcome of infection; the role of the infection in dermatitis conditions or syndromes such as FCGS and CRD; how to confirm the diagnosis; and how to manage infected cats. In the epidemiological context of leishmaniosis the role of infected cats as reservoir needs to be better defined. This latter matter is of great importance because in endemic European countries such as France, Italy, and Spain there is a high number of pet cats which may exceed the number of dogs[45]. Where stray cats live in huge numbers and with no sanitary control such as in Italy, it is increasingly important to determine if cats are a reservoir for infection.
It may be assumed that as effective preventive measures are increasingly applied to dogs in Europe the epidemiological impact of other minor reservoirs of zoonotic visceral/cutaneous leishmaniosis caused by *L. infantum* such as cats will grow in importance.

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REPRINT PAPER (SVK)

The clinical use of Deslorelin acetate (Suprelorin®) in companion animal medicine

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SUMMARY

In 2009 Suprelorin® was released in Switzerland for the temporary suppression of fertility in male dogs. However, in practice it has also been used for the treatment of other conditions in male dogs as well as in bitches. These include the management of benign hyperplasia of the prostate, induction or suppression of oestrus and the treatment of side effects of gonadectomy. In feline reproductive medicine GnRH-agonists are also gaining importance. These various applications are listed in terms of treatment success and possible side effects after administration about which owners must be fully informed.

Keywords: GnRH-agonist, oestrus induction, hormonal castration, dog, cat

Keywords: urine sediment, microscopic evaluation, dog, cat

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Introduction

In June 2009 Suprelorin®, a product of Virbac AG became licensed for the temporary suppression of fertility in male dogs in Switzerland. Since its release to the Swiss market, it has already been used in females and in species other than the dog by many practitioners. Suprelorin® is a subcutaneous implant that, after its one-time insertion, continuously releases a GnRH agonist substance, deslorelin acetate (DA). DA initially exerts a stimulatory effect on the GnRH receptors in the hypophysis leading to an increase in the concentrations of FSH and LH and subsequently sexual steroids. In the long-term however, it leads to the down regulation of GnRH receptors and consequently the suppression of the pituitary-gonadal axis. The observed clinical effects are similar to those after surgical castration but with a temporary duration of at least 6 months. In order to maintain suppression of fertility a new implant has to be inserted; the removal of the previous implant is not necessary. The recommended dosage is 4.7 mg DA (1 implant) independent of the animal's body weight.

Applications in the dog

Males

In male dogs treatment with 3, 6 or 12 mg DA induced a quick, short increase in blood testosterone (T) concentrations followed by a decline below the detection limit of the assay (<0.2 ± 0.1 ng/ml) within 3 weeks [Junaidi et al., 2003; Junaidi et al., 2009]. Due to the loss of T, testicle size decreases by approximately 65% within 14 weeks of application. As early as 4-5 weeks after implant insertion a lower ejaculate volume and poorer semen quality was detected. Even though libido was still present, no ejaculate could be collected between

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5 to 7 weeks after treatment [Junaidi et al., 2009]. Loss of libido was first seen in most males only after 8 weeks following the application of another GnRH agonist, 18.5 mg Azaglynafarelin [Goericke-Pesch et al., 2010]. The duration of action is dose-dependent and seems to be completely reversible. Physiological hormone concentrations were detected 394, 484 and 668 days after treatment with 3, 6 and 12 mg DA, respectively [Junaidi et al., 2009]. The re-establishment of male fertility after DA treatment was proven by successful matings and the ensuing pregnancies of the bitches [Trigg et al., 2001]. When long-term fertility suppression is desired, the insertion of a new implant is recommended when the effects of the previous one start to diminish, which can easily be detected by the increasing testicle size [Goericke-Pesch and Wehrend, 2009].

Suprelorin® can also be used for the treatment of benign prostatic hyperplasia (BPH) in the dog. Within 1 month of injection the prostate size was decreased by approximately 60%, prostatic cysts regressed in most cases and clinical symptoms e.g. haematuria subsided or disappeared (Jurczak et al., 2010). In the first two weeks after GnRH agonist administration clinical symptoms may worsen due to the initial "flare-up" effect. This can be prevented by the simultaneous application of finasteride (0.1-0.5 mg/kg SID p.o. for 3 weeks, Proscar, MSD Merck Sharp & Dohme-Chibret AG, Switzerland; Sirinarumitr et al., 2002) or cyproteronacetate (10 mg/animal SID p.o. for 3 to 4 days, Androcur, Schering AG, Switzerland; Riesenbeck et al., 2002]. Furthermore, with GnRH agonist treatment preputial discharge can be reduced [Goericke-Pesch et al., 2010] and unwanted, hormone-associated behavioural problems e.g. hypersexuality, territory marking and dominance towards other males can be controlled without initial relapse of symptoms [de Gier und Vinke, 2010; Goericke-Pesch et al., 2010].

Side effects of Suprelorin® include increased appetite with subsequent body weight gain, the development of urinary incontinence [Jurczak et al., 2010] and coat changes. Treatment of prepubertal animals under the age of 7 months should be avoided, because hormonal down regulation and subsequent reduction in testicle size may lead to cranial displacement of the testicles back into the inguinal canal [Goericke-Pesch and Wehrend, 2009]. For patients with testicular tumours or cryptorchidism castration is the only acceptable therapeutic option . Both conditions should be ruled out before Suprelorin® administration in order not to mask an oestrogen producing tumour as the primary cause [Goericke-Pesch and Wehrend, 2009]. The treatment of male dogs with GnRH agonists is a suitable alternative to gonadectomy and has the advantage of offering a reversible suppression of fertility. This is of particular importance for stud dogs and for males where possible unwanted side effects of castration could be checked first before proceeding with surgery.

Females(Bitches)

Oestrus induction

Based on their mechanism of action GnRH agonists can also be used for the induction of heat in females. Suprelorin® inserted during anoestrus in Beagle bitches initiated pro-oestrus in 5.6 (3-10) days [Walter et al., 2011]. In order to avoid ovulation failure or luteal insufficiency due to its down-regulatory effects, the implant should be removed in due time. When it was taken out on the first day of pro-oestrus, all treated bitches (n=11) ovulated and pregnancy was established in 63.6% of cases [Walter et al., 2011]. Implants can easily be removed when inserted either in the umbilical region, under the vestibular mucosa or in the medial region of the thigh [Kutzler et al., 2002; Fontaine et al., 2010; Walter et al., 2011]. In cases of intended breeding after heat induction, the clinician must be aware of that the time interval from the start of pro-oestrus until ovulation (8.2 days) is shorter than in a natural (normal) heat cycle [11 days; Walter et al., 2011] and therefore frequent examinations of the bitch should start early enough in order not to miss the optimal time for mating [Kutzler et al., 2009]. Luteal insufficiency followed by abortion despite implant removal can be a complication of Suprelorin® treatment and has been described in 4 out of 14 bitches [Kutzler et al., 2002]. In order to avoid this close monitoring of peripheral progesterone concentrations is necessary during gestation, so that luteal insufficiency is recognized early in the course of pregnancy and treated appropriately with a progesterone supplementation [Fontaine et al., 2010]. For induction of a fertile oestrus Suprelorin® should always be implanted in anoestrus, because during metoestrus histological repair of the uterine endometrium is not yet complete. When DA (1.05 mg or 2.1 mg, Ovuplant®, Fort Dodge, Overland Park, KS, USA) was given in combination with prostaglandin F2α (Dinoprost, Lutalyse®, Pfizer, USA) to induce luteolysis, the interoestrus interval was shortened and heat was induced,

but pregnancy after artificial insemination was achieved in only 2 out of 15 bitches [Volkmann et al., 2006].

Suppression of heat

As an alternative for gestagen treatment or ovario-hysterectomy, long-acting GnRH agonists may also be used for the suppression of heat in bitches. In a clinical study with one-time administration of DA (3, 6 and 12 mg) oestrus was postponed for 27 months [Trigg et al., 2001]. All dogs treated in anoestrus showed oestrus symptoms 4-8 days after implantation before subsequent down regulation, while bitches in metoestrus with a serum progesterone (P4) concentration >5ng/mL failed to show any heat signs [Trigg et al., 2001]. In contrast, in a study of Palm and Reichler (2011) Suprelorin® induced oestrus in 26 of the total 55 metoestrus dogs with P4 levels above 5ng/mL. Moreover, before suppression of ovarian function, the initial induction of heat is fertile and thus can result in pregnancy when bitches are mated [Wright et al., 2001]. In order to prevent oestrus occurring, pretreatment with gestagens for several days before and after administration of a GnRH analogue has been described in bitches and wild carnivores [Bertschinger et al., 2001; Wright et al., 2001; Corrada et al., 2006; Sung et al., 2006]. The results of this combination therapy are however variable. None of the 10 bitches showed heat when treated for 7 days with 2 mg megoestrol-acetate before and after GnRH agonist application [Wright et al., 2001], while with a similar protocol oestrus was induced in 4 out of 5 dogs in another study [Sung et al., 2006]. The concurrent administration of a GnRH-antagonist could not prevent the initial "flare-up" effect and only delayed heat [Hermo et al., 2006; Valiente et al., 2009].

When GnRH agonists were used in prepubertal bitches, the insertion of a 9.4 mg DA implant to 4-month-old puppies postponed puberty for 9 months, while implantation at 7 months of age induced oestrus in all dogs within 1-2 weeks [Trigg et al., 2006]. In this study 4 of 6 bitches treated between 5-8 months of age showed heat [Palm, Reichler 2011]. In a few cases, besides oestrus induction, other side effects associated following the application of Suprelorin® were ovarian cysts, persistent heat and/or uterine changes all of which may necessitate the removal of the implant or ovariohysterectomy [Romagnoli, 2009; Arlt and Heuwieser, 2011; Palm and Reichler, 2011]. Owners should be informed about the possible adverse effects of Suprelorin® before treatment starts. Age seems to play a role as older bitches have a significantly higher risk of persistent heat and the development of uterine abnormalities. Suprelorin® treatment can also cause coat changes, pseudopregnancy, urinary incontinence and behavioural problems [Palm and Reichler, 2011]. An advantageous aspect is its use in young bitches to test for possible spay-induced side effects. For example, in a case where Suprelorin® treatment leads to urinary incontinence (UI), it is likely that UI will also occur following gonadectomy. The advantages and disadvantages of spay can then be discussed with the owner in light of these findings when planning surgery. In order to make sure the implant can be removed when necessary, it should be inserted in the umbilical region under local anaesthesia.

Bitches with spay-induced UI could benefit from the administration of Suprelorin®, as 50% of dogs became continent after treatment [Reichler et al., 2003]. Successful therapy is probably due to an increase in the filling capacity of the urinary bladder, as it seems that DA does not influence urethral closure pressure [Reichler et al., 2006; Reichler et al., 2006]. Furthermore, Suprelorin® improved coat quality both in male and female dogs that had experienced coat changes after gonadectomy [Reichler et al., 2008].

Applications in the cat

Males(Tomcats)

In tomcats GnRH agonists have similar effects to those described in male dogs. Suprelorin® leads to a significant decline in testosterone (T) concentration within 28 days of administration, and within 12 weeks testicle size is reduced by approximately 60% [Goericke-Pesch et al., 2011]. In one out of 10 tomcats complete down regulation (T< 0.1 ng/ml) occurred only 28 weeks following implantation. As a consequence of decreased testosterone secretion, secondary male characteristics e.g. penile spikes disappear, and a significant increase in feed intake can often be noticed. Following an initial increase in male sexual behavior characteristics, it clearly diminishes thereafter and urine marking also stops [Goericke-Pesch et al., 2011]. A temporary infertility is achieved only after six weeks of successful down regulation, prior to which viable spermatozoa can still be released. Therefore mating within 20 days of Suprelorin® administration can result in pregnancy of the mated queen [Goericke-Pesch et al., 2011]. The duration of action varies between 6-24 months [Goericke-Pesch et al., 2010]. Similarly to male dogs,

infertility is completely reversible in the tomcat which is demonstrated by successful matings following a period of down regulation [Goericke-Pesch et al., 2010]. Urine marking can also be successfully be treated with GnRH depot analogues in castrated tomcats.[Goericke-Pesch und Wehrend, 2009].

Females (Queens)

In the queen, the administration of a DA-implant (6 mg) leads to an initial increase in oestrogen concentrations followed by a decrease within 30 days to levels significantly lower than those of untreated controls [Munson et al., 2001]. Ovarian activity can effectively be suppressed with GnRH agonists in the cat and signs of behavioural oestrus can also be prevented. Even though queens may show oestrus signs despite implant administration, mating does not lead to pregnancy [Toydemir et al., 2008]. Oestrus occurring in the first couple of weeks after treatment is probably due to induction by the implant, which is followed by ovulation and a long anovulatory phase thereafter [Rubion und Driancourt, 2009]. Oestrus induction is more likely to happen during treatment in anoestrus compared to treatment in interoestrus. The duration of action in queens is individually very variable and can last 6-24 months [Goericke-Pesch et al., 2010]. The only side effect described to date is a minimal local oedema, present for 3-5 days at the injection site, which is clinically not relevant [Munson et al., 2001]. Queens mated at the first oestrus after the abolishment of down regulation became pregnant and bore healthy kittens [Goericke-Pesch et al., 2010].

Conclusion

Suprelorin® has a wide range of successful applications in small animal practice. Licensed for the temporary suppression of fertility in male dogs, DA can also be used to treat benign prostatic hyperplasia, induce or suppress oestrus and treat castration/spay-induced conditions. In the case of administration for reasons other than those for which it is licensed, the owner must be fully informed about possible side effects and their consequences in advance.

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REPRINT PAPER (GR)

Urine sediment evaluation in the dog and cat

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SUMMARY

Microscopic examination of the urine sediment is an integral part of urinalysis and a useful, cost-effective diagnostic tool in the everyday practice. Diagnostic investigation of urinary diseases and several systemic metabolic diseases make the microscopic evaluation of urine sediment a priority along with the physical and biochemical examination of urine. The different methods of urine collection (midstream voiding, catheterisation or percutaneous cystocentesis) have advantages and disadvantages and the clinician has to decide on the best fitted method of urine collection, according to the medical background of the animal and the case-specific objectives of urinalysis. Proper handling and timely analysis of urine samples are essential for a valid microscopic evaluation. The microscopic examination of urinary sediment is usually conducted on stained or unstained "wet-mounted" preparations; occasionally, air-dried Giemsa-stained sediment smears are examined. Normal urine is sterile and may contain small numbers of cells (white and red blood cells, epithelial cells), few crystals, occasional casts, fat droplets and spermatozoa (male animals). In contrast, large numbers of cells or casts, presence of unusual types of crystals, neoplastic cells, parasites and microorganisms comprise abnormal findings, necessitating a more specialised diagnostic approach. This review focuses on the technical aspects pertaining to the proper sediment microscopic examination, the normally expected elements of sediment and the clinically relevant interpretation of abnormal findings.

Keywords: urine sediment, microscopic evaluation, dog, cat

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Introduction

Microscopic urine sediment examination is a useful diagnostic aid that, when combined with the findings of routine urinalysis, contributes significantly to the diagnosis of urinary tract and even systemic disorders[1, 2, 3]. Moreover, urine sediment findings (ie, haematuria, pyuria, bacteriuria) can alert the clinician to important problems while the patient is still asymptomatic[4, 5].

The purpose of the present review is to report the common conditions that may alter the results of urine sediment examination, to describe the technique for its preparation and microscopic evaluation, as well as normal and abnormal findings and their interpretation in dogs and cats.

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Factors that may influence urine sediment examination

The method and timing of urine collection, prior medical treatments and sample handling can influence the results of urine sediment examination[6].

A urine sample can be collected during midstream voiding, by aseptic catheterisation or by cystocentesis. The midstream voiding collection is a practical technique that excludes iatrogenic haematuria. However, these samples are often subject to contamination by the urethra and genital tract transit (bacteria, white or red blood cells, spermatozoa) or from the environment (foreign bodies, pollen grains), even when precautions are taken, thus reducing the reliability of this sampling method, especially when a urinary tract infection is suspected[7]. Catheterisation is a simple technique to perform in most male dogs, with a lower risk of sample contamination than a midstream voided sample. However, there is a significant risk of iatrogenic haematuria or introducing urinary tract infection[2, 8]. Cystocentesis may cause iatrogenic microscopic haematuria[9] and is contraindicated in animals with bleeding disorders; however, it is the preferred method of urine collection when a urinary tract infection is suspected[10]. Samples obtained by manual bladder expression have the same disadvantages as midstream voided samples. Moreover, there is an increased risk of bladder rupture, or vesicoureteral reflux and ascending urinary tract infection [2, 3]. The collection of urine voided on surfaces (ie, examination table) is an acceptable method, especially in animals with urine incontinence, provided that it is used only for sediment examination (ie, haematuria, pyuria, crystalluria) and not microbiologic testing. Nevertheless, the results should be verified using a sample obtained by a more appropriate method[7].

Morning samples are preferred for sediment analysis as they are concentrated and acidic, thus preserving structures like casts, white and red blood cells, and facilitating their detection[6, 11]. Fresh urine samples are preferred for cytologic examination because cells often deteriorate when urine samples are stored for an extended period of time[6].

The sample should be collected in a sterile container or syringe, labelled properly with patient identification data and method of urine collection. Ideally, urinalysis should be performed within 60 minutes of specimen collection [8, 12]. When this is not feasible, the sample may be refrigerated for up to 12 hours and then re-warmed to ambient temperature before analysis, in order to redissolve precipitates that form at lower temperatures [8]. If the sample remains at ambient temperature for more than 1-2 hours there is a risk of in vitro growth or death of microorganisms, while cell, cast and crystal morphology may be altered[2]. Oral or parenteral administration of fluids, diuretics or corticosteroids may affect sediment findings through urine dilution[9] and treatment with various drugs (ie, sulphonamides, allopurinol) may induce crystal formation[8, 13].

Preparation and examination of the urine sediment

Ideally a 5 ml sample should be collected, but in the clinical setting its volume is usually lower[3]. Initially, the sample is placed in a conical tube and centrifuged for 3 to 5 minutes at approximately 1500 to 2000 rpm[9]. The supernatant is aspirated and saved for chemical analysis, allowing a standard volume (approximately 0.5 ml) of fluid, containing the sediment, to remain in the conical tube. A drop of resuspended sediment is transferred to a clean microscope slide with a transfer pipette and a coverslip is placed over it[8, 14]. To obtain a stained wet mount preparation one drop of New Methylene Blue stain is added and mixed with the sediment before a drop is placed on a glass slide and covered with a coverslip. A stained preparation, useful for evaluation by oil immersion light microscopy, can be prepared by placing a drop of urine sediment on a clean glass slide, allowed to dry and using a commercially available stain (eg Diff-Quik)[1].

Microscopic examination of wet preparations should be done immediately. Proper lighting is achieved by partially closing the iris diaphragm and moving the substage condenser downward. Initially, the entire area under the coverslip is scanned with the aid of the low power objective (x10, low power field-LPF) in order to identify and semi-quantify casts and sizeable crystals. Examination under high power magnification (x40, high power field-HPF) is useful for the detection of microorganisms and to quantify epithelial cells, white and red blood cells and small crystals[3, 6]. Air-dried stained preparations facilitate evaluation by oil immersion light microscopy (x100, oil immersion field-OIF)[6].

Findings of microscopic urine sediment examination and their interpretation

Normal urine is sterile and may contain small numbers of cells (white and red blood cells, epithelial cells), few crystals, occasional casts and fat droplets. The presence of spermatozoa is a normal finding in intact male animals, even in samples obtained by cystocentesis, while in females it indicates recent mating[3].

Red blood cells (RBC)

A small number of RBC (<5/HPF) is considered a normal finding in the urine sediment[3, 9]. Higher numbers may be observed in samples obtained by catheterisation or cystocentesis. In that case, in order to exclude iatrogenic haematuria, it is recommended to re-evaluate these patients by performing sediment examination in urine collected during midstream voiding at least 24 hours later[15]. The presence of erythrocytes even in small numbers (<5/HPF) in serial microscopic examinations of midstream voided samples, necessitates further diagnostic investigation in order to rule out specific conditions of the urogenital tract (eg neoplasm, urolithiasis) or bleeding disorders[15]. Normally, erythrocytes appear round to biconcave, slightly refractile, lacking internal structures[8, 14] (Figure 1). They often appear shrunken or crenated in concentrated urine (specific gravity-SG >1020) (Figure 1), while they may lyse in dilute (SG <1010) or alkaline urine[9, 15]. Fat droplets may be mistaken for RBC, but, contrary to the erythrocytes, they are variably sized and float in a different plane of focus from the rest of the sediment[6]. Lipiduria (ie, fat in

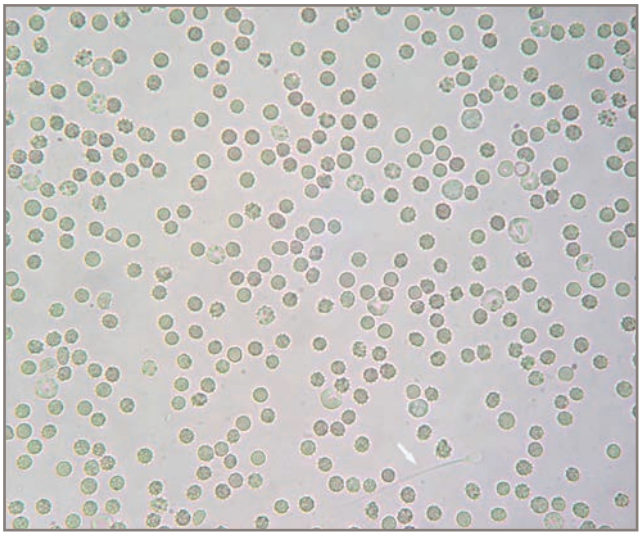

Figure 1: Haematuria. Several red blood cells appearing as pale discs or with crenation due to hypertonic urine exposure. A spermatozoon is also seen (arrow). (Unstained smear, x 400).

*Table 1: Common causes of canine and feline haematuria and pyuria**

Haematuria	Pyuria
Urinary tract	**Urinary tract**
Traumatic urine sampling (eg catheterisation, cystocentesis, renal biopsy, car accident)	Inflammation (eg bacterial or fungal urinary tract infections, feline lower urinary tract disease, cyclophosphamide-induced cystitis)
Urolithiasis (eg struvite, calcium oxalate)	Non-inflammatory causes (eg neoplasia, urolithiasis, trauma)
Neoplasia (eg transitional cell carcinoma)	
Inflammation (eg urinary tract infections, hyperplastic urethritis, feline lower urinary tract disease, therapy with cyclophosphamide)	
Disorders of primary or secondary haemostasis (eg ehrlichiosis, immune-mediated thrombocytopenia, anticoagulant rodenticide intoxication)	
Acute tubular necrosis	
Vascular abnormalities (eg renal infarction, idiopathic renal haematuria)	
Genital tract	**Genital tract**
Oestrus	Inflammation (eg prostatitis, vaginitis, prepuce contamination)
Inflammation, neoplasia, trauma	

* Modified from: DiBartola 2011 and Forrester 2004

urine) is more frequent in cats than in dogs, due to the fact that feline kidneys contain a large amount of lipid. In any case, fat droplets are considered a normal finding in both species. Haematuria should always be confirmed by microscopic examination of the urine sediment, because dipsticks do not differentiate haematuria from haemoglobinuria or myoglobinuria[15]. Haematuria usually suggests bleeding or urogenital disorders (Table 1).

White blood cells (WBC)

The presence of WBC in urine sediment (>5/HPF in samples obtained by cystocentesis, >8/HPF in midstream voided samples) is defined as pyuria and indicates inflammation[5, 16, 17] (Table 1).

In wet preparations WBC appear round and granular, about twice the size of RBC and, usually, smaller than epithelial cells (Figure 2). Leukocytes degenerate in aged urine and, may rarely lyse when urine is dilute or alkaline [8]. The WBC found in sediment are most commonly neutrophils and their differentiation from renal tubular epithelial cells may necessitate examination of stained preparations[9].

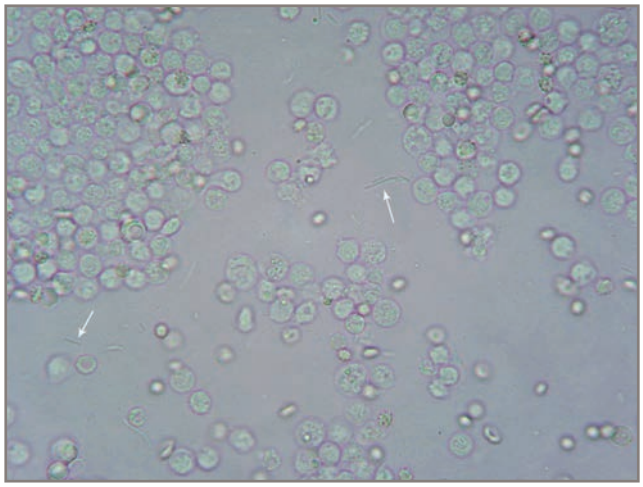

Figure 2: Bacterial cystitis. Note several granular white blood cells and rods (arrows). (Unstained smear, x 400).

The presence of WBC in sediment indicates septic or aseptic inflammation, without localising its site, unless they form casts, indicating renal origin[17]. Animals unable to mount an inflammatory response may develop urinary tract infection without significant accompanying inflammation. Conditions in which urinary tract infection may be present without significant pyuria include treatment with high doses of glucocorticoids, Cushing's disease and diabetes mellitus[6, 18, 19]. The diagnosis of pyuria should be based on sediment examination because the respective dipstick indication is not reliable in dogs and cats[20, 21].

Microorganisms

The most common microorganisms found in urine sediment are bacteria, with fungi, yeasts and parasites appearing rarely[6].

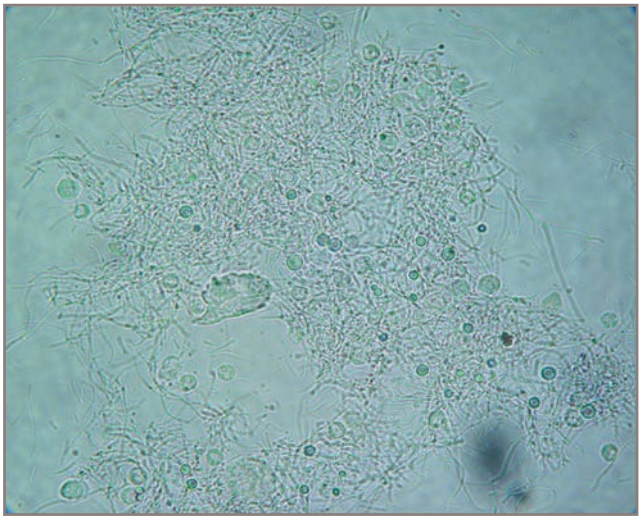

Figure 3: Bacteriuria in a dog. Note several bacteria aggregates (unstained smear, x 400).

Bacilli (Figure 3) and cocci should be present in numbers exceeding 10,000/ml and 100,000/ml respectively, to be detected microscopically. Brownian motion of small particles (eg phosphates) should not be misidentified as cocci in wet preparations[5, 8, 22]. Although detection of bacteria in urine sediment suggests urinary tract infection, it should be verified by urine culture[5, 8, 9]. Confirmation of bacteriuria is easier when microscopic examination of urine sediment is done in air-dried and stained sediment smears[1, 6]. When a sample remains at room temperature for an extended period of time bacterial overgrowth is possible[2].

Yeast organisms are a rare finding and are usually contaminants in urine samples. Fungal hyphae are rarely found in urine (Figure 4) and are considered evidence of systemic mycoses, because primary fungal infections of the urinary tract are infrequent[6]. Parasites, such as Capillaria sp. and Dioctophyma sp. can rarely affect dogs and cats living in areas with aquatic organisms[23]. Microfilaria of Dirofilaria immitis occasionally may be observed in urine sediment of infected dogs, presumably as a result of haemorrhage into the excretory pathway of the urinary system or due to iatrogenic haematuria[9].

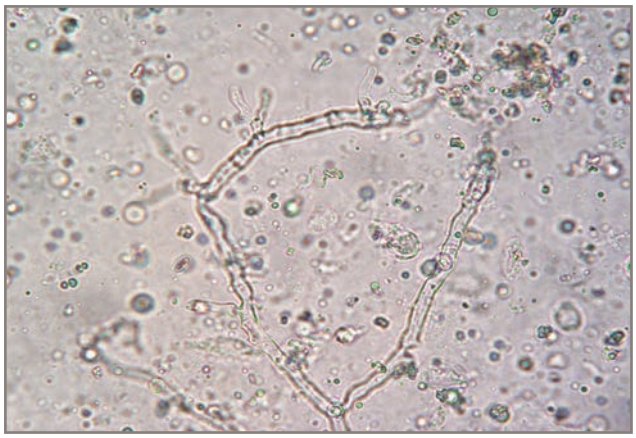

Figure 4: Fungal cystitis in a cat. Note several fungal hyphae (unstained smear, x 400).

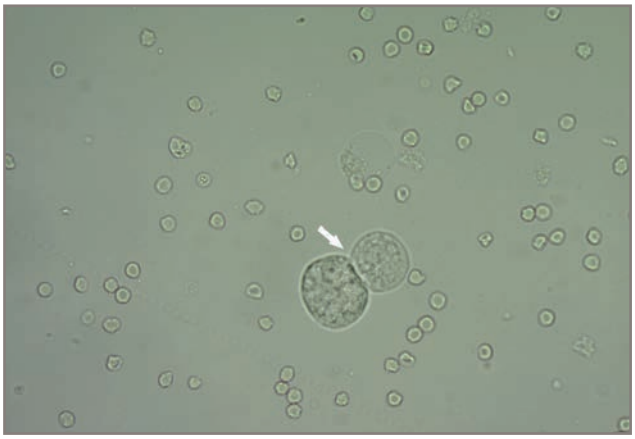

Figure 6: Two transitional epithelial cells amidst numerous red blood cells (unstained smear, x 400).

Epithelial cells

Detection of small numbers (0-1/HPF) of transitional or squamous epithelial cells in urine sediment is considered to be a normal finding as a result of normal exfoliation of epithelial cells. Epithelial cells vary markedly in size depending upon their origin and, generally, are larger in the lower portions of the urinary tract. They may occur in increased numbers in midstream voided samples or samples obtained by catheterisation[9]. Squamous epithelial cells originate from the distal urethra, vagina, vulva or prepuce and are the largest of the normal cells found in urine sediment[14]. They usually have a small, round nucleus, although occasionally a nucleus cannot be seen, straight edges and obtuse, angular corners (Figure 5)[6]. Normally, they are not detected in samples obtained by cystocentesis.

Transitional epithelial cells, derived from the proximal urethra, bladder, ureters and kidneys, appear round or oval with round nuclei and granular cytoplasm (Figure 6). Increased numbers of this type of cell can be seen in association with inflammation, infection, chemical (eg cyclophosphamide) or mechanical irritation and transitional cell carcinoma. In this latter case, evaluation of air-dried sediment smears may show severe cellular abnormalities[5, 24].

Casts

Casts are cylindrical moulds of the renal tubules composed of aggregated mucoprotein matrix (Tamm-Horsfall mucoprotein), secreted by the distal tubular epithelial cells, with or without embedded cells and other formed elements. Their formation is favoured in concentrated and acid urine, while they may dissolve in alkaline urine. High speed or protracted centrifugation and delay in examination may disrupt fragile casts[9]. Presence of abnormal numbers of casts in the urine sediment is called cylindruria[17]. They are classified as

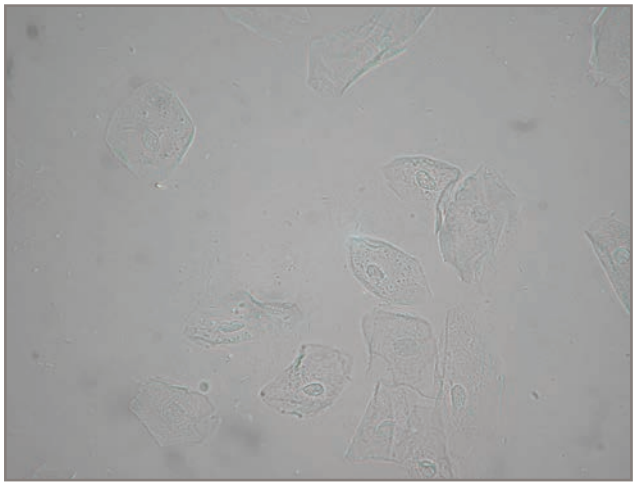

Figure 5: Squamous epithelial cells in the urine sediment of a dog (unstained smear, x 400). Courtesy: Dr E. Leidinger.

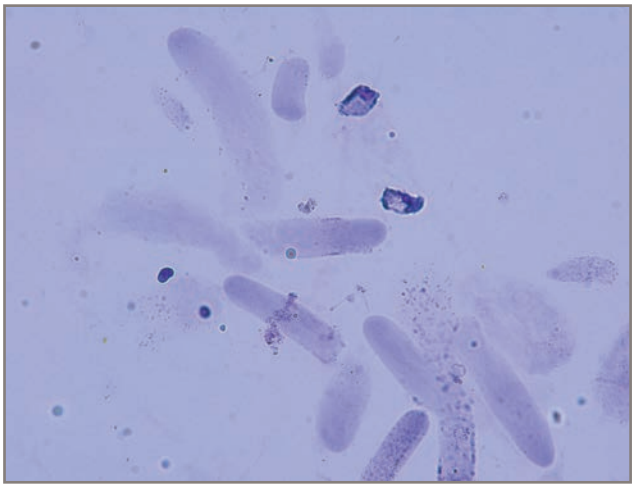

Figure 7: Numerous hyaline casts in the urine sediment of a dog (new methylene blue, x 400). Courtesy: Dr E. Leidinger.

hyaline, granular, cellular, waxy and fatty casts[2, 3]. Hyaline casts are considered to be the precursor form of all types of casts, composed mostly of Tamm-Horsfall mucoprotein. In unstained sediment they appear semi-transparent and colourless, but they are best detected in New Methylene Blue-stained preparations (Figure 7) [25]. Zero to 2 hyaline casts per LPF are considered normal. Increased numbers may be found in patients with pre-renal (ie, fever, general anaesthesia, exercise) or renal (glomerulonephritis due to immune complex deposition, amyloidosis) causes of proteinuria[10].

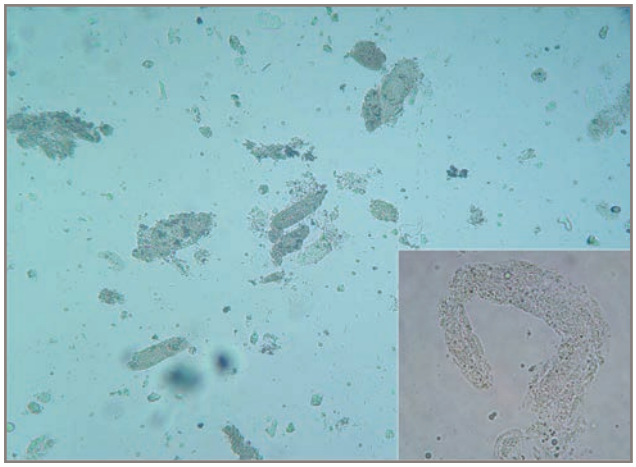

Figure 8: Cylindruria in a dog. Numerous intact and fragmented granular casts (unstained smear, x100). Inset: intact granular cast in higher magnification (unstained smear, x 400).

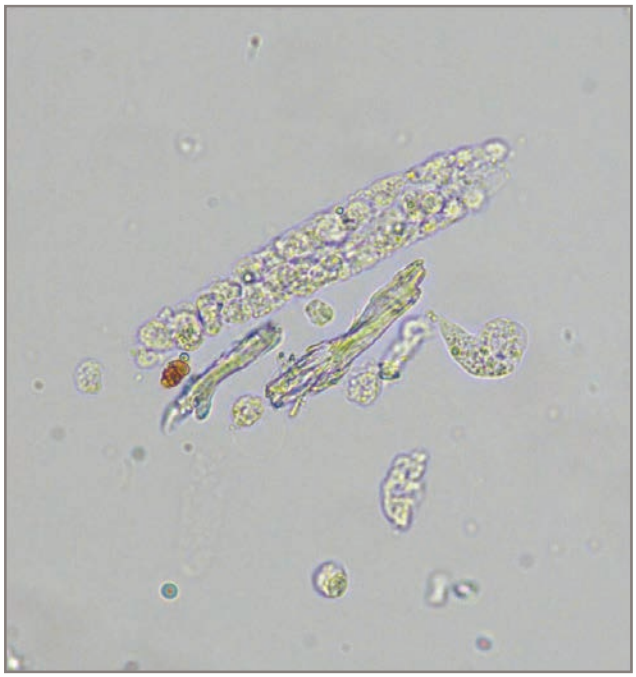

Figure 9: Cellular cast of unidentified origin in dog urine (unstained smear, x 400). Courtesy: Dr E. Leidinger.

Granular casts (Figure 8) originate from degenerated epithelial and WBC, entrapped in the mucoprotein matrix[9]. Small numbers of these casts (<2/LPF) comprise a normal finding, while large numbers suggest acute renal damage[6].

Normally, urine sediment does not contain cellular casts[2, 17]. According to the cell type present, they are classified as epithelial (ie, acute tubular necrosis or pyelonephritis), WBC (ie, pyelonephritis) and RBC (renal haemorrhage) casts (Figure 9). As the cellular component of these casts degenerates, they convert into granular casts[14].

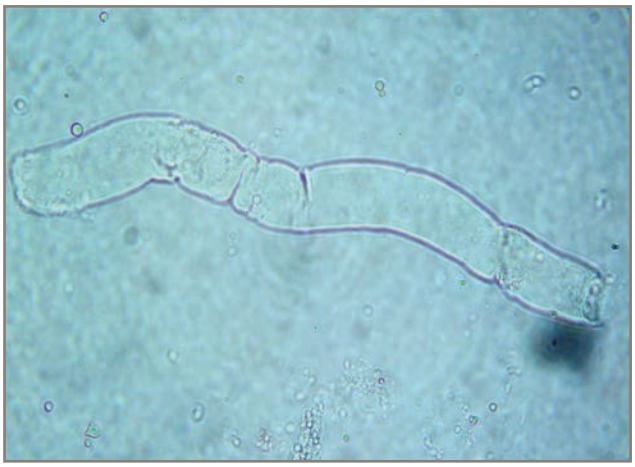

Figure 10: Waxy cast with square ends and fissures along its surface (unstained smear, x 400).

Waxy casts (Figure 10) represent the final stage of degeneration of granular casts, indicating chronic tubular damage[6]. Although they appear similar to hyaline casts, they have square instead of round edges, are dull and homogeneous and may have fissures along their surfaces[6].

Fatty casts contain many small fat droplets and are frequently seen in cats, due to the increased fat content of their kidneys, but are occasionally seen in dogs with diabetes mellitus[17]. Increased numbers (>1/LPF) of these casts suggest renal tubule degeneration[6].

Crystals

Crystalluria (detection of crystals in urine sediment) is a frequent finding in dogs and cats, usually considered clinically insignificant[6]. Crystalluria indicates saturation of urine with a crystalloid material but is seldom

indicative of urolithiasis. Additionally, uroliths may be present without crystals[26, 27]. However, detection of specific types of crystals (ammonium urate, cystine) and presence of common crystals in large numbers during consecutive examinations of urine sediment (in order to exclude in vitro crystalluria) are clinically significant[27, 28]. The type of crystals formed depends on urine pH, concentration and temperature[6]. To avoid the in vitro formation of crystals, immediate evaluation of a fresh urine sample (within 15-20 minutes from the collection) is mandatory[27].

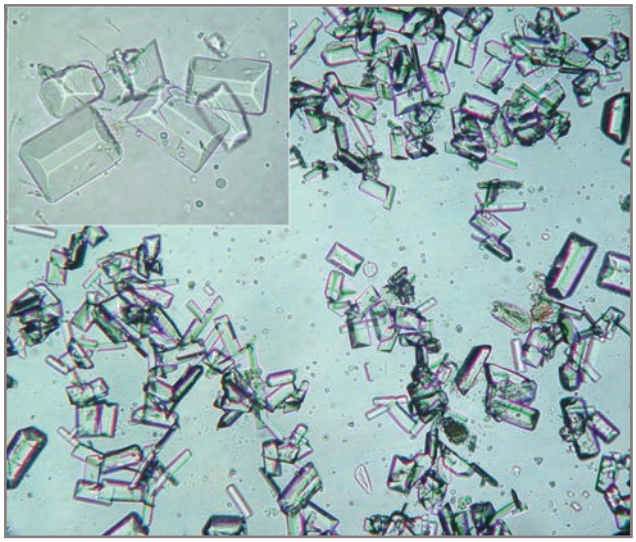

Figure 11: Magnesium ammonium phosphate (struvite) crystalluria. Note several variably shaped crystals (unstained smear, x100). Inset: Amidst spermatozoa, crystals with "coffin-lid" appearance are illustrated (unstained smear, x 400).

Struvite crystals (magnesium ammonium phosphate) are found in alkaline urine[29] and have a coffin-lid appearance, but also other shapes (Figure 11). Struvite crystalluria is a frequent urine sediment finding in healthy animals, however canine struvite uroliths are often associated with Staphylococcus sp. urinary tract infections, while in cats struvite uroliths are usually sterile[27, 30, 31]. These crystals are reported as occasional, moderate or many per LPF or HPF[10].

Calcium oxalate monohydrate crystals appear as elongated structures with pointed ends, while calcium oxalate dihydrate crystals form an envelope-like structure but may also have a rhomboid shape with internal Maltese cross pattern (Figure 12). They are found in acidic urine[12]. Animals poisoned with ethylene glycol often have large numbers of calcium oxalate crystals, especially the monohydrate form, in their urine, while

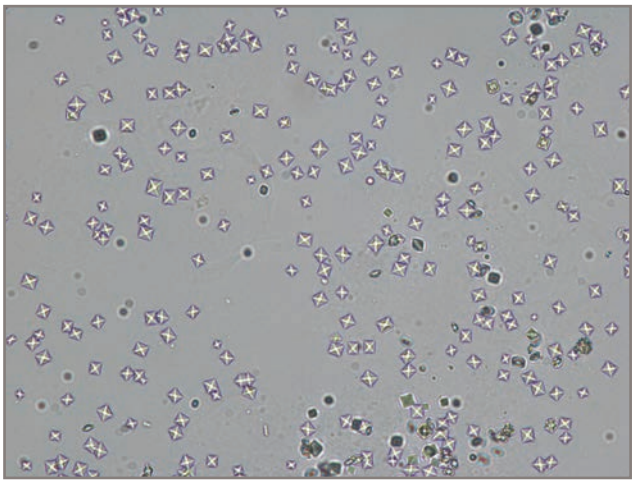

Figure 12: Numerous envelope-shaped calcium oxalate dihydrate crystals (unstained smear, x 400). Courtesy: Dr E. Leidinger.

calcium oxalate dihydrate crystals may be seen in small numbers in the urine of healthy animals. However, when they are repeatedly observed in serial urine sediment examinations, further diagnostic investigation is recommended to rule out systemic disorders (eg hypercalcaemia of malignancy)[6, 27]. Calcium oxalate crystals are semi-quantified per LPF or HPF.

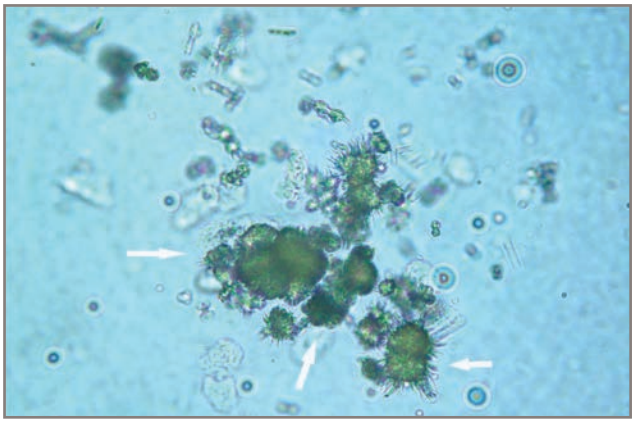

Figure 13: Thorn apple- like ammonium urate crystals in the dog (arrows). (Unstained smear, x 400).

Ammonium urate and amorphous urate crystals (Figure 13) are commonly detected in Dalmatians and English bulldogs due to the altered purine metabolism in these breeds. Their presence in other canine breeds and in cats indicates liver disease (eg congenital or acquired portosystemic shunts)[9].

Bilirubin crystals are yellow to amber needle-shaped structures (Figure 14) indicating bilirubinuria, which is a common finding in healthy dogs, whereas feline bilirubinuria is an abnormal finding associated with

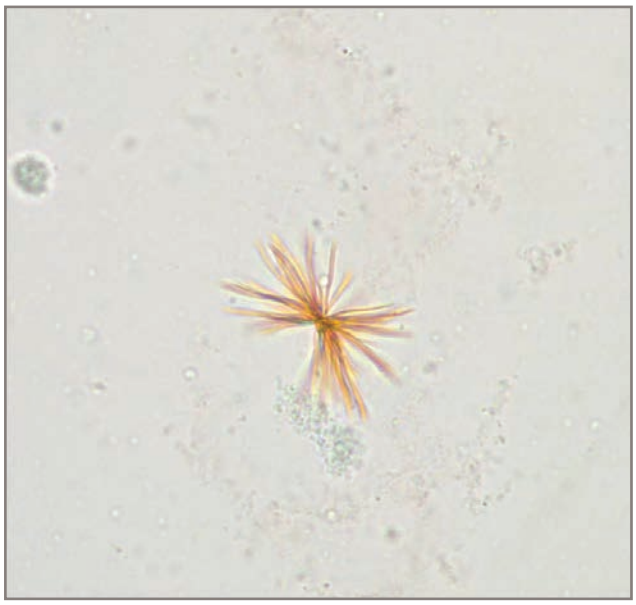

Figure 14: Needle-shaped bilirubin crystal (unstained smear, x 400). Courtesy: Dr E. Leidinger.

a variety of diseases[27]. Cystine crystals appear as hexagonal, flat plates and usually occur in acidic urine [27]. They are not found in normal animals but in those with cystinuria, a congenital metabolic defect concerning the transport of cystine and other amino acids across the renal tubules[6].

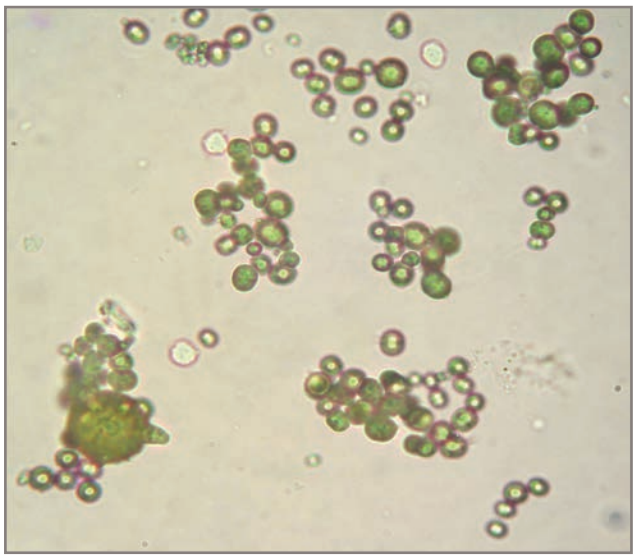

Figure 15: Xanthine crystals in a dog treated with allopurinol (unstained smear, x 400).

Infrequently, crystalluria is associated with drug administration (eg sulphonamides, allopurinol)[8]. In cases of long-term allopurinol treatment (eg dogs with leishmaniosis) detection of xanthine crystals is a usual, clinically insignificant finding[13] (Figure 15). Amorphous crystals, lacking a specific structure, occasionally found in urine sediment, are usually a variation of struvite crystals[27].

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REPRINT PAPER (D)

Haemophilia A and B in dogs

Reinhard Mischke[1]

SUMMARY

Based on our own clinical experiences and the literature, this review article elaborates on aspects of the aetiology, pathogenesis, clinical signs, diagnosis and therapy of haemophilia A and B in dogs. When compared to humans, dogs show more severe haemorrhagic symptoms at a residual factor activity (e. g. subcutaneous, intramuscular, and intraarticular haemorrhages after inappropriate trauma resulting in lameness and paralysis, excessive haemorrhage during second dentition, venal puncture, and surgery). Fortunately, genetic tests are now commercially available in Germany for selected breeds (haemophilia B in Rhodesian Ridgebacks; haemophilia A in Havaneses), which complement the conventional individual factor activity measurements and facilitate the detection of female carrier dogs. Treatment of bleeding crises is still mainly based on substitution therapy with fresh or fresh frozen plasma in addition to local haemostatic measures. Expectations regarding the clinical availability of gene therapy (including humans) have not yet been fulfilled.

KEY WORDS

Haemophilia A, haemophilia B, dog, breeds, diagnosis, genetic testing, therapy, literature review

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Introduction

This review article gives an overview on several clinically relevant aspects of haemophilia A (classical haemophilia) and haemophilia B ("Christmas disease") in the dog. Both diseases are X-chromosome-linked coagulation disorders characterized by a functional and/or quantitative factor (F) VIII or FIX deficiency [9, 41].

Epidemiology

Haemophilia A is, particularly with a view to its severe clinical bleeding complications, the most important hereditary coagulation disorder in both animals and in humans [8, 40], and the second most common hereditary haemostatic disorder to the (often subclinical) von Willebrand disease [34]. This is partially due to the relatively high mutation rate of the FVIII gene [22]. However, severely affected individuals rarely reach sexual maturity, so that these mutations show a tendency for a natural elimination [40].

In humans the prevalence of haemophilia A is 100 per million and the prevalence of hemophilia B is 10-20 per million [47]. In the dog, incidence of haemophilia A is also higher than incidence of haemophilia B (3-4 times higher

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Presented by GSAVA (D)

as an estimate [8]), which reflects the smaller size of the FIX gene and the resulting lower frequency of mutations. However, the exact incidence of the different types of canine haemophilia is unknown [48].

Haemophilia A has been reported on a global scale in case reports, case series and reviews [8, 12, 16, 34, 41] for a great number, if not the majority, of pure dog breeds and also in mixed-breed dogs. For many years, a high incidence has been reported particularly in the German Shepherd [20, 21, 34, 39]. The incidence, however, seems to have declined in this breed. In Germany, classical haemophilia in the Havanese dog is presently spotlighted [65]. In the Small Animal Clinic of the University of Veterinary Medicine Hannover, Foundation, haemophilia A was also diagnosed in the Wirehaired Dachshund, Shiz-Tzu, Jack Russell Terrier, Hungarian (Magyar) Vizsla, Weimaraner and the Great Dane.

Although the list of breeds affected by haemophilia B does not quite reach the breed range of haemophilia A, haemophilia B has been described for quite a number of dog breeds such as the Airedale Terrier, Alaskan Malamute, American Cocker Spaniel, Beagle, St. Bernard, Bobtail, Bichon Frisé, Cairn Terrier, Coonhound, Chow Chow, German Wirehaired Pointer, German Shepherd, Dobermann, French Bulldog, Foxterrier, Golden Retriever, Jack Russell Terrier, Labrador Retriever, Pit Bull Terrier, Rottweiler, Scottish Terrier, Sealyham Terrier, Sheltie, Shih-Tzu, Maltese Terrier and Weimaraner [8, 14, 19, 43, 74, 79, 83]. In the author´s client-owned dog pool, haemophilia B was diagnosed in the German Wirehaired Pointer, St. Bernard, Fila Brasileiro, and Rhodesian Ridgeback.

At the moment haemophilia B in the Rhodesian Ridgeback is attracting particular interest [47, 48, 63]. In the literature, a report regarding a family of French Bulldogs with both haemophilia A and B can also be found [77].

Aetiology, inheritance

Both forms of haemophilia show an independent, X-linked inheritance due to their location on the long arm of the X chromosome [9, 47]. This means that the disease becomes manifest in male animals with a defective X chromosome [4, 48]. Affected female dogs, however, show a normal X chromosome in addition to a defective one. Those heterozygotic animals are clinically normal but carry the defect [34]. Outside inbred colonies [5, 75] homozygous, and hence clinically affected female dogs as a result of successful breeding of a haemophilic male and a carrier female, are extremely rare [23, 66]. Such matings may occur in small dog breeds and in dog breeds with a narrow gene pool, and/or in mild forms of haemophilia [33, 34]. The author has, however, never seen such a case, which is in keeping with the reportedly low incidence.

Phenotypically affected males are offspring of carrier females. Half of the ova carry the defect so that mating of a carrier female with a healthy male leads to offspring where half of the males will be affected, and half of the females will be carriers. In contrast, all female offspring of an affected male are obligatory carriers, whilst male offspring is always free of the defect. [20, 41] (Fig. 1).

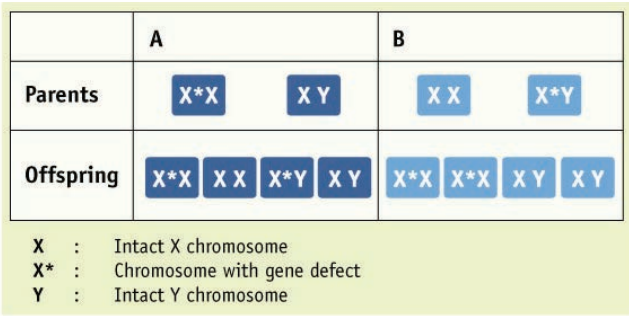

Fig. 1: Scheme of transmission of haemophilia from a haemophilic male dog (A) or a female carrier (B) to their offspring.

In the case of haemophilia B the defect has been specified on a molecular level in several breeds such as the Airedale Terrier, German Wirehaired Pointer, Labrador, Lhasa Apso, Pit Bull Terrier, Rhodesian Ridgeback, and also in the mixed-breed dogs of a haemophilic dog colony owned by the University of North Carolina (Chapel-Hill-Colony). Mainly point mutations [18,63)] and deletion mutations were identified [10, 29, 57]. An insertion mutation was also found [11]. For haemophilia A, a smaller number of mutations have been described due to the more complex analysis of the markedly larger FVIII gene [31, 44, 64, 65].

Pathogenesis

The effects of a FVIII- and FIX deficiency on the coagulation system are similar, since both factors act at the same location within this system [41]. Both factors are the most important components of the tenase enzyme complex which forms on the activated thrombocytes during the propagation phase of coagulation. Activated FVIII (FVIIIa) which is activated by initially small amounts of thrombin, functions as an important coenzyme of serinprotease FIXa which is activated by FVIIa. This perpetuates the coagulation process and hence large amounts of FXa, thrombin and finally fibrin

are generated which are needed for thrombus formation [30, 78]. Both coagulation factors (FVIII and FIX) are therefore essential for adequate formation of fibrin [34]. In patients suffering from haemophilia, delayed generation of FIXa and thrombin early cause disturbances of platelet plug formation and, due to a disturbed formation of fibrin, finally resulting in a reduced thrombus stability [32, 82]. This instable platelet plug can perhaps initially control bleeding. It will however be flushed away when vascular contraction diminishes and blood pressure rises, resulting in new bleeding which is often serious and prolonged [32, 34, 40].

Clinical presentation

Since the function and site of action of FVIII and FIX in the coagulation system are close together, haemophilia A and B cannot be differentiated based on their clinical presentatation [34, 40]. Clinical signs include extended subcutaneous, inter- or intramuscular haematomas (or haematomas in other soft tissues) which occur either spontaneously or after inadequate (minimal) trauma [16, 23, 33, 34, 55, 66, 71, 72, 76] (Fig. 2, 3). Furthermore, recurring lameness of changing location can be observed [13, 34, 35, 71, 72] as well as pareses and reduced reflexes [27, 36] (Fig. 4a).

Fig. 2: Bilateral subconjunctival haemorrhage in an 11-month-old male Havanese dog with clinically moderately severe haemophilia A (factor VIII: 15%). The dog also showed paraparesis, probably due to a haemorrhage in the area of the spinal cord. A trauma was suspected but had not been observed.

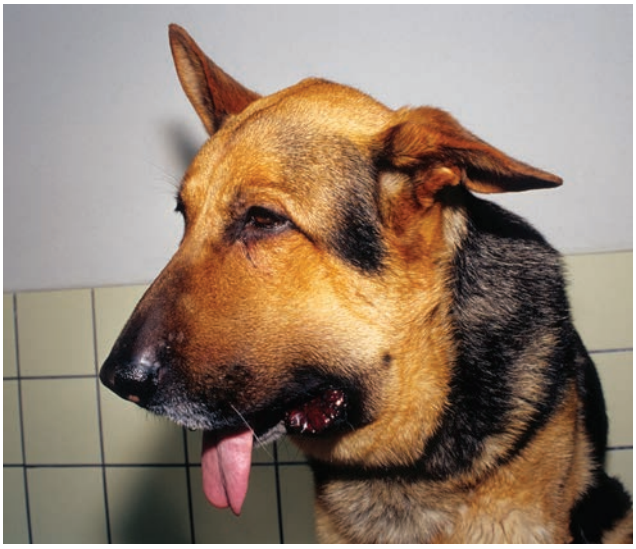

Fig. 3: Haemorrhage to the head and neck in a German Shepherd dog with severe haemophilia A (factor VIII < 1%) without known trauma.

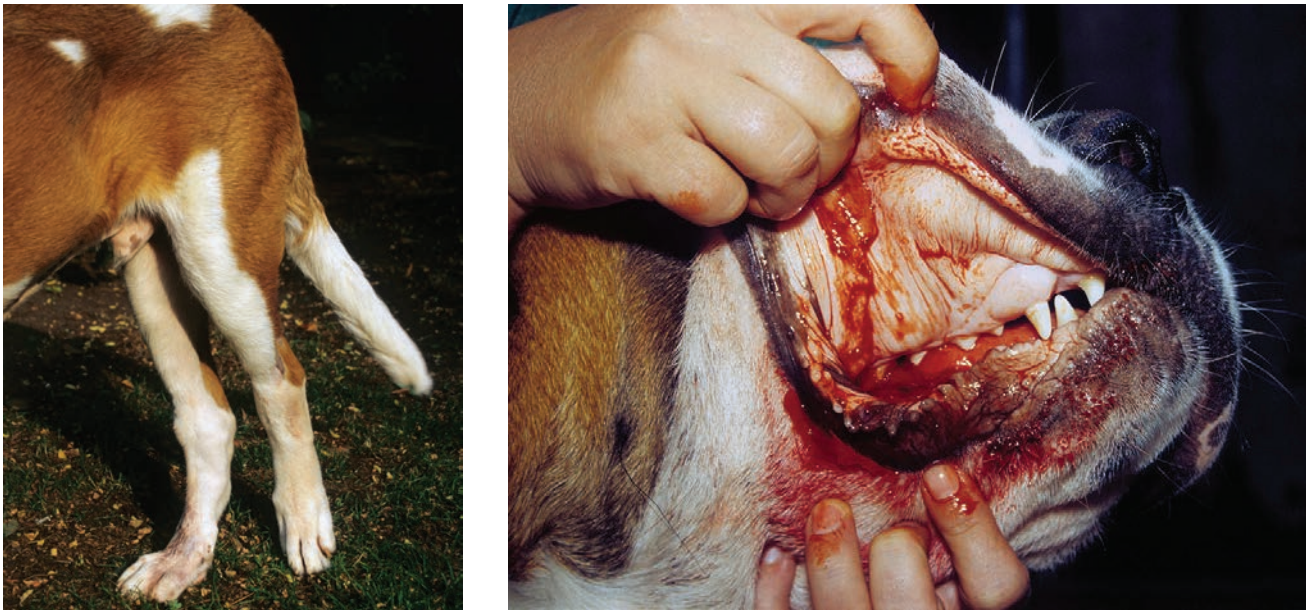

Fig. 4 : 6-month-old St. Bernard with haemophilia B; a) severe lameness of the left hind limb resulting from an acute haemorrhage into the knee joint; b) long-lasting massive bleeding during second dentition. The dog was euthanised at the request of the owner.

Such movement disorders are a consequence of haemorrhage into soft tissues (degeneration, fibrosis and contraction of muscles, nerve injuries) [27, 71], and also of bleeding into joints [3] and into the spinal cord [75]. Haemorrhage into joints can induce degenerative arthropathies [35, 41, 43]. This is particularly important in large dog breeds due to the higher joint loading [33]. Grøndalen et al. [28] described enostoses in 3 young German Shepherds which were possibly related to haemorrhage. The author of this manuscript made a similar observation in a German Shepherd suffering from haemophilia A. Excessive to life-threatening haemorrhages may occur during the change of teeth [2, 55, 61] (Fig. 4b), during oestrus [33], after trauma or injury [43], venipuncture [66], too vigorous nail clipping, and after surgery such as neutering or tail amputation [16, 34, 37, 71, 73]. More rarely observed are epistaxis [2, 61], gastrointestinal bleeding and haemoptysis [16, 61], and marked bleeding into the visceral cavities [27, 33, 61, 76], which can be potentially lethal [34, 39]. In addition, fatal haemorraghes into the central nervous system have been described in cases of severe haemophilia A [17, 33, 75]. Interestingly, a particularly high incidence of life-threatening bleeding into the central nervous system has been reported in the homozygous females of a colony of labrador mongrels with haemophilia A [75].

In severe cases, mortality can be high in puppies [76]. Marked bleeding during the change of teeth is often the first sign of a bleeding disorder [2, 23, 55, 61]. In many cases, haemophilia first becomes manifest as a consequence of veterinary interventions so that, unfortunately, an adequate supplementation of the deficient coagulation factor was omitted in advance of and/or during the procedure (Fig. 5–7). In less severe cases, the age of the patient at time of diagnosis can be up to 2 years [39].

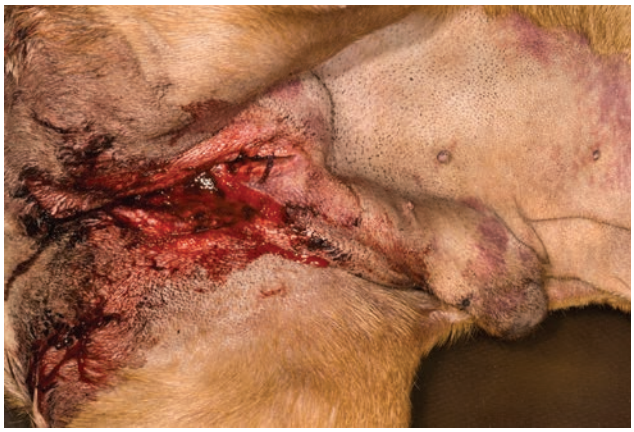

Fig. 5: Massive haemorrhage and impaired wound healing after castration of a 2.5-year-old male Rhodesian Ridgeback mixed breed dog with severe haemophilia B (factor IX: 1%). Despite repeated plasma transfusion and revision surgery, the dog had to be euthanised.

Fig. 6: Severe haematoma at the neck following a microchip implantation in a 5-month-old male Hungarian(Magyar) Vizsla with haemophilia A.

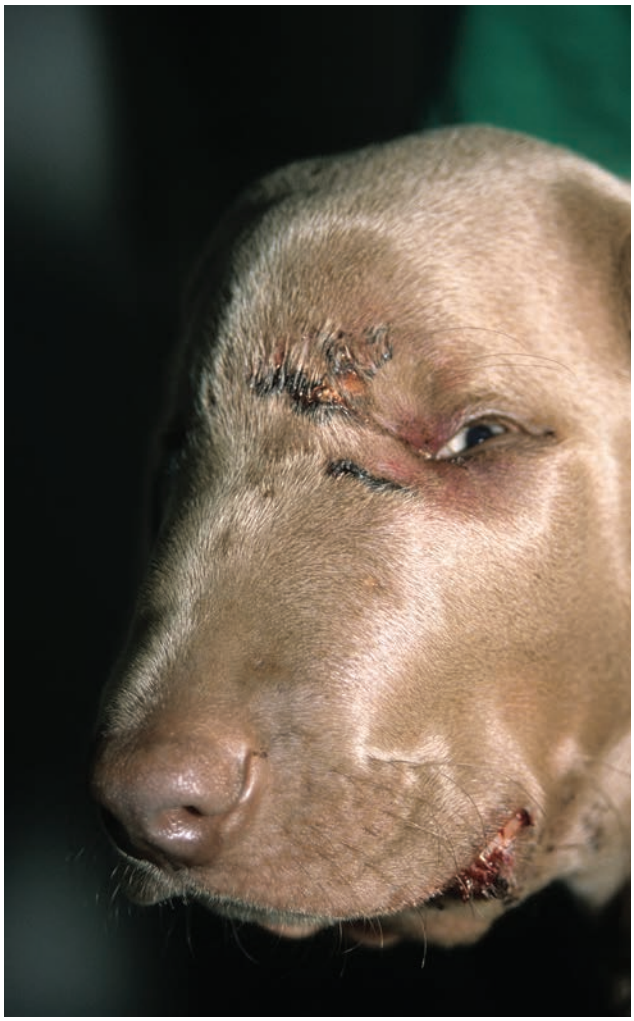

Fig. 7: Massive haemorrhage and impaired wound healing after surgical treatment of a forehead haematoma in a 3-month-old Weimaraner suffering from haemophilia A.

Table 1: Severity levels of haemophilia, depending on the activity of factor VIII or factor IX and associated bleeding manifestations in humans [4, 48]

Severity level	FVIII-/FIX residual activity (%)	bleeding manifestations
severe	< 1	frequent bleeding into muscles and joints, partly without clear cause (spontaneous bleeding), early onset
moderate	1–4 (5)	bleeding after surgery, injury or tooth extraction, but also minor trauma
mild	5 (> 5)–25	possible bleeding after surgery or major trauma/injury

Different degrees of severity of haemophilia A and B have been described in dogs. The severity of clinical presentation depends on residual activity of the respective coagulation factor, and also on size and activity of the animal [39, 48]. In humans, severe (< 1% FVIII), moderate (1–4 (5)% FVIII) and mild (> 5)–25% FVIII) forms of haemophilia A are differentiated [4] (Tab. 1). In severe, and less often also in moderate forms of haemophilia A, patients that do not receive therapy or prophylactic measures develop extensive bleeding into the subcutis, muscles and joints, whereas in mild cases enhanced bleeding only occurs following trauma or surgery [47, 48]. Some investigators chose to follow the criteria used in humans to classify severity degrees in dogs based on residual activity of FVIII or FIX [3, 15, 21]. However, in the author`s experience, bleeding is much more pronounced at a defined residual activity of the haemophilia factor even in small dog breeds as compared to human patients, which is in agreememt with other references [17, 35, 48]. This means that classification in humans does not provide a reliable orientation for the canine patient. For example, a “mild” form – according to classification in humans - of haemophilia A is observed in the German Shepherd at residual activities of FVIII between 4 and 10%, more rarely even up to 20%. The severity of clinical symptoms however rather suggests the presence of at least the moderate form [34, 35, 39, 61, 72]. This is possibly a reflection of greater physical activity (e.g. playing with other dogs, jumping around) and the less cautious behavior of the canine patient [61]. Haemorrhage at a defined residual factor activity is tendentially more severe in haemophilia A than in haemophilia B [48, 70].

Laboratory diagnosis

Basic Diagnosis

Both factors FVIII and FIX are part of the classical intrinsic pathway of the coagulation system. Hence, respective screening tests such as activated partial thromboplastin time (APTT) (with sensitive reagents) and/or activated coagulation time (ACT) or reaction time of the non-activated or intrinsic-activated thromboelastogram (TEG) are prolonged, whilst prothrombin time, thrombin time, platelet count, and bleeding time are within reference intervals [24, 27, 34, 35, 48, 55, 61, 62, 66, 71]. The extent of prolongation of APTT at a certain residual factor activity level depends on the reagent. In haemophilia A, APTT is prolonged by approximately 1.5 to 2.5 times [59, 60]. Deficiency of FIX results in a more pronounced prolongation of APTT compared to a FVIII decifiency of identical severity [59, 60]. Female carrier dogs cannot be reliably identified with this screening test [20, 35]. In dogs with hypovolaemic shock, sometimes other tests (and also single factor activities) are abnormal, which can mask haemophilia [61].

Coagulation factor tests

Measurement of FVIII or FIX activity (using human factor deficient plasma) against a canine standard (pooled plasma whose factor activity is defined as 100%) which is usually performed using coagulometer tests by a specialised laboratory is necessary for a specific diagnosis [34, 48, 55, 66, 71, 73]. Reported reference intevals are e.g. 72–136% for FVIII activity [61] and 75–140% for FIX activity [63]. Alternatively, photometric tests with chromogenic substrates can be used [20]. Affected male dogs mostly show activities of < 10% (of the activity of the pooled plasma). More rarely (mild forms), activity of the haemophilia factor is higher (10–25%). Moderate forms of haemophilia A are found, for example, in the affected Havanese dog with FVIII activities between 10 and 15 % in affected males [65], and in a recently reported family of Malinois from Italy [45].

Identification of clinically asymptomatic female carriers is essential for effective breeding programmes [35, 52]. For such breeding programmes, pedigree studies and genetic testing (see below) are more important than FVIII measurements [52]. Females that carry the defect often

show slightly subnormal plasma activities of FVIII/FIX of approximately 50% (approx. 30-65%) as compared to canine pooled plasma, however also values within the lower reference interval can be found [16, 20, 34, 35, 39, 63]. Partial overlapping of range of FVIII and FIX activities in carriers with the reference interval does not allow a reliable diagnosis solely based on activity of coagulation factors [34, 35, 63]. Repeated tests and consideration of test results of offsprings can increase probability of a "non carrier status" [39] (Tab. 2).

Table 2: Likelihood of "non-carrier status" of haemophilia in daughters of obligatory carriers, depending on factor VIII activity measurement [39].

Test result (plasma factor VIII activity)	Likelihood of "non-carrier-status"
w/o factor VIII measurement	50%
15–60%	11%
one test result > 60%	82%
two test results > 60%	98%
one test > 60%, one test < 60%	64%
two tests > 60%, one test < 60%	93%

As haemophilic males during a haemorrhagic crisis [27, 72], many carriers display a disproportionally high concentration of the von Willebrand factor (vWF) compared to the activity of FVIII, so that the vWF : FVIII ratio can be helpful in detecting carrier females (which mostly show a ratio of > 2) [34, 35, 56]. However, in a different study with 16 female German Shepherds which were all heterozygous carriers for haemophilia A, neither FVIII activity nor vWF:FVIII ratio yielded sufficient distinction from the control group of 18 healthy female German Shepherds [56]. When measuring FVIII in puppies it is important to consider that, among others, FVIII activity is lower during the first 3 months of life compared to that of adult dogs [53]. Interestingly, FVIII activity does not markedly change during sedation with acepromazine or xylazine, so that blood of dogs sedated with these agents can be used for diagnosis [54]. It is not necessary to cool or to freeze samples for dispatch to a specialised laboratory, since FVIII is stable at room temperatures in citrated blood and citrated plasma [50]. (Note: similar data is not available for FIX, however an analogous stability can be assumed).
Parallel immunological determination of the concentration of the respective coagulation factor (FVIII/FIX) allows the detection of dysfunctional molecules [16, 48], although it must be considered, that the binding affinity of antibodies to structurally altered coagulation proteins may also be subject to change.

Genetic analysis

DNA analyses using tissue samples such as EDTA blood or mucosal swabs are especially helpful to exclude the carrier status in female dogs intended for breeding [52, 63]. After discovery of the mutations which trigger haemophilia A and B, genetic tests became available for several dog breeds in the USA. Since the detection of the genetic defects responsible for haemophilia in certain dog breeds in Germany [63, 65] genetic tests for routine diagnostics are also available in our country (haemophilia B in the Rhodesian Ridgeback, haemophilia A in the Havanese dog, tests provided by Laboklin GmbH, D-Bad Kissingen). 1 ml EDTA-blood or a swab of the buccal mucosa should be sent to the laboratory (sampling sets and instructions are available from the above mentioned laboratory). For genetic analysis it has always however to be considered that rarely, even within a dog breed, different mutations might be present within various dog families [8, 12].
Indirect detection of haemophilia A in female carrier dogs via a combination of FVIII microsatellite markers has proven relatively reliable. 37 of 39 female carrier dogs of different breeds could be identified by using a panel comprising three markers (intron 6, 10, and 21) [12].

Prognosis

Both haemophilia A and B are incurable. Male dogs with severe haemophilia, especially haemophilia A, and patients belonging to large dog breeds show a markedly reduced life expectancy as compared to healthy individuals [61]. Among other reasons, this is due to a more pronounced bleeding tendency in the dog as compared to humans (see above) and to very limited options for prevention and therapy of bleeding crises, but is also significantly influenced by housing conditions [39]. The author published a case series including 14 dogs suffering from haemophilia A with the following results: two dogs died during bleeding crisis, nine dogs were euthanised (often due to chronic lameness) at ages ranging from 9 months to 4 years (median: 1 year), and three dogs were still alive at the age of 4-11 years [61].
Very severe forms of haemophilia are normally incompatible with life and affected dogs seldom reach

puberty [40]. Many dogs severely affected with haemophilia A die shortly after birth due to major haemorrhage from the umbilical cord or other haemorrhagic crises, or have to be euthanised during puppyhood [2, 17, 27, 34, 35, 37, 71]. The guarded to poor prognosis must be discussed with the dog owner before starting an elaborate factor replacement therapy [61, 74].

Therapy

Treatment of patients with haemorrhages due to haemophilia comprise various measures:

- Patient care and local haemostatic measures,
- Factor replacement therapy (fresh plasma, fresh frozen plasma, fresh whole blood, recombinant human FVIIa),
- Other drug therapy (antifibrinolytics),
- (Gene therapy).

Patient management and local haemostatic measures

It is often attempted with variable success to control haemorrhage by keeping the patient at rest, if necessary by sedation, in combination with local haemostatic measures (compresses, haemostyptics, electrocoagulation, vessel ligation) [16, 20, 74]. In some cases, for example bleeding lesions of the oral cavity, electrocoagulation is preferable to blood vessel ligation, since suture exit holes can be sources of new haemorrhages. Large external haematomas should remain untouched even after bleeding has been controlled by factor replacement therapy where it was necessary. Generally, drainage of such haematomas is not advisable, because this can result in secondary haemorrhage and infection (Fig. 7) [16]. Pressure bandages can help to reduce the dimensions of such haematomas [34]. Resorption of larger haematomas may take weeks [16].

Drugs should be administered orally wherever possible. Unavoidable intravenous or subcutaneous injections should be administered using a small gauge needle [34]. Intramuscular injections are absolutely contraindicated, since they often result in deep muscle haematomas [16, 34]. Antibiotics are often administered in dogs with extended haemorrhages [23, 27, 43, 73] to control bacterial growth in haematomas. They are the mandatory drugs in case of infected haematomas [16].

Further supportive measures for patient care of haemophiliacs include a thorough control of ecto- and endoparasites [16]. A high quality, soft diet should be fed particularly after bleeding crises, whereas bones, biscuits and hard toys must be avoided [16].

Factor replacement therapy

In cases of serious internal and/or uncontrollable bleeding episodes and/or haemorrhages with potentially life-threatening consequences (e.g. haemorrhages into the central nervous system; haematomas causing obstruction of the upper airways), factor replacement therapy with fresh plasma or fresh frozen plasma is the therapy of choice [34]. In addition, surgery-associated replacement therapy using blood- and plasma products enables control of normal surgical bleeding [73]. In some countries, canine fresh whole blood and blood products such as fresh frozen plasma are commercially available. Administration of fresh whole blood (possibly in combination with fresh plasma or fresh frozen plasma) is mainly indicated when a considerable loss of red blood cells occurs due to bleeding [16, 34, 43, 73, 74, 76]. However, transfusion volume is limited (20 ml/kg body weight (BW), with an exemption in hypovolemia) and hence the amount of coagulation factor replacement. In addition, transfusion of red blood cells carries the risk of patient sensitization and of incompatibility reactions. In cases of emergency, however, no alternative to fresh whole blood transfusion is often available in veterinary practice [23, 27, 67, 73, 76].

The aim of factor replacement therapy is to increase the activity of the deficient coagulation factor to 25-30% (as compared to canine pooled plasma) in order to ensure sufficient haemostatis [61, 62, 80]. To achieve this approximately 15 ml/kg BW in mild and moderate cases of haemophilia [80] and 20 ml/kg BW in severe cases with insignificant residual activity of the FVIII or FIX [61, 81], of fresh plasma or fresh frozen plasma are needed (Fig. 8). An infusion of 15 or 20 ml plasma/kg BW results in an average increase of FVIII activity in the haemophilic dog by 20 or 33%, respectively [61]. In a model of canine haemophilia A, it was shown that the cuticle bleeding time is within the reference interval after substitution therapy with cryoprecipitate (see below) at FVIII activities of > 25%, whereas values are still considerably prolonged at FVIII activities < 20% [25].

Usually, repeated administration of 10(6-12) ml plasma/kg BW at intervals of (8-)12 hours is recommended [15, 41, 76] due to the short half-life of FVIII and FIX [34, 81]. The half-life of canine FVIII is reported to be 6-14 hours [21],

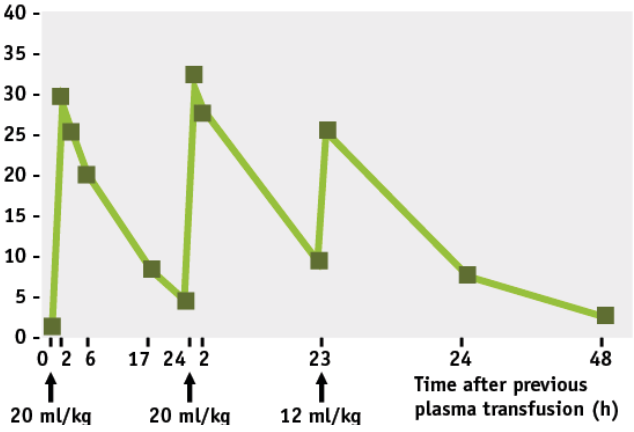

Fig. 8: Factor VIII activity in a 3-year-old male Siberian Husky with haemophilia A after repeated transfusion of fresh plasma or fresh frozen plasma.

8-10 hours [27], approx. 13 hours (based on measurement of APTT) [1], and up to 16.5 hours [61]. The half-life of FIX is only slightly longer after replacement therapy with canine fresh frozen plasma [62]. With the exception of major trauma, in some cases one single application of 15-20 ml/kg BW plasma is sufficient [61].

Cryoprecipitate can be prepared by slow thawing of fresh frozen plasma; during thawing, fibrinogen precipitates with FVIII, vWF and fibronectin - the precipitated proteins are then re-suspended in a small volume of the supernatant fluid. Due to its small volume, cryoprecipitate ensures an efficacious therapy of a FVIII deficiency (however not of a FIX deficiency) [1, 26, 34]. Cryoprecipitate is also favourable in view of moderate side effects which can occur after plasma transfusions (e.g., pruritus, oedema) [80], however, it is rarely available for the veterinary practice due to its elaborate production.

Heterologous, commercial single factor concentrates, especially those of human [7], but also of porcine origin [46] can be used. In principal, they are haemostatically effective, and have been proven efficacious for treatment of haemorrhages in the haemophilic dog [41]. Their pharmacokinetics resemble those of homologous clotting factors. For example, the mean half-life of recombinant human FIX concentrate and human F IX concentrate derived from plasma is 19 and 18 hours, respectively, when given to dogs with haemophilia B [7]. However, the immense costs of such preparations often preclude their use in veterinary medicine. Commercially available human FVIIa concentrates (NovoSeven, Novo Nordisk) [6, 58] are efficacious, but their even higher price also makes them an unrealistic alternative. Infusion of heterologous proteins induces antibody formation [42] which can cross react against homologous coagulation factors. After repeated administration, sensitisation occurs with a resulting risk of anaphylactic reactions [7]. However, in a family of Miniature Schnauzers which had received canine cryoprecipitate in cases of haemorrhagic events, antibodies directed against canine and human, but not against porcine FVIII were observed [26].

Other drug therapy

Unlike in the case in humans [49], desmopressin (DDAVP) is not effective in treating canine FVIII deficiency [34]; it does not result in an increase in FVIII activity [51, 61]. In contrast, administration of inhibitors of fibrinolysis can support fibrin formation at sites of vascular injury. Tranexamic acid (Cyclokapron, e.g. Media Pharma GmbH, Bad Homburg, Germany; not registered for veterinary use) has been described as being effective in the dog when given orally at a dosage of 15-20 mg/kg BW every 6-12 hours [41].

Gene Therapy

Interestingly, dogs with haemophilia B were the first "non-laboratory" animals in which a long-lasting significant increase (for several months) of activity of the FIX could be achieved by gene therapy (genetic transfer e.g. via adenovirus) [38, 69]. Its practical application would be of great benefit, since only a moderate increase of residual activity of the affected factor by a few per cent should result in a marked reduction of the grade of severity of clinical symptoms. However, it cannot be expected that this treatment option will become available for the clinical use in dogs in the foreseeable future.

Prevention

Individual patient

For the individual patient suffering from haemophilia, avoiding trauma is the most important prophylactic measure. Since also playing with other dogs can cause problems, affected dogs should be kept alone [16]. Uses where there is a high exposure to trauma (e.g., as in the case of hunting dogs) should be totally avoided [20]. Mandatory surgical procedures necessitate timely preventive factor replacement therapy; elective surgeries should be avoided as far as possible. Preoperative coagulation screening, particulary of young males of affected breeds, is always indicated.

Drugs that negatively affect haemostasis (e.g. platelet function inhibitors such as acetylsalicylic acid and clopidogrel; non steroidal anti-inflammatory drugs such as phenylbutazone, and also phenothiazine derivatives, antihistamines and sulfonamides) are contraindicated [16, 20, 34]. Thrombocytopenia can develop during the viraemic phase following vaccination with a live vaccine, so live vaccines are better avoided in haemophilic patients [16, 33]. Drugs should be, as already mentioned, be preferably administered orally, and only if necessary intravenously or subcutaneously [20].

The frequency of bleeding episodes can possibly be reduced by permanent oral administration of antifibrinolytics (tranexamic acid, see above.). In an experimental study, long-term prophylactic replacement therapy with recombinant human FIX in dogs with haemophilia B resulted in a 49% reduction of bleeding events [68]. Similar to gene therapy, however, treatment with recombinant human FIX is unavailable for veterinary clinical practice.

Breeding programmes

Breeding programmes are important in the elimination of haemophilia, especially the identification and exclusion of female carries [16]. The Veterinarian`s efforts should therefore go beyond the medical care of the patient. Using clients as messengers, breeders should be informed about the diagnosis in order to initiate coagulation and/ or genetic tests in breeding dogs and littermates of the patient to limit distribution of the defect[16, 45,70].

Conclusions

From a clinical viewpoint, haemophilia A and B are the most relevant coagulation factor deficiencies in the dog. Due to X-chromosomal transmission it is predominantly male dogs which present with clinical symptoms. Haemophilia should be considered particularly when unexplained haemorrhage (e.g. prolonged bleeding during change of teeth, haematomas without adequate trauma) or recurring lameness is observed in young male dogs. In Germany, haemophilia B in the Rhodesian Ridgeback and haemophilia A in the Havanese dog have currently the greatest clinical relevance, however, many other dog breeds are affected and spontaneous mutations must be taken into account. A suitable screening test is the activated partial thromboplastin time, followed by measurements of the coagulation factors VIII and IX to confirm the diagnosis. In addition, gene tests are available for the above mentioned dog breeds which are essential for the reliable detection of carrier status. When haemophilia is diagnosed in a male dog, if possible its dam and siblings should also be tested, and breeders should be involved in the consultation in order to prevent distribution of the defect and the disease. Severe haemorrhagic crises often require replacement therapy with fresh plasma or fresh frozen plasma. Treatment possibilities however, especially regarding availability of blood products, are limited in veterinary medicine.

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REPRINT PAPER (UK)

Influence of signalment on developing cranial cruciate rupture in dogs in the UK

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SUMMARY

OBJECTIVES: To investigate risk factors associated with cranial cruciate ligament rupture in dogs.

METHODS: Retrospective case-control study: medical records of a first-opinion veterinary practice were searched for dogs diagnosed with cranial cruciate ligament rupture (1995 to 2007). For each case, six unaffected dogs were randomly selected from all dogs presenting that day for comparison. Multi-variable binary logistic regression was performed to assess the association of variables on likelihood of cruciate rupture.

RESULTS: Frequency of cranial cruciate ligament rupture was 1.19% [95% confidence interval (CI) 1.02 to 1.36%]. West Highland white terriers (n=17), Yorkshire terriers (n=14) and Rottweilers (n=11) were at significantly increased risk of cranial cruciate ligament rupture (P≤0.002). Rottweilers were at five times greater risk compared with other pure breeds (OR 5.12, 95% CI 2.281 to 11.494, P<0.001), obesity quadrupled the risk of cranial cruciate ligament rupture (OR 3.756, 95% CI 1.659 to 8.502, P=0.001) and females were twice as likely to suffer cranial cruciate ligament failure compared to males (OR 2.054, 95% CI 1.467 to 2.877, P<0.001). Dogs less than two years old were statistically less likely to sustain cranial cruciate ligament rupture than dogs older than eight years (OR 0.246, 95% CI 0.127 to 0.477, P<0.001). There was no significant difference in median weights (in kilograms) of neutered dogs, compared to their entire counterparts in either the case group (P=0.994) or in the control group (P=0.630). There was also no significant difference in body condition (underweight/normal weight/over-weight/obese) of neutered versus entire dogs among the cases (P=0.243), or the controls (P=0.211).

CLINICAL SIGNIFICANCE: Cranial cruciate ligament rupture is more likely in Rottweilers and in female dogs, older dogs and obese dogs. Following multi-variable analysis, it was established that neutering was not associated with increased risk of cranial cruciate ligament rupture.

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Introduction

Cranial cruciate ligament (CrCL) rupture is one of the most common orthopaedic conditions of the dog and the major cause of degenerative joint disease of the canine stifle [Brinker and others 1997]
The CrCL undergoes long-term degenerative changes secondary to mechanical loading as well as micro-injury directly related to structural failure under normal physiological conditions [Hayashi and others 2003]. Vasseur and others (1985) reported that by the age of five years, there was microscopic evidence of degenerative disease in the CrCL of dogs weighing more than 15 kg and these changes progressed with age. It has been reported [Vasseur and others 1985, Bennett and others 1988] that the most frequent degenerative changes, which may be related to hypovascularity, occurred in the central region of the CrCL, with the caudal ligament being less severely affected.

Cranial cruciate rupture is considered to have a complex and multi-factorial aetiology, with many predisposing risk factors previously reported [Hayashi and others 2004]. A smaller number of publications, mostly from the USA, have investigated risk related to breed, age, gender, reproductive status and weight specifically [Whitehair and others 1993, Duval and others 1999, Slauterbeck and others 2004]. Only one regional study has been carried out in the UK, which involved 28 cases over 3 months was conducted over 30 years ago [Barnes 1977]. These studies have produced some contrasting results and many of them do not include a control population, making interpretation of results difficult.

Whitehair and others (1993) reported that female dogs have a higher prevalence of CrCL rupture than male dogs, and neutered animals when compared to their entire counterparts were also found to be at increased risk. Whitehair and others (1993) calculated a ratio of prevalence for neutered to entire animals as 2:1. Age at the time of neutering was not found to have any effect on prevalence of CrCL rupture. This study also reported dogs between 7 and 10 years of age, regardless of gender to be at increased risk of CrCL rupture and larger dogs suffered ruptures at younger ages. Duval and others (1999) looked only at dogs less than or equal to two years old, and concluded that large breeds were over-represented for that age category, supporting the theory that large dogs rupture CrCL at younger ages.

A small study of 28 dogs with CrCL rupture conducted 30 years ago in Kent subjectively assessed bodyweight (on a scale of – to +++) and found that half the cases were overweight [Barnes 1977]. No control group was included and no data were available for comparison to the normal canine population, so no conclusions could be made. Duval and others (1999) found that the mean bodyweight of affected dogs was greater than that of control dogs. Whitehair and others (1993) also took bodyweight into account in their study and found that dogs weighing more than 22 kg had a higher prevalence of rupture compared to dogs weighing less than 22 kg. However, both of these studies simply evaluated the size of the dog rather than their body condition, meaning they could not conclude whether the higher prevalence was due to the increased body size alone or related to the animals being overweight. Breeds shown to be at increased risk of CrCL rupture include: Akita, American Staffordshire terrier, Chesapeake Bay retriever, Labrador retriever, mastiff, Neapolitan mastiff, Newfoundland, Rottweiler, Staffordshire terriers and St Bernards. Those found to be at decreased risk were: basset hounds, dachshunds and old English sheepdogs [Whitehair and others 1993, Duval and others 1999]. To the authors' knowledge, no similar study has been conducted in the UK.

The purpose of this study was to investigate the role of breed, age, gender, reproductive status and weight on the risk of developing CrCL rupture in a case-controlled canine study in a first-opinion practice. Elucidation of these risks allows current UK and USA populations to be compared and also identify changes in the UK risk factors over the last 30 years.

Materials and methods

A retrospective case-controlled study, investigating risk factors leading to CrCL rupture, was performed. Medical records of an RCVS-accredited Small Animal Hospital (2010; http://www. rcvs.org.uk/) in Wolverhampton were searched for dogs diagnosed with CrCL rupture between January 1995 and May 2007. Inclusion criteria were pelvic limb lameness with ipsilateral positive cranial drawer sign at presentation. For each case, breed, gender and reproductive status (entire or neutered), age (in years), weight (kilograms) and limb affected were all recorded. For all cases of bilateral rupture/later rupture to contralateral limb, only one limb/first episode was counted. To choose between limbs for bilateral injuries for each animal, limbs

were assigned a number (1 or 2) and one was selected via a random number generator [True Random Number Service 1998; http://www.random.org/].

For each new case recruited to the study, six dogs were randomly selected from all cases seen that day using a random number generator (http://www.random.org/). Any repeated animals or those with recorded lameness were excluded. Information recorded for each dog comprised breed, sex, reproductive status, weight and age.

Statistical analysis

All statistical analyses were completed using a standard computerised statistical software package (SPSS, version 17.0, Chicago, IL, USA).

Breed associations were explored for breeds with 10 or more incidences of CCL rupture. Age was categorised as less than or equal to two years, over two to less than or equal to eight years and over eight years. Investigation of the effect of obesity on risk of cruciate rupture was completed by comparison of study animal bodyweights with average weights in relation to breed and sex of a reference population [Diez and Nguyen 2006]. The difference in bodyweight for each animal below the lower range or above the upper range of the reference value was calculated as a percentage. All dogs were then categorised as either "underweight", "normal", "overweight" or "obese" based on the percentage difference from the reference population (Table 1). Where no reference weight or gender was available and for cross-breeds, the case/control was excluded from the evaluation of body condition.

Table 1. Categorisation of dogs according to their weight as a percentage of BIW

	% of reference weight
Underweight	55≥BIW<85
Normal	85≥BIW<115
Overweight	115≥BIW<145
Obese	145≥BIW

BIW = breed and sex ideal weight

The chi-squared test and univariable logistic regression were used to compare categorical variables between the case and control groups. Normality of quantitative variables was assessed graphically, and via the Kolmogorov-Smirnov statistic and the Mann-Whitney U test was then used. Variables significant at a level of P less than 0·2 were taken forward for evaluation in the multi-variable model (see below).

Multi-variable binary logistic regression was performed to assess the association of variables on likelihood of cruciate rupture. A forced entry method was used. Collinearity between independent variables was assessed using collinearity diagnostics including tolerance and variance inflation factor values. Presence of first-order interactions between variables in the final model was also investigated. Reliability of the multi-variable model was assessed using the Hosmer-Lemeshow test and the DFBeta and deviance diagnostic statistics. Statistical significance was set at the 5% level.

Results

Over the study period, 189 dogs presented with CrCL rupture. There were 1179 controls with a case-control ratio of approximately 1:6. Twenty-four patients (11%) presented twice during the study period after rupturing their contralateral CrCL and four dogs (1.8%) presented on a single occasion with bilateral failure. Ninety-seven out of 189 (51.3%) of cruciate ruptures concerned the left limb and 92 of 189 (48.7) the right. There was no significant difference in the proportion of cruciate ruptures that affected the left versus the right hind limb (P=0.716). The most frequent pure breeds observed in the case group included West Highland white terriers (n=17), Yorkshire terriers (n=14), Labradors (n=14), Rottweilers (n=11) and golden retrievers (n=10). There was evidence of an overall breed association with rupture in the univariable analysis (P<0.001, Table 2). Within this variable certain pure breeds individually had a significant association with cruciate rupture. These were Rottweilers (P<0.001), West Highland white terriers (P=0.001) and Yorkshire terriers (P=0.002) (Table 2). Females were significantly more likely to experience a cruciate rupture than males (P<0.001, Table 2). The case group comprised 124 females (66.0%) and 64 males (34.0%) (1 missing) compared to 584 (49.5%) females and 595 males (50.5%) in the control group. Table 3 summarises the sex and neutering status of cases and controls. Neutered animals suffered significantly more cruciate ruptures (P=0.004, Table 2). Cases were statistically significantly older than controls (median 8.08, range 0.50 to 15.83 years and median 7.17, range 0.08 to 19.17, respectively, P=0.025). When animals were categorised according to age there

Table 2. Results of univariable logistic regression

Variable	Category	Number of cases	Number of controls	OR	95%CI Lower	95%CI Upper	P
Breed	—	—	—	—	—	—	<0.001
	Rottweiler	11	23	3.99	1.874	8.492	<0.001
	WHWT	17	53	2.676	1.477	4.846	0.001
	Golden retriever	10	41	2.035	0.981	4.22	0.056
	Yorkshire terrier	14	41	2.848	1.487	5.456	0.002
	Labrador	14	85	1.374	0.745	2.533	0.309
	Cross breed	43	277	1.295	0.871	1.926	0.202
	Other pure breed	79	659	Reference	—	—	—
	Missing	1	—	—	—	—	—
Sex	Female	124	584	1.974	1.43	2.725	<0.001
	Male	64	595	Reference	—	—	—
	Missing	1	—	—	—	—	—
Neutering status	Neutered	99	488	1.575	1.157	2.145	0.004
	Un-neutered	89	691	Reference	—	—	—
	Missing	1	—	—	—	—	—
Age (years)	—	—	—	—	—	—	<0.001
	Age ≤2 years	11	233	0.259	0.136	0.494	<0.001
	Age >2≤8 years	81	410	1.086	0.784	1.502	0.621
	Age >8 years	93	511	Reference	—	—	—
	Missing	4	25	—	—	—	—
Condition	—	—	—	—	—	—	0.009
	Underweight	2	14	0.537	0.117	2.456	0.422
	Overweight	11	41	1.008	0.476	2.132	0.984
	Obese	14	14	3.756	1.659	8.502	0.001
	Normal	41	154	Reference	—	—	—
	Missing	121	956	—	—	—	—

OR = Odds ratio, 95% CI 95% Confidence interval for odds ratio, WHWT West Highland white terrier

was a significant difference between cases and controls (P<0.001, Table 2).

One hundred and twenty-six cases and 517 controls had a recorded weight with a median value in kilograms of 18.4 (range 1.4 to 70 kg). There was no statistical significance between the weights of the case and control groups (P=0.747). Data on weight compared to a reference population (Diez and Nguyen 2006) were available for 68 cases and 223 controls. Animals less than one year of age were excluded from this particular analysis,

Table 3. Sex and neutering status of case and control animals

	Cases				Controls			
	FE	FN	ME	MN	FE	FN	ME	MN
n	37	87	52	12	206	378	485	110
% of Category	29.8	70.2	81.3	18.8	35.3	64.7	81.5	18.5
Total n	124		64		584		595	

FE = Female entire, FN = Female neutered, ME = Male entire, MN = Male neutered

as they may not yet have attained mature bodyweight. There were 41 "normal", 11 "overweight", 14 "obese"

Table 4. Results of multi-variable binary logistic regression

Variable	Category B	Number of cases	Number of controls	OR	95%CI Lower	95%CI Upper	P
Breed	—	—	—	—	—	—	<0.001
	Rottweiler	1.633	0.413	5.12	2.281	11.494	<0.001
	WHWT	0.958	0.312	2.605	1.414	4.8	0.002
	Golden retriever	0.704	0.380	2.022	0.961	4.256	0.064
	Yorkshire terrier	1.045	0.341	2.843	1.456	5.551	0.002
	Labrador	0.264	0.319	1.301	0.697	2.43	0.408
	Cross breed	0.087	0.212	1.091	0.72	1.655	0.681
	Other pure breed	—	—	Reference	—	—	—
Sex	Female	0.720	0.172	2.054	1.467	2.877	<0·001
	Male	—	—	Reference	—	—	—
Age (years)	—	—	—	—	—	—	<0.001
	≤2 years	1.401	0.337	0.246	0.127	0.477	<0.001
	>8 years	—	—	Reference	—	—	—

Neutering status not included in final model. In earlier model B=0.080, SE=0.187, OR=1.083, 95% CI=0.751 to 1.563, P=0.669
B Unstandardised regression coefficient, SE Standard error of the regression coefficient, OR Odds ratio, 95% CI 95% Confidence interval for odds ratio, WHWT West Highland white terrier

and 2 "underweight" cases. Among the controls 154 were "normal", 41 were "overweight", 14 were "obese" and 14 were "underweight". There was a significant difference in these weight categories between the cases and controls (P=0.009, Table 2). In univariable logistic regression analysis, dogs that were "obese" were almost four times as likely to sustain a cruciate rupture than those of "normal" weight (OR 3.756, 95% CI 1.659 to 8.502, P=0.001, Table 2). There was no significant difference between the median weight in kilograms of neutered versus entire animals in the case group (P=0.994) or in the control group (P=0.630). There was also no significant difference in body condition (underweight/normal weight/overweight/obese) of neutered versus entire dogs among the cases (P=0.243), or the controls (P=0.211).

Multi-Variable logistic regression analysis

The frequency of CrCL ruptures seen in this study was 1.19% (95% CI 1.02 to 1.36%). Breed was significantly associated with cruciate rupture in specifically the Rottweiler, West Highland white terrier and Yorkshire terrier, all demonstrating increased odds of CrCL failure (Table 4). Rottweilers had the greatest significant association and were approximately five times more likely to sustain a cruciate rupture compared with "other" pure breed dogs after adjusting for other factors in the model (OR 5.12, 95% CI 2.281 to 11.494, P<0.001, Table 4). Females were significantly associated with having a cruciate failure and were twice as likely to present with rupture as males (OR 2.054, 95% CI 1.467 to 2.877, P<0.001, Table 4). Dogs less than or equal to two years of age were significantly less likely to sustain a cruciate rupture than animals over eight years of age (OR 0.246, 95% CI 0.127 to 0.477, P<0.001, Table 4). Neutering status was no longer significant in the multi-variable analysis (P=0.669). Obesity was not included in the multi-variable model because of a high percentage of missing values (64% of cases, 81% of controls missing). Model fit was assessed as good as indicated by the Hosmer-Lemeshow test (P=0.822). There were no DFBeta values greater than 1 and the maximum deviance value was 2.75, also suggesting good model performance.

Discussion

CrCL ruptures are commonly seen in first-opinion veterinary practice. The purpose of this study was to perform a fresh appraisal of risk factors: breed, age, gender, reproductive status and weight, associated with rupture of this ligament in the UK.

The frequency of CrCL ruptures seen in this study was 1.19%. Data for cases withdrawn from the population were unavailable, so an overall incidence could not be calculated; therefore, this figure cannot be compared directly to the reported incidence of 3.48% found in one USA study [Slauterbeck and others 2004]. There is a paucity of literature comparing the lifestyles of dogs in the UK and USA, so it is only possible to speculate that a difference in breed genetics, husbandry or exercise could explain any difference that may have existed between the two studies. Neither study was able to distinguish between partial tears and complete ruptures, so no conclusions can be drawn as to the incidence of each of these individually.

Animals that ruptured CrCL were significantly older than control animals. Whitehair and others (1993) previously reported the peak incidence of CrCL rupture to be 7 to 10 years of age. Our results parallel theirs, with a median CrCL rupture of 8.08 years. Young dogs do not have the same degree of degeneration of the CrCL as older dogs [Vasseur and others 1985]. This explains the finding that dogs under the age of two years were statistically less likely to sustain CrCL rupture compared to animals over eight years of age. Older dogs will have a greater degree of degeneration, which means the CrCL is more likely to rupture during normal activity than in younger dogs [Vasseur and others 1985].

Breeds found to be at risk in this study parallel the findings of both Whitehair and others (1993) and Duval and others (1999). However, in contrast to these studies, no correlation was found between breed and age. This finding could reflect population selection bias (tertiary referral compared to first-opinion practice) or case age selection (dogs less than two years compared to those of all ages).

No previous study has compared a "CrCL ruptured" population with a control population to assess obesity (bodyweight as a percentage of recommended breed weight) as a risk factor for CrCL. Previous studies have only assessed this subjectively [Barnes 1977] or compared weight of affected dogs against their non-affected control population [Whitehair and others 1993, Duval and others 1999]. No significant difference was found between bodyweight of either group; however, when weights were compared to breed and sex of a reference population [Diez and Nguyen 2006] and univariable analysis performed, "obese" dogs were almost four times as likely to sustain a cruciate rupture as those of "normal" weight. Theoretically, in obese animals, there will be increased loading of the limbs and increased tension on the ligaments within the joints, which could predispose these ligaments to rupturing. In human beings with obesity, an increased risk of osteoarthritis of certain non-weight-bearing joints in the hand suggests that metabolic factors are likely to play a role in the relationship between obesity and OA. Recent investigations into the role of adipokines in the development of OA have demonstrated the negative impact of leptin and adiponectin on chondrocyte health [Gualillo 2007, Simopoulou and others 2007]. Leptin levels have been shown to be higher in females compared to males even after adjustments for BMI have been made and could account for the sex bias of certain diseases such as osteoarthritis [Teichtahl and others 2005]. These findings warrant further investigation into the role of obesity in cruciate rupture.

Morphometric techniques that combine stature and bodyweight (e.g. body condition scoring) would be a preferred method of obesity assessment; however, this study was limited by its retrospective nature and this was not possible but our findings following univariable analysis, suggest that correlation between obesity and CrCL rupture exists.

In the initial analysis neutered animals were almost twice as likely to sustain a CrCL rupture than entire animals (OR 1.575, 95% CI 1·157 to 2·145, P=0·004; Table 2). However, neither weight (in kilograms) nor body condition was significantly different between entire and neutered dogs in the case or control groups. This implies that there was no association observed between neutering status and obesity. Female dogs were twice as likely to suffer cruciate rupture than their male counterparts. Although female sex and neutering status individually were significant in the initial analysis, the multi-variable model indicated that an amalgamated female neutered (FN) category was not significant.

Duval and others (1999) found that 21% of dogs had bilateral rupture at presentation and 16% of dogs with unilateral failure later developed rupture of the contralateral CrCL. Doverspike and others (1993) report that as high as 37% of patients rupture the contra-lateral ligament, but this increases to 59% if radiographic changes are visible in the "uninjured" joint. Stifle radiographs were not a prerequisite for inclusion into this study as

they were not taken in all cases. Because of the retrospective nature, the clinical examination could not be standardised and data regarding other orthopaedic conditions were not always reported at the time of presentation, preventing the inclusion of criteria such as medial patella luxation being included in statistical analysis. This was not a paper investigating surgical outcome, so number of cases treated medically or surgically was beyond its scope. A recent paper evaluating tests used to diagnose canine CrCL failure concluded that the cranial drawer test was highly specific for CrCL failure. However, patella tendon palpation and radiographic assessment of the infrapatellar fat pad had better sensitivities, although they only detect joint swelling, which is not specific to CrCL failure [Carobbi and Ness 2009]. There is a possibility that by only including dogs with cranial drawer sign, those animals with full or partial tears but no cranial drawer were excluded, thus underestimating the frequency of CrCL ruptures over the study period.

Of the 189 dogs in this study, just under 2% had bilateral rupture at time of first presentation and 11% developed CrCL rupture of the contralateral limb at a later date; however, no phone survey or other follow-up was conducted, so this value may have been higher as the author cannot rule out cases that may have presented to a different clinic with a contralateral CrCL failure, and this is a limitation of this retrospective study. Bilateral rupture is likely to be due to degenerative changes, and may be due to the increase in loading and force on the contralateral leg following unilateral failure. This may explain why many occur within 12 months of the initial presentation. By acquiring cases from general practice it is probable that cases included better represent the canine population within the UK as compared to the skewed population seen within referral institutes. As this study drew cases from a single hospital, there is a risk that local preference for breeds and local practices may cause geographical bias. All attempts were made to negate any bias by drawing conclusions against a control population from the same hospital. Assumptions that dogs that varied from their mean breed weight were truly over/underweight rather than small/large for their breed and in good condition have to be made with caution; however, with the inclusion of a 15% tolerance on either side of the mean breed weight average, it is likely that this possibility has been minimised.

Elucidating predisposing factors would be beneficial as it would allow owners to be better educated when purchasing a dog and reduce the chance of CrCL rupture occurring with both welfare and economic implications for owners and allow a veterinarian to dispense better advice to their clients.

This study showed that the risk factors for CrCL rupture included being a Rottweiler (five times greater risk than "other" breeds), being obese (four times increased risk compared to dogs of normal bodyweight) and being female (twice as likely as males). Dogs aged less than two years were less likely to sustain CrCL rupture than dogs older than eight years and neutering was not a significant risk factor in the development of CrCL rupture following multi-variable analysis.

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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 (SVK)

Primary hyperaldosteronism in cats: a series of seven cases

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SUMMARY

Primary hyperaldosteronism is a disease characterised by elevated aldosterone secretion by the adrenal glands. The present case series describes seven cats with primary hyperaldosteronism presented between 2002 and 2011. Common clinical signs were weakness, anorexia, cervical ventroflexion and blindness. All cats showed hypokalaemia. Blood pressure measurement in six cats revealed hypertension in five; four of the five cats were blind because of retinal detachment. Ultrasonographic examination showed unilateral adrenomegaly in six cats and normal adrenals in one. The serum aldosterone concentration exceeded the reference range in four cats.
Five cats underwent unilateral adrenalectomy, which was without complications and led to normalisation of the electrolyte concentrations. Histological examination of the adrenal glands revealed adrenocortical adenoma in four cats and adrenocortical carcinoma in two; the cat with ultrasonographic normal adrenals had bilateral nodular adrenal hyperplasia.

Key words: primary hyperaldosteronism, cat, hypertension, hypokalaemia, aldosterone

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Introduction

Primary hyperaldosteronism is a condition characterised by overproduction of aldosterone by the adrenal cortex. The first report of this disease in cats was published in 1983 and 36 feline cases have been described since [Eger et al., 1983, Flood et al., 1999, MacKay et al., 1999, Moore et al., 2000, Rijnberk et al., 2001, Ash et al., 2005, DeClue et al., 2005, Javadi et al., 2005, Reimer et al., 2005, Rose et al., 2007, Djajadiningrat-Laanen et al., 2008, Briscoe et al., 2009, Renschler and Dean, 2009]. Causes include neoplasia and hyperplasia of the adrenal cortex that lead to an autonomous aldosterone secretion that is independent of the renin concentration [Fig. 1; Schulman, 2010]. Aldosterone increases tubular secretion of potassium and resorption of sodium and water in the distal nephron of the kidney. Increased losses of potassium in the urine result in hypokalaemia and manifest primarily as muscle weakness. Another effect of hyperaldosteronaemia is pronounced hypertension caused by the elevated intravascular volume and direct effects

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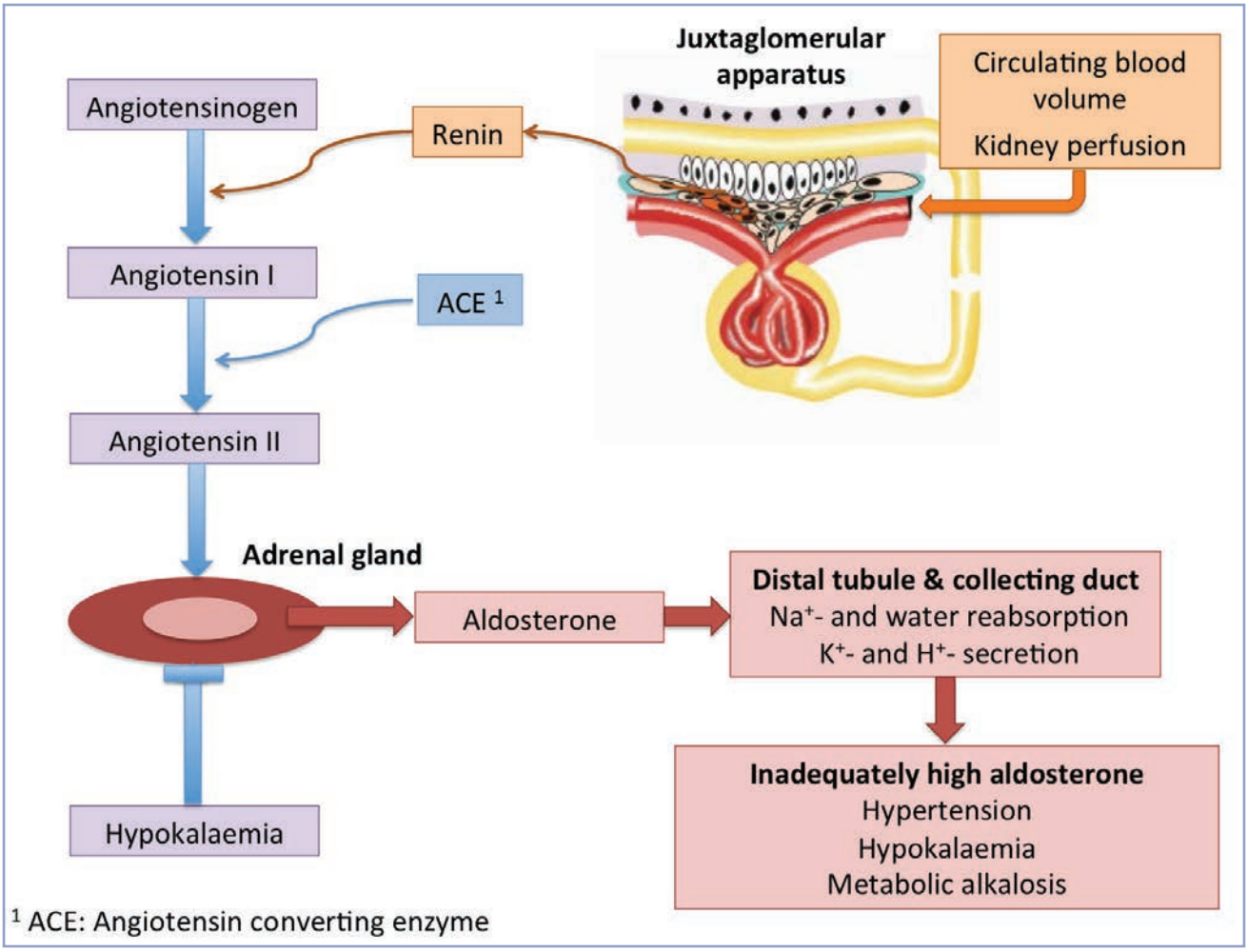

Fig.1. Schematic representation of the renin-angiotensin-aldosterone system and the effects of an inadequately high aldosterone concentration on serum electrolytes, acid-base and hydration status. Renin is secreted from cells of the juxtaglomerular apparatus of the kidney in response to a decrease in circulating blood volume or reduced renal perfusion. Renin carries out the conversion of angiotensinogen, which is released by the liver, to angiotensin I. Angiotensin I in turn is converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor and effects secretion of aldosterone from cells of the zona glomerulosa of the adrenal cortex. Hypokalaemia inhibits aldosterone secretion. Aldosterone stimulates the reabsorption of sodium and water and the secretion of potassium and hydrogen ions in the distal tubules and collecting ducts of the kidney. In primary hyperaldosteronism, inadequately high aldosterone levels increase reabsorption of water and loss of potassium and hydrogen ions, resulting in hypertension, hypokalaemia and metabolic alkalosis.

of aldosterone on the cardiovascular system [Schulman, 2010]. Hypertension in turn leads to damage of various organs, particularly the eye. Hypertensive retinopathies, retinal haemorrhage, retinal detachment and intraocular haemorrhage are common in cats with primary hyperaldosteronism [Schulman, 2010, Jepson, 2011].

The diagnosis is based on diagnostic imaging and hormone measurements. In most of these patients, ultrasonography reveals abnormalities in the shape or size of the adrenal glands. The detection of an elevated serum aldosterone concentration is characteristic of primary hyperaldosteronism [Schulman, 2010]. However, in dehydrated patients, the concentration of aldosterone may also be elevated as a physiological response to hypovolaemia. The measurement of plasma renin activity and calculation of a quotient (aldosterone concentration: renin activity) is therefore superior to the measurement of a random aldosterone concentration; cats with primary hyperaldosteronism usually have a higher ratio than healthy cats [Javadi et al., 2005].

The goal of this report was to describe the clinical presentation, laboratory abnormalities, treatment and outcome of seven cats with primary hyperaldosteronism and to discuss various tests for diagnosis of this disease.

Table 1. Signalment and clinical signs in 7 cats with primary hyperaldosteronism.

Cat No.	Breed[1]	Age (years)	Sex[2]	Clinical signs				
				Anorexia	Muscle weakness	Cervical ventroflexion	Blindness	Retinal detachment
1	Persian	17	FN	yes	yes	no	yes	yes
2	DSH	7	MN	no	yes	yes	no	retinal folds
3	DSH	15	FN	yes	no	yes	no	n.d.[3]
4	DSH	15	FN	yes	yes	no	yes	yes
5	DSH	16	MN	yes	no	no	yes	yes
6	DSH	unknown	FN	no	yes	no	yes	yes
7	Siamese	16	MN	no	yes	no	no	n.d.[3]
Total	**5 DSH 1 Persian 1 Siamese**		**4 FN, 3 MN**	**4/7**	**5/7**	**2/7**	**4/7**	**4/7**

[1] DSH, Domestic Shorthair, [2] FN, female neutered; MN, male neutered, [3] n.d., no ophthalmologic examination

Materials and methods

Animals

This retrospective study included seven cats with primary hyperaldosteronism (Table 1). Six cats were presented to the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty of the University of Zurich between 2002 and 2011, one cat was presented to a private veterinary practice near Zurich in 2008. Criteria for inclusion in this study were the presence of typical clinical signs, such as weakness, cervical ventroflexion or acute blindness, and a complete diagnostic work-up including haematological and serum biochemical analyses, aldosterone measurements, abdominal ultrasonography and histological evaluation of adrenal glands.

Haematological and serum biochemical examinations

Haematological and serum biochemical examinations were conducted at the Clinical Laboratory, Vetsuisse Faculty of the University of Zurich, using the Cell-Dyn 3500 System (Abbott, Baar, Switzerland) or Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan) for the former and the Cobas Integra 700 System (Roche Diagnostics, Rotkreuz, Switzerland) for the latter.

Ultrasonographic examination of the abdomen

Ultrasonographic examination was carried out by radiologists in the Division of Diagnostic Imaging of the Vetsuisse Faculty, University of Zurich, in six cats and by an external veterinary radiologist in one case. Published reference ranges were used for evaluating adrenal gland size [Zimmer et al., 2000].

Ophthalmological examination

Five cats underwent ophthalmological examination by clinicians in the Division of Ophthalmology of the Vetsuisse Faculty, University of Zurich, using slit-lamp (Kowa SL 14; Kowa Company Ltd., Tokio, Japan) and indirect ophthalmoscopy (Heine Omega 500, Heine Optotechnik, Herrsching, Germany). A diagnosis of retinal detachment was made when the fundic examination revealed complete or localised detachment of single or multiple bullous or flat retinal sections.

Blood pressure measurement

The blood pressure was measured in six cats using the Doppler technique (Model 811-B; Parks Medical Electonics, Aloha, USA) as described [Reusch et al., 2010]. A systolic blood pressure ≥ 150 mmHg was defined as hypertension [Brown et al., 2007].

Endocrinological examinations

In all cats, serum aldosterone concentration was measured by a commercial laboratory (Unilabs Dr. Weber, St. Gallen, Switzerland) using a direct

radioimmunoassay (RIA, Coat-A-Count Aldosterone, Siemens Medical Solutions Diagnostics, Los Angeles, USA). Established reference ranges for cats were used for interpretation [Zimmer et al., 2000]. In one cat (No. 6), the aldosterone-to-renin ratio was measured and a fludrocortisone suppression test carried out. The renin activity was determined using an enzymatic assay at the Centre Hospitalier Universitaire Vaudois in Lausanne [Nussberger et al., 1987]; the renin activity of three healthy cats was measured for comparison because reference ranges were not available. For this purpose, one ml of blood was collected into a chilled EDTA tube and centrifuged at 4°C. The harvested plasma was stored at -20°C and shipped to the laboratory on dry ice. In the same plasma sample, the aldosterone concentration was measured using a fluid-phase assay [Nussberger et al., 1984] and the aldosterone concentration (pmol/l)-to-renin activity (fmol/l/s) was calculated [Javadi et al., 2004]. For the fludrocortisone suppression test, fludrocortisone acetate (Florinef, 0.05 mg/kg) was administered orally twice daily for four days and the urine aldosterone-to-creatinine ratio determined before the first and 12 hours after the last administration (Unilabs Dr. Weber). Urine was collected via cystocentesis, chilled and sent to the laboratory in an insulated cooler (Sarstedt cooling transport container, Sarstedt, Sevelen, Switzerland). The urine aldosterone concentration was measured using a direct RIA (Coat-A-Count Aldosterone, Siemens Medical Solutions Diagnostics, Los Angeles, USA) and the creatinine concentration was determined using the Jaffé method (ARCHITECT® c Systems™, Abbott, Baar, Switzerland).

Results

Signalment and clinical presentation

The signalment, clinical signs and blood pressure of the cats are summarised in Tables 1 and 2. There were five Domestic shorthair, one Persian and one Siamese cats that ranged in age from seven to 17 years (median, 16 years). They included four neutered females and three neutered males. The most frequent clinical signs were anorexia, weakness, cervical ventroflexion and blindness.

In six cats, systolic blood pressure was measured: five cats had hypertension with systolic blood pressures ranging from 180 to 240 mm Hg, one cat had a systolic blood pressure of 140 mm Hg. Four cats with hypertension were blind and had retinal detachment, which was bilateral in three patients and unilateral in one. The latter cat showed multifocal retinal haemorrhages in the contralateral eye. One other cat had bilateral retinal folds.

Table 2. Blood pressure, biochemical results and serum aldosterone concentration in 7 cats with primary hyperaldosteronism

Cat No.	Blood pressure	Potassium (mmol/l)	Sodium (mmol/l)	Blood urea nitrogen (mmol/l)	Creatinine (umol/l)	Creatine kinase (U/l)	Aldosterone (pmol/l)
Reference range	**<150 mm Hg**	**3.8–5.5[1]**	**158-165[1]**	**7.4–12.6[1]**	**98-163[1]**	**77 – 355[1]**	**19 – 1579[1]**
1	240	3.2	159	11.2	123	n.d.[2]	1238
2	n.d.[2]	2.7	156	12.5	185	16400	670
3	140	2.9	165	9.7	142	n.d.[2]	2737
4	240	3.0	158	11.0	101	182950	1634
5	240	2.4	152	13.7	201	n.d.[2]	706
6	185	2.8	182	32.3	321	n.d.[2]	3546
7	180	2.2	161	11.7	136	9205	7853
Total	**increased 5/6**	**decreased 7/7**	**increased 1/7**	**increased 2/7**	**increased 3/7**	**increased 3/3**	**increased 4/7**

[1] 5th and 95th percentiles, [2] not done

Haematological and biochemical examinations

All cats were hypokalaemic (2.2 – 3.2 mmol/l, median 2.8 mmol/l, Table 2) at the initial examination. One cat showed hypernatraemia and three were azotaemic. The activity of creatine kinase was significantly increased in the three cats in which this parameter was measured.

Ultrasonographic examination of the adrenal glands

Six cats had unilateral adrenomegaly (Table 3). The enlarged adrenal glands were spherical in three cats (Fig. 2) and rounded and lobular in one other. The echogenicity of the glands was heterogeneous in four and homogeneous in two cats. The adrenal glands appeared ultrasonographically normal in one cat. For none of the abnormal adrenal glands was vascular invasion evident.

Endocrinological examinations

The serum concentration of aldosterone ranged from 670 to 7,853 pmol/l (median 1634); it was above the normal range in four cats, in the high normal range in one cat and in the middle normal range in the remaining two cats (Table 2). The aldosterone-to-renin ratio in cat No. 6 was 29.4, which was ten times the ratio of the three healthy control cats (1.9 – 3.5). The fludrocortisone suppression test revealed a markedly increased baseline urinary aldosterone-to-creatinine ratio of 1600 x 10^{-9} (cut off < 46 x 10^{-9}) and no notable suppression after fludrocortisone (1200 x 10^{-9}, cut off, < 6 x 10^{-9}; [Djajadiningrat-Laanen et al., 2008].

Table 3. Ultrasonographic and histological examinations, treatment and outcome in 7 cats with primary hyperaldosteronism.

Cat No.	Adrenomegaly		Histological diagnosis	Adrenalectomy	Outcome	Survival time[1]
	left	right				
1	no	no	Nodular hyperplasia	no	Euthanasia because of deteriorating condition	3 months
2	no	yes	Adenoma	no	Euthanasia because of decompensated cardiomyopathy	3 months
3	no	yes	Adenoma	yes	Euthanasia because of multiple abdominal masses	23 months
4	yes	no	Carcinoma	yes	Normal electrolytes, stable kidney values, no hypertension	No follow-up after 7 months
5	no	yes	Carcinoma	yes	Normal electrolytes, stable kidney values, no hypertension	No follow-up after 5 weeks
6	no	yes	Adenoma	yes	Euthanasia because of severe renal azotaemia	3 months
7	yes	no	Adenoma	yes	Recurrence of a mass in the region of the removed left adrenal gland	Alive at the time of writing (7 months)

[1] *Interval between first presentation and euthanasia or last follow-up examination*

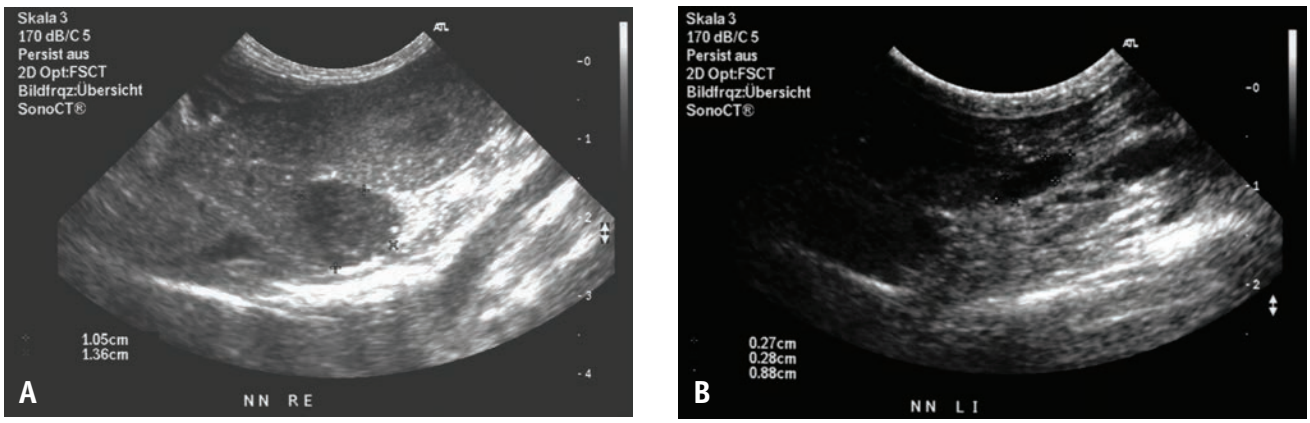

Fig.2. Ultrasonographic representation of the neoplastic right (a) and unremarkable left adrenal gland (b) of cat No.6.

Treatment and outcome

All cats were treated medically, and five underwent unilateral adrenalectomy after stabilisation (Table 3). Medical treatment included infusion therapy, parenteral (KCl, 20 - 60 mmol/l in Ringer's lactate solution as constant rate infusion via indwelling catheter) or oral potassium substitution (potassium gluconate, 2 - 6 mmol/cat twice daily) and spironolactone (Aldactone®, 5 - 12.5 mg/cat, orally once or twice daily). The four cats with a systolic blood pressure >180 mmHg also received amlodipine (Norvasc®, 0.625 – 1.25 mg/cat, orally once daily), which lowered the blood pressure to 140 - 170 mm Hg; amlodipine was discontinued in two cats after adrenalectomy. Cat No. 7 with a systolic blood pressure of 180 mmHg did not receive antihypertensive treatment before adrenalectomy. The three azotaemic cats were treated with benazepril (Fortekor®, 1.25 - 2.5 mg/cat, orally once or twice daily). Cat No. 2 with advanced hypertrophic cardiomyopathy received furosemide (Lasix®, 2 mg/kg, orally twice daily).

Adrenalectomy was without complications and led to normalisation of the electrolyte concentrations in all five operated cats and to normalisation of blood pressure in three of four hypertensive cats. Persistent hypertension in the remaining cat was attributed to chronic renal insufficiency. Blood pressure was not measured after adrenalectomy in one cat. Abdominal ultrasonography carried out one month postoperatively in one cat and five months postoperatively in one other showed no changes in size of the remaining adrenal. However, a 1.3 cm x 2.4 cm hypoechoic mass with a hyperechoic centre was seen in the region where the adrenal gland had been removed in one of the cats. Recurrence of the aldosteronoma was considered unlikely because the potassium and aldosterone concentrations were normal at that time point and a tentative diagnosis of granuloma or abscess was made. The owner declined further diagnostic work-up.

The survival time was available for four of the cats and ranged from three to 23 months (Table 3). Two other cats (Nos. 4 and 5) were followed for seven months and five weeks, respectively, after initial presentation. The remaining cat (No. 7) was still alive at the time of this writing, which was seven months after initial presentation.

Histological examination

Histological examination of the adrenal glands revealed adrenocortical adenoma in four cats and adrenocortical carcinoma in two (Table 3, Fig. 3). The cat with normal adrenal glands on ultrasonography had bilateral nodular adrenal hyperplasia.

Discussion

Primary hyperaldosteronism is a well-known condition in people, particularly in hypertensive individuals, but has only started to attract attention in veterinary medicine in the last few years. This series describes seven cases of primary hyperaldosteronism in cats in Switzerland. With the exception of one cat, all were 15 years of age

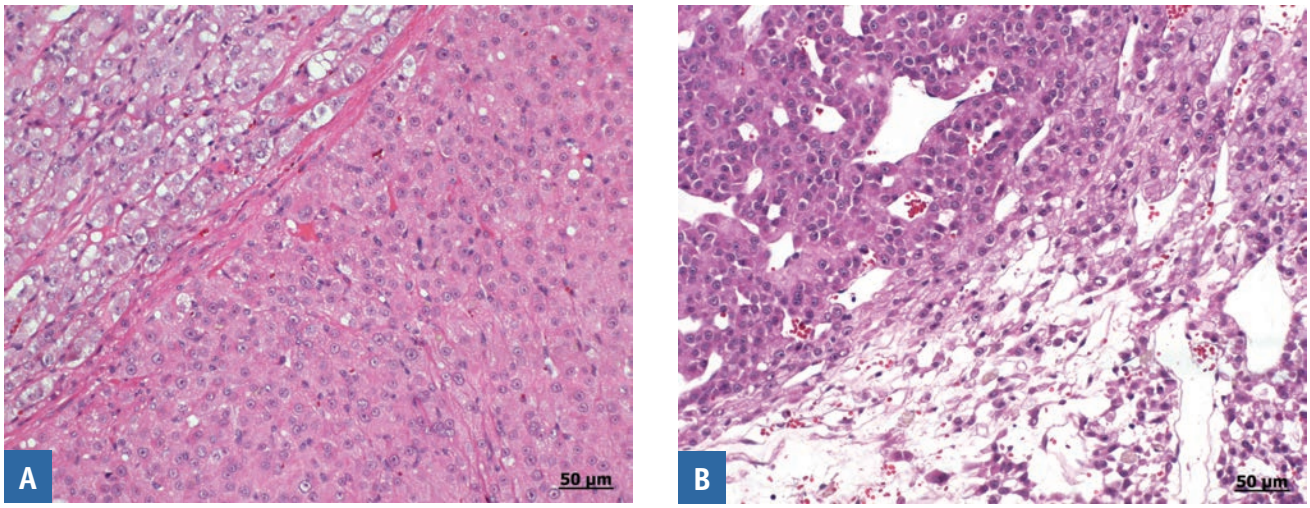

Fig.3. Histological sections of adenoma (a) and carcinoma (b) of the adrenal cortex of cats Nos. 6 and 4, respectively. (a) Adenoma of the adrenal cortex: proliferation of adrenal cortical cells delimited by a thin capsule of connective tissue. A section of non-neoplastic adrenal medulla is visible in the upper left. (b) Carcinoma of the adrenal cortex: there is a solid infiltrative proliferation of adrenal cortical cells.

or older, which was in general agreement with previous reports involving a total of 36 cats ranging in age from five to 18 years (median, 13 years)[Eger et al., 1983, Flood et al., 1999, MacKay et al., 1999, Moore et al., 2000, Rijnberk et al., 2001, Ash et al., 2005, DeClue et al., 2005, Javadi et al., 2005, Reimer et al., 2005, Rose et al., 2007, Djajadiningrat-Laanen et al., 2008, Briscoe et al., 2009, Renschler and Dean, 2009].

The most common clinical signs of hyperaldosteronism are anorexia, muscle weakness, cervical ventroflexion and blindness. While the first two signs are nonspecific in old cats, acute blindness is not and a possible association with hypertension should always be investigated. Hypertension is among the most common causes of retinal detachment in cats [Maggio et al., 2000] and primary hyperaldosteronism was associated with hypertension in 30 of the 36 cases published in the literature to date. Five cats of this case series had hypertension and four had irreversible blindness attributable to retinal detachment.

All cats of this study were hypokalaemic, which is the principal biochemical abnormality in cats with primary hyperaldosteronism [Schulman, 2010]. Other common causes of hypokalaemia include chronic anorexia, chronic diarrhoea and polyuria, especially due to chronic renal insufficiency. Differentiation of primary hyperaldosteronism and chronic renal insufficiency is not straightforward because both conditions occur mainly in older cats and both may be accompanied by hypertension, hypokalaemia and muscle weakness. Moreover, about 60% of cats with primary hyperaldosteronism have azotaemia at the time of first presentation, which may further complicate differentiation of the two conditions. The authors of a recent study postulated that primary hyperaldosteronism is involved in the development of chronic renal insufficiency, possibly via fibrotic and sclerotic vascular changes caused by elevated aldosterone concentration and hypertension which finally lead to kidney damage [Javadi et al., 2005].

The detection of an adrenal mass supports the diagnosis of primary hyperaldosteronism: six of the seven cats of the present study had unilateral adrenomegaly. A tentative diagnosis of primary hyperaldosteronism based on adrenomegaly and persistent hypokalaemia should be confirmed by hormonal analysis. Measurement of serum aldosterone concentration can be easily accomplished and is offered by commercial laboratories. Reference ranges vary between laboratories and with the assays used. An elevated aldosterone concentration in a cat with persistent hypokalaemia and adrenomegaly makes primary hyperaldosteronism very likely. However, the aldosterone concentration can be affected by several factors. Dehydration causes a physiological increase in aldosterone levels and measurements in sick dehydrated patients not suffering from the disease could lead to a spurious diagnosis of primary hyperaldosteronism. Hence, patients should be rehydrated before aldosterone measurements. Furthermore, aldosterone must be interpreted in relation to the potassium concentration; an aldosterone concentration in the reference range along with pronounced hypokalaemia should be judged as inadequately high because adrenal aldosterone secretion is suppressed by hypokalaemia (Fig. 1). The aldosterone concentration exceeded the reference range in four cats and was within the reference range in the remaining three cats of this study.

Because of the difficulty interpreting concentrations of single hormones, the measurement of hormone pairs is common in diagnostic endocrinology. This also applies for the diagnosis of primary hyperaldosteronism in the form of the aldosterone-to-renin ratio. Whereas under physiological conditions, an increase in the aldosterone concentration is accompanied by an elevated renin activity (Fig. 1), cats with primary hyperaldosteronism exhibit an autonomous aldosterone secretion that occurs independently of the renin activity. Therefore, the aldosterone-to-renin ratio is higher in cats with primary hyperaldosteronism than in healthy cats (Javadi et al., 2005). However, it is critical that the blood be collected into chilled tubes and the plasma shipped on dry ice to specialised laboratories that use enzymatic assays to measure feline renin activity. Suppression tests offer an alternative for the diagnosis of over-function of hormone-secreting organs. The fludrocortisone suppression test is based on the fact that individuals with primary hyperaldosteronism exhibit an autonomous aldosterone secretion that cannot be suppressed with fludrocortisone. The test requires the oral administration of fludrocortisone tablets by the owner for four days, and is contraindicated in patients with severe hypokalaemia. To date, results of this test have only been published for healthy cats and one cat with primary hyperaldosteronism [Djajadiningrat-Laanen et al., 2008]. Because of the inconvenience of renin activity measurement and the

fludrocortisone suppression test, these diagnostic procedures were limited to a single cat (No. 6) in the present study; as expected, the aldosterone-to-renin ratio was much higher (ten-fold) than in healthy cats and fludrocortisone did not suppress the urinary aldosterone-to-creatinine ratio.

A recent case series has described 11 cats with primary hyperaldosteronism that lacked adrenomegaly and were diagnosed with nodular hyperplasia of the adrenal cortex [Javadi et al., 2005], similar to cat No. 1 of the present series. This underlines that although unilateral adrenomegaly supports the diagnosis, adrenal glands of normal size do not rule out primary hyperaldosteronism. In these cases, a definite diagnosis has to be based on the results of hormone measurements.

In summary, primary hyperaldosteronism is an important differential diagnosis in cats with muscle weakness, retinopathy, hypertension and persistent hypokalaemia. A tentative diagnosis can be made based on hypokalaemia, hypertension and the ultrasonographic detection of an adrenal mass. The diagnosis is confirmed by hormone measurements. Concurrent diseases such as chronic renal insufficiency or cardiomyopathy are common. The prognosis of primary hyperaldosteronism is relatively favourable in cats, particularly after adrenalectomy.

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REPRINT PAPER (A)

Chronic nasal disease in cats - A retrospective study

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SUMMARY

In this retrospective study of 41 cats with chronic nasal disease, the following disorders were diagnosed: neoplasia (n = 19), idiopathic chronic rhinosinusitis (ICRS) (n = 12), nasopharyngeal polyps (n = 3), foreign bodies (n = 2), nasopharyngeal stenosis (n = 1) and nasal aspergillosis (n = 1). In three cats, diagnosis could not be established despite thorough diagnostic work-up. Sex, indoor or outdoor housing, quality and quantity of nasal discharge, bacteriological findings of nasal flushes as well as radiological and CT findings did not differ significantly between cats with neoplasia and cats with ICRS. Cats with neoplasia were older (3 – 15 years, median age 11 years) and had displayed clinical symptoms for a shorter period of time (1 – 8 months, median 2 months) than cats suffering from ICRS (age: 1 – 13 years, median age 7.5 years; symptoms: 1 – 36 months, median 5 months). In all cats with neoplasia, rhinoscopy detected a mass, while this was only seen in 30 % of the cats with ICRS. In these cases, examination of biopsy samples is essential to establish an exact diagnosis. Combining the physical examination with imaging techniques, rhinoscopy and cytological/histopathological examination of biopsy samples increases the likelihood of achieving a correct diagnosis.

Keywords: Cat, nose, rhinitis, rhinoscopy, nasal discharge.

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Introduction

Chronic nasal disease in cats is a frequently encountered problem in small animal practice. Typical signs include nasal discharge, sneezing, nasal stridor, dysphagia, breathing through the mouth and coughing (Henderson et al., 2004; Michiels et al., 2003). Chronic nasal discharge can be due to many different diseases e.g. nasal neoplasia, infectious rhinitis (of mycotic, viral or bacterial origin), foreign body rhinitis, dental disease, congenital malformations (cleft palate), oronasal fistulae, nasopharyngeal polyps, nasopharyngeal stenosis or trauma (Van Pelt and Lappin, 1993; Michiels et al., 2003; Demko and Cohn, 2008). If an aetiological diagnosis cannot be established despite a thorough diagnostic work-up, the disease is classified as idiopathic chronic rhinosinusitis (ICRS) (Michiels et al., 2003; Demko and Cohn, 2007). As a targeted therapy is dependent on the underlying disease, systematic diagnostic work-up is crucial.

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At present, opinions differ on the specific value of the different diagnostic procedures. Physical examination, imaging techniques and rhinoscopy as well as cytological and histological examination of biopsy samples may shed light on the aetiology of the disease. In a recently published study (Demko and Cohn, 2007), an aetiological diagnosis could be established in only 36 % of the cats with chronic nasal discharge. This may, however, be due to the fact that in 43 % of the patients the diagnostic work-up was limited to a physical examination and no further diagnostic procedures were carried out. It is the aim of the present study to give an overview of the most common aetiological diagnoses, typical clinical signs and the diagnostic value of the different diagnostic procedures.

Materials and Methods

Animals

For the retrospective analysis, we evaluated patient records of cats that were presented to the Clinic for Internal Medicine at the Department/Clinic for Companion Animals of the University of Veterinary Medicine Vienna for chronic nasal discharge between January 2005 and February 2010.

Clinical signs

Data of 41 cats pertaining to five different diagnosis groups (neoplasia, ICRS, polyps, foreign bodies and other diseases) were evaluated. Age, sex, breed and housing conditions of the cats were documented. Case history , clinical symptoms, diagnoses and therapies were reviewed for each group. Special attention was given to the presence and type of unilateral or bilateral nasal discharge, sneezing, nasal stridor, cough, dysphagia, inappetence and behavioural changes as well as to the duration of symptoms and former treatments.

Diagnostic procedures

All results of haematological and blood chemistry analyses, radiographic examinations, CT images, bacteriological examinations of samples obtained by endoscopic nasal flushes (Johnson et al., 2005) as well as results of endoscopic examination of the nasal cavity and evaluation of histological examination of obtained biopsies were used for the present retrospective study. Evaluation of radiographic findings focused on the presence and localisation of opacities, signs of turbinate destruction, nasal septum deviation and osteolytic lesions. Analysis of the CT findings was based on the same criteria. Rhinoscopy was performed to look for hyperaemia of the nasal mucosa, turbinate destruction, large amounts of mucoid to purulent discharge and presence of solid masses, foreign bodies or fungal mycelium. Histologically confirmed chronic rhinitis without any proof of a specific underlying disease was classified as ICRS. All results of additional diagnostic procedures (FeLV antigen test, FIV antibody test, calicivirus or herpesvirus PCR) were also documented.

Statistics

Data of cats with neoplasia (n = 19) were compared with those of cats suffering from ICRS (n = 12). A Mann-Whitney-U-Test was used to compare age distribution and duration of symptoms. For evaluation of sex distribution, housing conditions, clinical signs and other findings, a Fisher's Exact Test was performed. Due to the reduced number of animals in the remaining four groups, patient data of these animals were not included in the comparison.

Results

Patient records of 41 cats showed the following diagnoses summarized in Figure 1: nasal neoplasia (n =

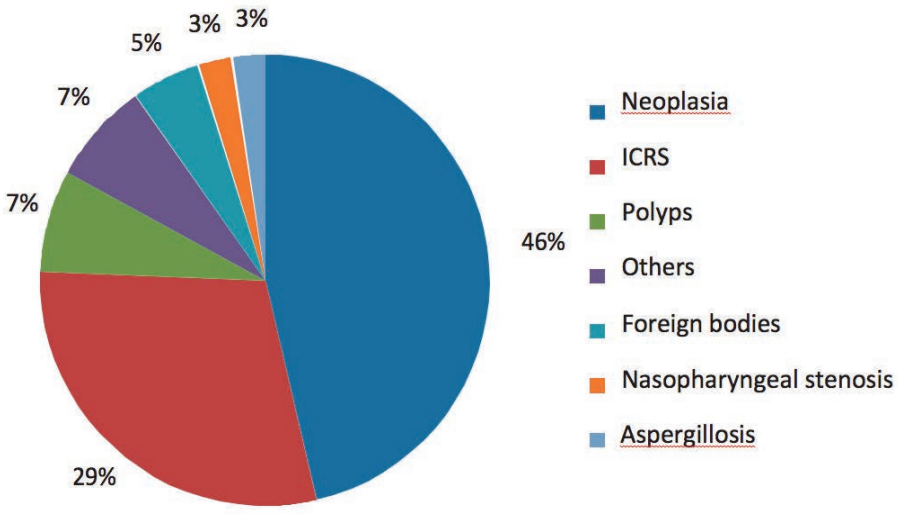

Figure 1: Frequency of diagnoses in cats with chronic rhinitis (ICRS/ Idiopathic chronic rhinosinusitis)

19), idiopathic chronic rhinosinusitis (ICRS) (n = 12), nasopharyngeal polyps (n = 3), foreign bodies (n = 2), nasopharyngeal stenosis (n = 1) and nasal aspergillosis (n = 1). In three cats, no definite diagnosis could be established.

Neoplasia

Nineteen cats were diagnosed with neoplasia. The average age of the patients was 3 to 15 years (median = 11 years). Of these patients, 10 cats were female (9 neutered, 1 intact) and 9 male castrates, meaning that the sex distribution was equilibrated. Both outdoor and indoor cats were affected. According to the history, some animals had already received treatment with corticosteroids or antibiotics.. The most frequent clinical symptoms included nasal discharge, nasal stridor and sneezing. The observed nasal discharge was both unilateral and bilateral and of haemorrhagic or purulent type (Table 1). Haematology did not reveal any particular pathological changes.

Table 1: Housing conditions, pretreatment, clinical signs and bacteriological findings in the different groups of patients.

	Neoplasia (n = 19)	ICRS (n = 12)	Foreign body (n = 2)	Polyps (n = 3)	Others (n = 5)
Indoor cat	11(19)	5(12)	0(2)	2(3)	3(5)
Outdoor cat	8(19)	7(12)	2(2)	1(3)	2(5)
Pretreatment with antibiotics	15(19)	7(12)	1(2)	2(3)	3(5)
Pretreatment with corticosteroids	3(19)	4(12)	0(2)	0(3)	1(5)
Nasal discharge, unilateral	11(14)	5(11)	0(1)	0(1)	2(5)
Nasal discharge, bilateral	3(14)	6(11)	1(1)	1(1)	2(5)
Nasal discharge, haemorrhagic	9(14)	4(11)	1(1)	0(1)	2(5)
Nasal discharge, purulent	8(14)	6(11)	1(1)	1(1)	1(5)
Sneezing	7(19)	5(12)	2(2)	0(3)	4(5)
Nasal stridor	10(19)	9(12)	0(2)	3(3)	3(5)
Open-mouth breathing	1(19)	1(12)	0(2)	2(3)	1(5)
Dysphagia	2(19)	1(12)	1(2)	2(3)	1(5)
Loss of body weight	2(19)	0(12)	0(2)	0(3)	0(5)
Facial deformity	2(19)	0(12)	0(2)	0(3)	0(5)
Apathy	0(19)	1(12)	0(2)	0(3)	0(5)
Cough	0(19)	1(12)	0(2)	0(3)	1(5)
Pseudomonades (bacteriology, nasal samples)	2(5)	5(10)	NA	NA	NA
Staphylococci (bacteriology, nasal samples)	0(5)	3(10)	NA	NA	NA
Pasteurella (bacteriology, nasal samples)	0(5)	2(10)	NA	NA	NA
Escherichia coli (bacteriology, nasal samples)	0(5)	2(10)	NA	NA	NA
Mycoplasma (bacteriology, nasal samples)	0(5)	2(10)	NA	NA	NA
Enterococci (bacteriology, nasal samples)	0(5)	1(10)	NA	NA	NA
Chlamydia (bacteriology, nasal samples)	1(5)	0(10)	NA	NA	NA

ICRS = Idiopathic chronic rhinosinusitis; NA = not assessed

In two cats, the ELISA test for FeLV was positive (n = 5). In one of these cats, a multicentric lymphoma with renal involvement was suspected based on the abdominal sonography findings. Bacteriology of the samples obtained by nasal lavage (n = 5) yielded positive results in three cats; in one patient, a mixed bacterial population was found (Table 1).
Lateral and ventrodorsal radiographs (n = 9) showed soft-tissue opacities in the nasal cavity and paranasal sinuses, turbinate destruction, osteolytic foci in the bones delimiting the nasal cavities as well as septum deviation. Similar findings were obtained by nasal computed tomography (n = 10) (Table 2). A solid mass was identified and biopsied in 19 cats during rhinoscopy. In 14/19 cats, lymphoma was diagnosed on the basis of cytological and histological examinations

Further diagnoses were adenocarcinomas (4/19), fibrosarcomas (2/19), squamous cell carcinomas (2/19), unclassified neoplasias of mesenchymal origin (2/19) and one neoplasia of histiocytic origin (1/19) (Table 2).

Idiopathic chronic rhinosinusitis

In 12 cats, the histologically confirmed inflammation of the nasal mucosa of unknown cause was classified as ICRS. The age of the patients varied between 12 months and 13 years (median = 7.5 years). Three of the affected cats, both indoor and outdoor, were female (2 neutered, 1 intact) and nine were male castrates. Some cats had already received prior treatment with antibiotics and corticosteroids. The most commonly observed clinical signs were nasal discharge, stertorous respiration and sneezing. Nasal discharge was bilateral in some cats and unilateral in others and of purulent or haemorrhagic type (Table 1). Haematology and blood biochemistry did not reveal any specific pathological changes. In five of the cats tested for FeLV antigen, the test result was negative. In one animal, the FIV antibody test brought a negative result. The Calicivirus PCR test was positive in one cat suffering from chronic bilateral purulent nasal discharge. Bacteriological examination of the fluid obtained by nasal lavage (n = 10) showed a mycoplasma infection in two cases, while the bacterial population was mixed in seven cats (Table 1).
In lateral and ventrodorsal radiographs taken in seven cats to evaluate nasal cavity and paranasal sinuses, soft tissue opacities and turbinate destruction were seen. Similar results were observed on the CT images (n = 5) (Table 2). Rhinoscopy revealed hyperaemia and swelling of the nasal mucosa as well as severe mucous accumulation within the nasal cavity in 8/12 patients. In 4/12 cats, a solid mass was identified. Mixed-cell inflammatory lesions represented the most common histological findings (11/12 cats). In one case (1/12), plasmacytic infiltration was diagnosed (Table 2).

Polyps

Nasopharyngeal polyps were identified by rhinoscopy in three cats aged between 3 and 24 months. The clinical signs displayed included nasal stridor, (3/3), open-mouth breathing (2/3) dysphagia (2/3) and bilateral mucopurulent nasal discharge (1/3). Radiographic images showed an opacity in the nasopharynx in 2/3 cats, while soft tissue opacities were identified in both nasal cavities and frontal sinuses in the third patient (1/3). The polyps were removed rhinoscopically by traction-avulsion.

Histological examination of the removed masses confirmed the macroscopically established tentative diagnosis of juvenile inflammatory polyps. One cat experienced recurrence of the symptoms two months after having been discharged from the clinic.

Foreign bodies

In two cats (one patient was presented twice in 6 months), nasal foreign bodies were found. These cats were aged 1 and 10 years, and both patients had been presented for sneezing. Bilateral purulent haemorrhagic nasal discharge was present in one cat, while another one suffered from dysphagia. Radiographs showed bilateral opacities in the nasal cavities of one cat; no pathological findings, however, were seen on the films of the other cat. Overall, three grass awns were removed rhinoscopically, one from the nasal cavity and two from the nasopharynx.

Nasal disease of other origin

Nasopharyngeal stenosis was diagnosed in one cat. Biopsies taken from both nasal cavities of this cat were histologically normal. In one cat, aspergillosis was suspected based on the typical intranasal white plaques observed macroscopically; this tentative diagnosis was confirmed by cytology. In another animal, rhinoscopy could not be completed due to anaesthesia complications. In two cases, a rhinoscopically identified mass was biopsied, but histological findings were ambiguous. The recommended recheck was declined by the cat owners.

Comparison between neoplasia and ICRS

Cats with nasal neoplasia were older (3 – 15 years, median

Table 2: Findings obtained by radiography, CT, cytology and histology in cats of the different groups

	Neoplasia (n = 19)	ICRS (n = 12)	Foreign body (n = 2)	Polyps (n = 3)	Others (n = 5)
Unilateral opacity Nc *R*	3(9)	4(7)	0(2)	0(3)	1(4)
Bilateral opacity Nc *R*	1(9)	3(7)	0(2)	0(3)	2(4)
Unilateral opacity Nc + Pns *R*	4(9)	0(7)	0(2)	0(3)	1(4)
Osteolysis *R*	3(9)	0(7)	0(2)	0(3)	1(4)
Septum deviation *R*	1(9)	0(7)	0(2)	0(3)	0(4)
Turbinate destruction *R*	4(9)	1(7)	0(2)	0(3)	2(4)
Destruction or retrobulbar bones *R*	1(9)	0(7)	0(2)	0(3)	0(4)
Unilateral opacity Nc *CT*	2(10)	1(5)	NA	NA	1(2)
Bilateral opacity Nc *CT*	1(10)	1(5)	NA	NA	0(2)
Unilateral opacity Nc + Pns *CT*	4(10)	1(5)	NA	NA	0(2)
Bilateral opacity Nc + Pns *CT*	3(10)	2(5)	NA	NA	0(2)
Osteolysis *CT*	5(10)	0(5)	NA	NA	1(2)
Septum deviation *CT*	1(10)	0(5)	NA	NA	0(2)
Turbinate destruction *CT*	7(10)	2(5)	NA	NA	1(2)
Destruction of retrobulbar bones *CT*	1(10)	0(5)	NA	NA	0(2)
Mass *E*	19(19)	4(12)	0(2)	3(3)	2(5)
Hyperaemia, swelling, hypersecretion *E*	5(19)	8(12)	1(2)	1(3)	4(5)
Lymphoma *C*	6(9)	0(2)	NA	0(2)	0(4)
Adenocarcinoma *C*	2(9)	0(2)	NA	0(2)	0(4)
Squamous cell carcinoma *C*	1(9)	0(2)	NA	0(2)	0(4)
Mixed inflammatory cell infiltrate *C*	0(9)	2(2)	NA	0(2)	0(4)
Plasmacytic inflammation *C*	0(9)	0(2)	NA	0(2)	0(4)
Lymphoma *H*	9(17)	0(12)	NA	0(3)	0(4)
Adenocarcinoma *H*	2(17)	0(12)	NA	0(3)	0(4)
Squamous cell carcinoma *H*	1(17)	0(12)	NA	0(3)	0(4)
Histiocytic neoplasia *H*	1(17)	0(12)	NA	0(3)	0(4)
Mesenchymal tumour *H*	2(17)	0(12)	NA	0(3)	0(4)
Fibrosarcoma *H*	2(17)	0(12)	NA	0(3)	0(4)
Mixed inflammatory cell infiltrate *H*	0(17)	11(12)	NA	0(3)	0(4)
Plasmacytic inflammation *H*	0(17)	1(12)	NA	0(3)	0(4)

ICRS = Idiopathic Chronic Rhinosinusitis; Nc = Nasal cavity; Pns = Paranasal sinuses; *R* = Radiology; *CT* = Computed tomography; *E* = Endoscopy; *C* = Cytology; *H* = Histology; NA = not assessed

age 11 years) than those diagnosed with ICRS (1 – 13 years, median age 7.5 years; p = 0.03). No significant difference was observed between the two groups regarding sex (p = 0.13), indoor vs. outdoor cat (p = 0.3), type of pretreatment (p = 0.2) and presence of unilateral (p = 0.1) or haemorrhagic (p = 0.16) discharge. At the time the cats were presented to the clinic, duration of clinical signs had been significantly longer in ICRS cats (1 – 36 months, median 5 months) than in the patients with neoplasia (1 – 8 months, median 2 months; p = 0.004). Results of

bacteriological examinations ($p = 0.09$) and frequency of pathological alterations observed in radiographs and CT images did not differ significantly between the two groups (data not specified). Significantly more masses were identified by rhinoscopy in the group of cats with neoplasia than in patients with ICRS ($p < 0.001$). A definite diagnosis of neoplasia could only be established based on cytological or histological findings.

Discussion

The results of the present study are largely in line with those of previous studies (O'Brien et al., 1996; Michiels et al., 2003; Henderson et al., 2004; Demko and Cohn, 2007). The most common diagnosis in this study was nasal neoplasia, with lymphoma being the most frequently identified mass. This corresponds with the results of earlier studies (O'Brien et al., 1996; Henderson et al., 2004). The second largest patient group were cats with ICRS. The aetiology of this disease is by definition unknown. It is therefore important to rule out other causes of chronic rhinitis. Although herpes virus and calici virus infections may cause acute rhinitis, they do not presumably play a role in chronic nasal disease (Johnson et al., 2005). However, previous infections with herpes virus and the resulting anatomical alterations in the nasal cavity with partial loss of mucociliary clearance capacity are under discussion as a possible cause of ICRS development (Michiels et al., 2003; Henderson et al., 2004). Histological examination of samples obtained from ICRS patients mostly reveals a mixed-cell and in rare cases a lymphoplasmacytic infiltration of the nasal mucosa (Michiels et al., 2003). Cats with neoplasia were older than cats with ICRS; this result is in line with those of other studies (Henderson et al., 2004; Demko and Cohn, 2007). At the time the patients were presented to the clinic, clinical signs had been present for a longer time in cats with ICRS than in animals with neoplasia. This may be due to the fact that ICRS progresses more slowly and takes a milder course. The most common clinical sign shown by the cats included in our study was nasal discharge. Unilateral nasal discharge was present in the majority of cats with neoplasia and was also observed in almost 50 % of the ICRS cats. Regarding haemorrhagic nasal discharge, the situation was similar. As already described in an earlier study (Demko and Cohn, 2007), signs did not differ significantly between the different groups of patients. Results of bacterial culture of samples obtained from the nasal cavity are not easy to interpret, as they may be influenced by the physiological mucosal bacterial flora as well as by secondary or primary infections. The majority of the spectrum of microorganisms observed in our patients can also be identified in healthy cats. As described in a former study (Johnson et al., 2005), mycoplasma was only found in the ICRS cats of our study, while tests were negative for the cats in the other groups. This means that mycoplasma cannot be ruled out as a primary pathogen responsible for ICRS in cats. Findings of radiological and CT imaging did not differ significantly between ICRS cats and cats with neoplasia. Literature also contains references to substantial overlappings of radiological findings between cats with nasal neoplasia and those with ICRS (O'Brien et al., 1996; Lamb et al., 2003). Whether the use of CT imaging is of any advantage in the diagnosis of feline nasal disease is controversially discussed (Karnik et al., 2009; Schoenborn et al., 2003). In the present study, imaging techniques could not clarify the aetiology of nasal disease, but gave a good overview of the extent and localisation of pathological alterations and were an aid to orientation for rhinoscopical examination and taking of biopsies. Solid masses were encountered significantly more frequently in cats with neoplasia than in patients with ICRS. However, a definitive diagnosis of neoplasia could be established only on the basis of cytological or histological findings from biopsies taken from our patients.

Inflammatory polyps, as found in three cats of our study are benign pedunculated masses originating from the Eustachian tube or the tympanic bulla, growing into the nasopharynx. Young animals are most commonly affected by polyps; congenital defects (Baker, 1982) or reactions to infections (Anderson et al., 2000) are being discussed as causes. Polyps can be removed by traction-avulsion, ventral bulla osteotomy or total ear ablation with lateral bulla osteotomy. Traction-avulsion is the most gentle procedure for removing a polyp. However, recurrence is possible (as was the case for one animal in our study), and a transient Horner´s syndrome may develop (Muilenburg and Fry, 2002, Veir et al. 2002). In one cat in our study, a marked stenosis of the nasopharynx was diagnosed by rhinoscopy. Nasopharyngeal stenoses may develop as a sequela of inflammatory diseases (Mitten et al., 1988) or be present as a congenital defect (Khoo et al., 2007). Nasal foreign bodies, which travel to the nasopharynx or the nasal cavities via the retrograde route, may also cause chronic rhinitis (Hender son et al., 2004). In our study, foreign bodies were diagnosed in two cats. While fungal rhinitis is rare in cats living in Central Europe, nasal cryptococcosis

is quite common in other geographic regions of the world (Demko and Cohn, 2007). Nasal aspergillosis is uncommon in cats (Schulz et al., 2003) and was diagnosed in only one cat of this study. Performing an endoscopic examination of the nasopharynx and nasal cavities enabled us to detect and remove or sample foreign bodies, fungal mycelium, polyps and masses.

In summary, no specific differences were observed between the groups representing the two most common causes of chronic nasal discharge (neoplasia and ICRS) except for the longer persistence of clinical signs before presentation in cats with ICRS, the older age of cats with neoplasia and the more frequent endoscopic diagnosis of solid masses in cats with neoplasia. Other causes of chronic nasal discharge like foreign bodies, polyps, stenoses and mycoses could be reliably identified by endoscopy. Taking biopsies, ideally under visual control, for cytological and histological examination is of particular diagnostic value in these patients. Combining a thorough physical examination with imaging techniques, rhinoscopy and cytological/ histopathological examination of biopsy samples increases the likelihood of achieving a correct diagnosis.

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