FECAVA
Federation of European Companion Animal Veterinary Associations

EJCAP ONLINE

Volume 24(2), Summer 2014

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Protecting travelling pets & their owners

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a Latvian piece to the puzzle

Crystalloids vs. colloids:
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Commissioned paper

Protecting travelling pets and their owners from disease – the role of the veterinarian

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SUMMARY

Clear changes in the distribution of vectors of disease in Europe have been evident over the past two decades. Climate change may be responsible for part of this, but changes in habitat management and host availability may also be influential. Coupled with the large increase in pet animal movements both due to the harmonisation of the non-commercial movement regulation across the EU and increased global travel, the risk of the spread of disease from endemic to non-endemic areas has been heightened significantly. Other important factors in the emergence of zoonotic and vector borne diseases include the increasing urbanisation and vicinity of human and animal populations along with social and cultural factors such as food habits, farming practices, international travel and trade (in particular in animal and animal products) which facilitate the global spread of emerging infectious diseases in a short space of time.

The effective control and management of vector-borne disease requires a comprehensive understanding of the biology and ecology of these vectors, the characteristic of the pathogens they transmit, the mechanisms of infection transmission and also the array of ecological and environmental factors that influence the interactions between them. The veterinary profession has a key role to play in the surveillance of disease, improving education and raising awareness of the risks associated with the travelling pet [9].

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Introduction

The major reduction of rabies in wildlife across Europe in the last 20 years together with reduced border restrictions has led to a dramatic increase in the movement of pets from country to country. This has resulted in an increase in the spread of exotic diseases, probably exacerbated by the effects of climate change, allowing disease transmitting vectors to spread further north from the Mediterranean basin. Animals are becoming more susceptible to diseases to which they had not been previously exposed. The role of the veterinarian in surveillance, screening for disease and educating the pet owner to help protect the health and welfare of travelling pets and also to warn of possible zoonotic infections is becoming increasingly important.

Trends in pet animal movements

In the 1990s, the rabies situation throughout the EU improved spectacularly following the implementation of oral rabies vaccination of foxes in regions affected by the sylvatic-rabies epidemic that had swept through mainland Europe since the 1960s.

In 2003, health requirements for the non-commercial

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movement of pets changed, and the United Kingdom, Republic of Ireland, Sweden, Finland and Malta were permitted extra controls for a five-year transition period. In January 2012, however, these countries were compelled to harmonise with the rest of the EU which meant that serology blood testing post rabies vaccination ceased, dogs cats and ferrets could travel 21 days after rabies vaccination and the requirement for tick treatment was dropped. However, the requirement to treat for the tapeworm *Echinococcus multilocularis* (EM) was retained for those countries where there was no evidence of EM in the wildlife, but the window for treatment prior to movement was extended from 24 to 48 hours to 1 to 5 days.

A new Regulation will come into force on the 29th December 2014, and this may have a knock-on effect on the movement of cats and dogs throughout Europe.

There is little data on the trends in pet animal movements but, as an example, it is estimated that in 2000 there were around 14,000 animals imported into the UK. By 2004 this had increased to more than 65,000 and in 2008 it was more than 100,000 animals. In 2012, the figure jumped to 154,000. The harmonisation of the rules across the EU in January 2012 then led to a 75% increase in pet movements into the UK over the following 12 months [1].

The non-commercial movement Regulation 998/2003 is specifically meant for owners to travel with their pets and not for the sale or transfer of ownership. However, increasingly the Regulation is misused for the purposes of trade in puppies across Europe.

Over recent years there has been a significant increase in the sale of puppies over the Internet, which has contributed to increased movement. This burgeoning demand for puppies has led to illegal movements of dogs, more recently becoming the target of organised crime. It is this illegal trade that has serious implications both for the welfare of these animals and for the risks of disease spread across borders.

There has also been an increasing trend for the adoption of stray street dogs by people who are travelling abroad both in Europe and beyond then bringing them back to their own country either legally or illegally. The health status of these dogs is often unknown so they pose a risk of disease incursion.

Most of these dogs will not have been socialised adequately, many have behaviour problems and therefore will not prove suitable as domestic pets. This often leads to abandonment, which further increases the risk of disease spread potentially into the wildlife.

The role of the veterinarian

Veterinarians in companion animal practice have an important and quite unique role to play in helping to detect, prevent and treat exotic diseases in the pet animals that travel from one country to another. An increasing number of owners are seeking to travel with their pets. In order to provide clients with the advice needed for taking their pet animals abroad there are significant benefits to the setting up of a pet travel clinic in a veterinary practice[2]. (Fig. 1).

The purposes of such a clinic are:

- To provide surveillance for the identification and screening of pets for emerging diseases that have recently entered the country.
- To assist those clients who wish to take their pets abroad, either on holiday or permanently, by ensuring that they have the appropriate preventative medicines.
- To explain about the potential risks from zoonotic infections.

Fig. 1 Setting up a travel clinic in veterinary practice (©BVA, with kind permission)

1. Surveillance

The local veterinary practice is often the first port of call for an owner with an ill animal that they may have recently brought into the country. If disease is suspected then appropriate diagnostic screening should be undertaken. Good history taking is essential to ascertain where the animal has come from and which countries it may have travelled through. Does the animal's description on the pet passport relate to the animal presented? Does the microchip number on the passport correspond with the microchip implanted in the animal? In the case of a puppy,

does the age match the age written on the passport? Veterinarians need to be familiar with the reporting mechanisms to the appropriate enforcement authorities both in the cases of non-compliance with pet passport regulations or suspected illegal importation. Imported companion animals provide a route via which exotic ticks and their associated pathogens can be introduced into a country where they are not presently endemic. Submitting these ticks for laboratory identification is important especially so in the Member States where the harmonisation of the pet travel regulation in January 2012 meant the requirement for treatment for ticks before importation ceased [3].

The illegal importation of dogs and cats significantly increases the risks of rabies incursion into Europe. Despite the best protections, France has seen regular outbreaks since 2000, mainly from animals brought in from North Africa. It will be local veterinarians who are most likely to identify any disease so anyone who suspects rabies should immediately quarantine the animal and inform the relevant authorities.

2. Screening

Veterinarians should ensure that they are familiar with the clinical signs, differential diagnoses and screening tests for emerging diseases that travelling pets potentially could transmit from countries where these diseases are endemic. There is an increasing trend for people to adopt street dogs from the southern and eastern regions of Europe. Any signs of disease should be investigated. The European Scientific Counsel Companion Animal Parasites (ESCCAP) and Canine Vector-Borne Diseases websites are an excellent resource for information and diagnostic tests of vector-borne diseases [4,5].

3. Prevention

Prevention can best be achieved by not exposing the animal to the risk of infection in the first place so it is important to discuss with the client whether they have considered the suitability of taking their pet on a long journey to another country. Aspects that need to be discussed include the age and health status of the animal, the region that is to be visited, environmental temperatures and endemic diseases to which the animal may be exposed, the length of the journey and the mode of transport. The client needs to inform the veterinarian which countries the animal will be travelling to so that preventative medication can be prescribed for the diseases to which that animal may be exposed. Clients should be encouraged to make an appointment with the travel clinic at least one month before the journey for the animal to undergo clinical examination, to check that the microchip is functioning, to discuss the welfare of the animal when travelling and then to prescribe appropriate preventative medication.

The ESCCAP website provides excellent advice on pet travel both for the veterinarian and the owner at http://www.esccap.org/travelling-pets-advice/

4. Zoonoses

Rabies continues to be a major public health concern, both in Europe and worldwide. Several vector-borne diseases are important zoonoses, such as leishmaniosis, borreliosis, rickettsiosis, bartonellosis, dirofilariosis and tick-borne encephalitis. People who are at risk of exposure to zoonotic parasites or any other zoonotic pathogen should be advised of the health risks and made aware that such risks are generally increased during pregnancy, or when there is an existing illness or immunosuppression. The two major species of tapeworm that are of medical and public health importance are *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause cystic echinococcosis and alveolar echinococcosis (AE) respectively. AE in humans is particularly serious with high mortality and a poor prognosis if managed incorrectly. People can become infected by ingesting tapeworm eggs after physical contact with dogs or foxes and/or their faeces [6].

Generally important preventive measures for pet owners include:

- reducing wherever possible the risk of a pet being exposed to ectoparasites
- controlling pet ectoparasite infestations through regular diagnostic testing and/or repeated application of appropriate ectoparasiticides, particularly for ticks and parasitic insects
- minimising exposure, especially of children, to potentially contaminated environments
- practising good personal hygiene

DISEASE RISKS

Rabies

Worldwide, rabies still accounts for some 55,000 human deaths annually, with 99% of these resulting from a dog bite. However, it is estimated that approximately 327,000

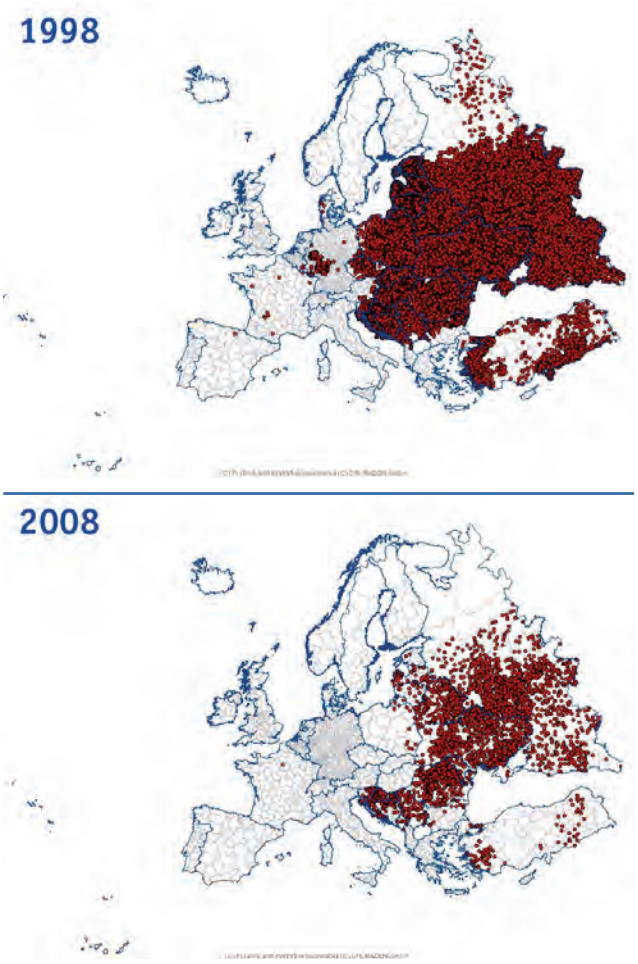

Fig. 2 Rabies Distribution in Europe (©WHO Rabies bulletin) 1998 / 2008

deaths are prevented by post-exposure prophylaxis.
Despite the substantial progress that has been made in reducing the burden of rabies, especially in central and Eastern Europe (Fig. 2), the disease remains endemic in animal populations in many European countries.
The principal reservoirs of classical rabies in Europe are the red fox (*Vulpes vulpes*) and the raccoon dog (*Nyctereutes procyonoides*). In addition, distinct epidemiological cycles occur in certain bat species involving different lyssaviruses. Although classical rabies has been eliminated in many Member States through the implementation of oral rabies vaccination programmes, rabies is still prevalent in wildlife in several eastern Member States and adjacent non-Member States [7]. The majority of western European countries are now free of classical rabies, with reported rabies restricted to the relatively rare bat cases (European bat lyssaviruses type-1 and -2). The raccoon dog plays a significant role in the epidemiology of rabies in the Baltic countries where numbers of infected raccoon dogs can exceed that of foxes. Rabies surveillance should target animals suspected of having contracted the disease and animals imported from endemic third countries showing signs suggestive of rabies.

Clinical signs

Initially, a dog may show extreme behavioural changes such as restlessness or apprehension, both of which may be compounded with aggression. A dog may bite or snap at any form of stimulus, attacking other animals, humans and even inanimate objects. They may be pyrexic at this stage. As the virus progresses, an infected dog may become hypersensitive to touch, light and sound. They may eat unusual things and hide in dark places. Paralysis of the throat and jaw muscles may follow resulting in foaming at the mouth. Disorientation and ataxia may occur. This can progress to weakness, seizures and death.

Vector-borne diseases

These are caused by a wide range of infectious agents including viruses, bacteria, and parasites (protozoa and helminths), which are transmitted by a variety of arthropod vectors such as ticks, *Diptera* (mosquitoes, sand flies, muscid flies), lice and fleas.
Vector-borne pathogens or diseases are important because:

- They may be highly pathogenic in dogs and cats
- Their transmission is often unpredictable
- Their diagnosis and control are difficult
- Variable clinical signs can develop after long incubation periods and these are rarely pathognomonic
- Animals may have persistent infections and thus act as reservoirs
- Several are important zoonoses, such as leishmaniosis, borreliosis, rickettsiosis, bartonellosis and dirofilariosis

Climatic and ecological changes, national regulations on the management of stray dogs and cats together with the increase in pet travel and translocation of pet animals can influence the epidemiological situation of vector-borne diseases in Europe. Rare diseases may increase in frequency in certain areas, either due to increased importation of infected animals or because the causative agents and their vectors spread to and establish in previously non-endemic areas. Such an expansion of endemic areas has been recorded for various parasitic diseases such as dirofilariosis, babesiosis and leishmaniosis. Babesiosis, for example, has been observed across central Europe in the past few years, emerging from previous endemic regions in Europe. Another important feature of these diseases is their increasing occurrence in wildlife, which act as reservoirs [8].

Canine leishmaniosis

This is endemic in more than 70 countries in the world. It can be found in regions of southern Europe, Africa, Asia, South and Central America and has recently emerged in the USA. Fig. 3 shows the approximate northern limit of the endemic area in Europe. Canine leishmaniosis is also an important concern in non-endemic countries where the imported disease may constitute a veterinary and public health problem.

Fig. 3 Approximate distribution of canine leishmaniosis in Europe. Solid blue: endemic area; light blue: individual, non-imported cases observed. (© ESCCAP, with kind permission)

Fig. 4 Phlebotome sand fly – sand fly (©CDC)

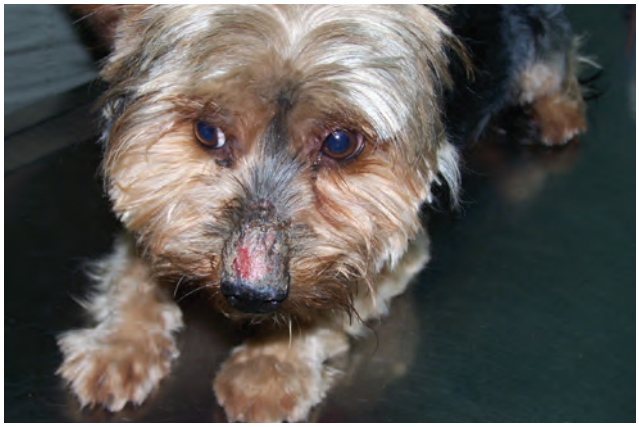

Fig. 5 Skin lesions in dogs with cutaneous Leishmania infection (Permission granted by Susan Paterson, with acknowledgment to Kevin Camelleri)

The disease is caused by the protozoa *Leishmania infantum*, which is transmitted to humans and animals by the bite of phlebotomine sand flies (Fig. 4) that usually feed around sunset and at night.

Clinical signs and diagnosis

It is a chronic disease with an incubation period of months to years. Once the disease becomes patent, progression is usually rapid and death occurs within a few weeks to months without treatment.

There are a wide variety of clinical presentations ranging from skin lesions (Fig. 5) including alopecia, scaling and ulceration, to weight loss and poor appetite, local or generalized lymphadenopathy, ocular lesions, epistaxis, lameness, anaemia, renal failure or diarrhoea.

Diagnosis is by fine-needle aspiration from bone marrow or lymph nodes for microscopic determination of the parasite or PCR. It is also recommended to blood sample for anti-Leishmania antibody assay and PCR.

Based on the seroprevalence data it has been estimated that 2.5 million dogs in France, Italy, Portugal and Spain are infected.

Treatment

Treatment is rarely, if ever, curative and dogs usually remain infected for life. Meglumine antimonite in combination with allopurinol is recommended and many dogs will respond and clinical signs will resolve.

Prevention is much the preferred option either by the use of deltamethrin collars that can provide up to 6 months of protection against sand fly bites and by vaccination, which is now available in Europe.

For more information, see the papers on Leishmaniasis in cats and dogs (EJCAP 23(2)).

Dirofilariosis

Filarial worms are nematodes infecting connective tissues and the vascular system of dogs and cats. Mosquitoes, but also fleas and ticks, act as vectors for the different species (Table 1).

Dirofilaria immitis, the canine and feline heartworm, is the most pathogenic species, while *D. repens,* which causes subcutaneous dirofilariosis, is the most important species responsible for zoonotic infections in Europe.

Table 1: Filarial species infecting dogs and cats in Europe [8].

Filarial parasite	Vectors	Prepatent period	Length of adult worms	Location of adult worms
Dirofilaria immitis	Mosquitoes *(Culicidae)*	120-180 days	M: 12-18 cm F: 25-30 cm	Pulmonary arteries/ right side heart
Dirofilaria repens	Mosquitoes *(Culicidae)*	189-259 days	M: 5-7 cm F: 10-17cm	Subcutaneous tissue/ muscular fasciae
Acanthocheilonema (former: *Dipetalonema reconditum)*	Fleas and ticks	427-476 days	M: 9-17 mm F: 21-25 mm	Subcutaneous tissue/muscular fasciae, peritoneal cavity, kidney
Acanthocheilonema (former: *Dipetalonema dracunculicoides*)	Fleas and ticks *(R. sanguineus)*	120 days	M: 15-31 mm F: 33-55 mm	Peritoneal cavity
Cercopithifilaria spp.	Ticks *(R. sanguineus)*	Unknown	M: unknown F: 23-24 mm	Subcutaneous tissue/muscular fasciae

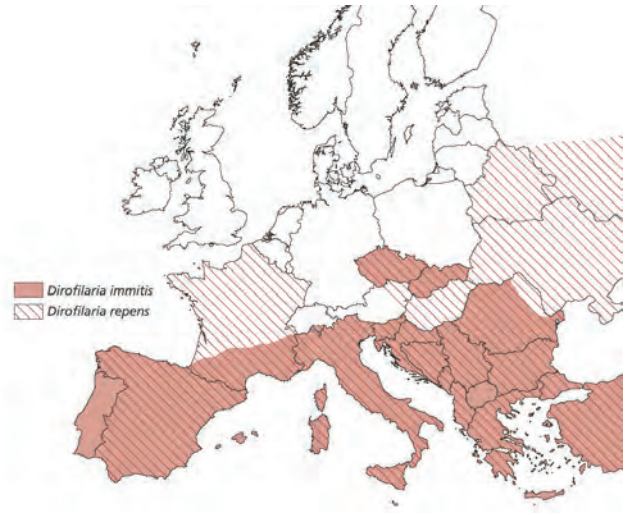

Fig. 6 Approximate distribution of endemic areas of Dirofilaria immitis and Dirofilaria repens in Europe (© ESCCAP, with kind permission)

Clinical signs and diagnosis

The clinical evolution of heartworm disease in dogs is usually chronic. Most infected dogs do not show any clinical signs for years. Signs of the disease develop gradually and may begin with a chronic cough that may be followed by moderate to severe dyspnoea, weakness, and sometimes syncope after exercise or excitement. At this stage, auscultation may reveal abnormal pulmonary sounds (crackles) over the caudal lung lobes, and a split second heart sound can often be heard. Later, when right congestive heart failure is developing, oedema of the abdomen and less often in the limbs may be observed together with anorexia, weight loss and dehydration. Arterial damage is usually more severe in dogs that perform intensive physical exercise; sudden death is rare and usually occurs following respiratory distress or progressive emaciation.
During the chronic stages of the disease, there may be a sudden onset of acute signs. For example, after severe spontaneous thromboembolism following the natural death of many heartworms, dogs may show acute life-threatening dyspnoea and haemoptysis.

Most cats show no clinical signs for a long time after infection. These cats may undergo spontaneous self-cure without showing any signs or they may suddenly show a dramatic acute syndrome usually with respiratory signs such as coughing, dyspnoea and haemoptysis; vomiting also frequently occurs. Sudden death in apparently healthy cats is not an infrequent consequence of infection.
Diagnosis in dogs is with blood tests to demonstrate the presence of circulating microfilariae or adult antigens in serum or plasma samples. X-rays and echocardiography can also be used as diagnostic aids. Detection of microfilariae in the blood of infected cats is unlikely to be successful. Tests to detect adult female heartworm antigens have a very high specificity and can thus provide definitive proof of infection.

Treatment

Treating adult heartworm infections is often unsatisfactory due to pulmonary thromboembolism being an inevitable consequence of successful adulticide therapy. Prevention of infection in the first place is recommended by the monthly administration of a topical (selamectin) or oral (milbemycin oxime) macrocyclic lactone throughout the mosquito transmission season from spring until late autumn. In southern Europe, protection against heartworm should be carried out from May until the end of November.

Bartonellosis

The most important species involved in bartonellosis is the bacterium *Bartonella henselae* that is of relevance mainly as the causative agent of cat scratch disease (CSD) in humans. Cats are considered the main reservoirs of, amongst others, *B. henselae* and *B. clarridgeiae*. The vectors of many Bartonella species, especially *B. henselae*, are fleas, mainly the cat flea *Ctenocephalides felis*.

Clinical signs and diagnosis

Most infections with *Bartonella spp*. in cats remain asymptomatic. Generally, a bacteraemia develops within one to three weeks after the initial infection with chronic recrudescences for up to 21 months. Clinical signs are observed only in immunosuppressed cats that might show fever, lymphadenopathy, gingivitis, uveitis, and endocarditis; transient anaemia and persistent eosinophilia have also been described. Infection has also been associated with diseases of the urinary tract as well as with reduced reproductive performance. In dogs, more than eight species of *Bartonella* have been associated with endocarditis, myocarditis, hepatitis and rhinitis, but *Bartonella*-associated disease is probably underdiagnosed. Diagnosis can be based on the presence of clinical signs that may be associated with bartonellosis and response to treatment with an antibiotic effective against *Bartonella* spp. The gold standard for the diagnosis of bartonellosis is blood culture. It is also possible to detect *Bartonella* DNA in samples of blood, tissue, cerebrospinal fluid or aqueous humor. Antibodies can be detected serologically from approximately 10 to 14 days after infection. For the diagnosis of clinical bartonellosis repeated testing of serum samples should show a rising antibody titre.

Treatment

The therapy of bartonellosis with currently available drugs only reduces the bacteraemia but does not eliminate the pathogen. Treatment is therefore only recommended for animals that show clinical signs and/or have contact with immunocompromised persons. Possible therapies include amoxicillin-clavulanic acid, doxycycline, enrofloxacin.
If the cat or dog responds to the therapy, this should be continued for at least 28 days or for 2 weeks after remission of the clinical signs.
Should the animal still show clinical signs after 7 days then treat with azithromycin for up to two weeks after the signs have subsided.

Babesiosis

Babesia spp are haemoprotozoa that exclusively infect erythrocytes and are transmitted by hard ticks (Table 2).

Clinical signs and diagnosis

Acute disease: Incubation period 1-3 weeks: moderate to severe clinical signs. Medium-high fever, lethargy, anorexia, jaundice, vomiting and in some cases, red coloured urine ("rusty urine"). Common clinicopathological findings are haemolytic anaemia, thrombocytopenia, neutropenia and sporadic haemoglobinuria. If untreated, a long recovery period may be followed by relapses that may lead to shock, icterus and severe or even fatal renal failure.
Chronic disease: Clinical signs may include moderate

Table 2. The common Babesia species of dogs and cats and their vectors in Europe [8].

Causative agent	Hosts	Tick vector	Distribution
Babesia canis	Dogs	*Dermacentor reticulatus*	Endemic in Northern Spain, Portugal, France, central & eastern Europe up to the Baltic region associated with the distribution of *Dermacentor* spp.
Babesia vogeli	Dogs	*Rhipicephalus sanguineus*	Southern Europe, associated with the distribution of *Rhipicephalus sanguineus*
B. (Theileria) annae	Dogs	*Ixodes hexagonus* & *I ricinus*	Northwest Spain and Portugal (in foxes found in Croatia and Germany)
Babesia gibson	Dogs	*Rhipicephalus sanguineus* *Haemaphysalis* spp. *Dermacentor* spp.	Sporadic and rare in Europe, imported from Asia
Babesia spp.	Cats	*Rhipicephalus* spp.	Southern Europe associated with the distribution of *Rhipicephalus sanguineus*.

depression, intermittent fever, anaemia, myositis and arthritis. Several *Babesia spp*. or subspecies have been reported in domestic cats from various parts of the world, particularly South Africa. Relatively few reports originate from Europe, and clarification of the species infecting cats in Europe is currently under investigation. Clinical cases of feline babesiosis reported are characterised by lethargy, anorexia, weakness and diarrhoea. Fever with icterus is not common, but signs may not be apparent until later stages of the disease.

Diagnosis of acute babesiosis can be confirmed with high sensitivity by the examination of thin blood smears (Giemsa-stain or Diff-Quick) to detect large or small *Babesia spp*. Specific antibodies can only be detected from two weeks after the first infection and acute infections will therefore be missed if relying on serology for diagnosis. Species- or subspecies-specific PCRs (including real-time PCRs) have been described and are being increasingly used in routine laboratory diagnosis.

Treatment

Chemotherapy should be initiated immediately after confirmation of a diagnosis of babesiosis. Imidocarb dipropionate, and in some countries phenamidine, are the drugs commonly used for the therapy of *B. canis* infection and in many cases treatment with these drugs will eliminate the parasite. However, in endemic areas, treated dogs do not develop a specific immune response capable of protecting against re-infection. In all cases, adequate supportive therapy is strongly recommended including rehydration and, if appropriate, blood transfusion.

Ehrlichiosis

This is caused by a Gram-negative obligate intracellular bacterium. In Europe, *Ehrlichia canis* is the aetiological agent of canine monocytic ehrlichiosis (CME). The main host is the dog (other canids can serve as reservoirs of infection) and the vector is the tick Rhipicephalus sanguineus (Fig. 7).

Clinical signs and diagnosis

During the acute phase of CME, which lasts around 1-3 weeks, dogs show apathy, depression, anorexia, dyspnoea, fever, lymphadenopathy, splenomegaly, petechiae, epistaxis and vomiting. Also typical are thrombocytopenia, leukopenia and mild to moderate normocytic, normochromic and non-regenerative anaemia. In the subclinical phase, which may last for weeks or months, the dogs appear clinically normal. Thrombocytopenia and

Fig. 7 Rhipicephalus sanguineus – the brown dog tick (©CDC)

hypergammaglobulinaemia are typical. Chronic CME is characterised by a very complex clinical picture. Noticeable symptoms are weakness, apathy, sustained weight loss, fever, lymphadenopathy, splenomegaly, peripheral oedema in the hind limbs and scrotum, pale mucous membranes, a predisposition for bleeding with haemorrhages in the skin and mucous membranes, mucopurulent ocular and nasal discharges, epistaxis, and haematuria. In addition, interstitial pneumonia with dyspnoea, renal dysfunction, glomerulonephritis, arthritis, polymyositis and lameness may occur. Typical changes in the eyes of the patients are anterior uveitis, corneal opacities and blood in the anterior chamber, subretinal haemorrhage, retinal detachment and blindness. With the involvement of the CNS, nystagmus, signs of meningoencephalomyelitis, paresis, ataxia and convulsions appear.

Diagnosis in dogs is generally based on the combination of a thorough anamnesis to assess the possibility of an exposure to ticks, the assessment of clinical signs, haematological and clinical chemistry findings, and serology and/or PCR.

Treatment

Treatment consists of the administration of anti-rickettsial agents together with symptomatic therapy. Tetracyclines are the most commonly used compounds.

Anaplasmosis

Trans-stadial but not transovarial transmission of *Anaplasma phagocytophilum* occurs in the *Ixodes* vector. Usually, tick feeding for 24-48 hours is required for the transmission of this agent to susceptible dogs.

With *Anaplasma platys* the natural mode of transmission has not been definitely established, but ticks and other arthropod vectors are likely to be involved. In experimental infections, the incubation period ranges from 8 to 15 days. Infections lead to cyclic thrombocytopenia, and the highest bacterial load is found during the initial peak. In subsequent cycles, only around 1% of the platelets are affected while thrombocytopenic episodes remain approximately the same. Over time, the severity of the thrombocytopenic response diminishes.
The geographical distribution in Europe of infections with *A. phagocytophilum* and *A. platys* generally correspond to the distribution of their respective (or supposed) tick vectors. With increasing travel of dogs with their owners, infections must also be expected to occur in previously non-endemic areas.

Clinical signs and diagnosis
Clinical signs are non-specific such as sudden onset of lethargy, anorexia and fever; lameness, pale mucous membranes, tense abdomen, diarrhoea, vomiting, petechial haemorrhages, tachypnoea, splenomegaly, enlarged lymph nodes, rarely cough, uveitis, limb oedema, polydipsia and neurological signs. Most common laboratory abnormalities are thrombocytopenia, anaemia, lymphopenia, monocytosis, leukopenia, and leukocytosis, hyperglobulinaemia, hypoalbuminaemia, increased liver enzymes and hyperbilirubinaemia.
The diagnosis of *Anaplasma spp*. infections in dogs is generally based on the combination of a thorough anamnesis, to assess the possibility of a previous tick infestation, the clinical signs, haematological and clinical chemistry findings and serology and/or PCR.

Treatment
The treatment of anaplasmosis consists of the administration of antirickettsial agents and symptomatic treatment. Tetracyclines are the most commonly used compounds. With correct treatment, the prognosis of *A. phagocytophilum* infections is fairly good.

Borreliosis – Lyme disease

There are currently 11 known species/genotypes of the *Borrelia burgdorferi* complex, which are spirochaetes that infect many mammals and birds and are transmitted by ticks (*Ixodes ricinus, I. hexagonus,* and *I. persulcatus*). Human infections are of major public health importance and although infections have been demonstrated in dogs, they are not of major clinical importance. Humans as well as dogs acquire *Borrelia* infection when exposed to infected ticks but there is no interdependency between dogs and humans in terms of transmission. Positive serology in cats has also been reported, but disease in cats, if it occurs at all, is poorly understood and there is therefore little data concerning the prevalence of infection, clinical appearance and treatment options for cats.
As one would expect, endemic areas of borreliosis are related to the distribution of the tick vectors. Over the past twenty years, a number of studies have been published on prevalence and genetic variability within the B. burgdorferi complex in Europe. Lyme borreliosis is present all over Europe, except in extremely hot southern or cold northern areas.

Clinical signs and diagnosis
Borreliosis is a well-recognised disease in humans but, as yet, is not clearly defined in dogs and most infected dogs are asymptomatic. “Lyme arthropathy” which is lameness in one or more joints has been described; puppies may be at higher risk of polyarthritis. “Lyme nephropathy”; there are many reports of dogs seropositive for Borrelia that have immune-mediated glomerulonephritis but further studies are needed to clarify if there is any association. In some clinical cases, dogs could present fever associated with lameness.
Diagnosis can be by detection of Borrelia by culture, cytology or PCR but this is time consuming and expensive. Positive serology will merely indicate exposure to the bacteria rather an existing disease.

Treatment
Response to antibiotic therapy should be evident within 1-2 days in the case of polyarthritis. The drug of choice is doxycycline. Antibiotic treatment may not clear the infection from all dogs.

CONCLUSION

Pet travel has the potential to change epidemiological situations with export or import of non-endemic parasite species, therefore veterinarians and pet owners must protect the pet population from the disease risks associated with travel and its consequences. Veterinarians, pet owners and the medical profession should work together to reduce the risks associated with zoonotic transmission of parasitic diseases. Veterinarians should inform the pet owner about parasites and enable them to act responsibly for their pet’s life and the pets and other animals and people in their communities.

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Commissioned paper

Controversies in fluid therapy

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SUMMARY

Intravenous fluid therapy has become a ubiquitous intervention in both human and veterinary medicine. The field of fluid therapy is characterised by numerous controversies, and despite their widespread use, fluids should be considered as drugs, as their use is associated with potential side effects and complications. This paper will review the differences between crystalloids and colloids, and how their clinical use has changed according to recent scientific evidence. Due to their theoretical advantages, hydroxyethyl starches (HES) have become the most commonly used colloids in both human and veterinary medicine. However, the results of human studies have revealed clear adverse effects on renal and haemostatic functions and an increase in mortality when comparing colloids versus crystalloids for fluid resuscitation. A quantitative toxicity has also been identified and excessive fluid resuscitation appears to be associated with an adverse outcome. These recent studies should prompt the veterinary profession to undertake an appraisal of current fluid therapy practices and recommendations that have thus far been largely based on theoretical benefits rather than clinical evidence. In this review we will focus on some common controversies and how our approach to fluid therapy may be adapted in light of the most recent veterinary and human data.

Key Words: synthetic colloids, hydroxyethyl starches, complications, coagulopathy, kidney injury

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Introduction

The first report in the medical literature of the intravenous administration of a salt-based solution was described in 1832 by Thomas Latta for the treatment of patients affected by cholera[1]. Since then, intravenous fluid therapy has become a ubiquitous intervention in both human and veterinary medicine and it represents a cornerstone in the treatment of ill patients. The field of fluid therapy is characterised by numerous controversies regarding whether there are optimal fluid types, optimal doses and even whether there is a preferable timing and rate of fluid administration. Despite widespread use, fluid therapy is associated with potential side effects and complications, as recently revealed by the results of several large human randomised clinical trials [2,3,4]. These recent studies in the human field should prompt the veterinary profession to undertake an appraisal of current fluid therapy practices and recommendations that have been thus far largely based on theoretical benefits rather than clinical evidence. In this review we will focus on some common controversies and how our approach to fluid therapy may be adapted in light of the most recent veterinary and human data.

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Basic principles of fluid therapy

To be able to appreciate the nuances of fluid therapy a basic understanding of normal body fluid physiology is required. Total body water (TBW) accounts for approximately 60% of total body weight. Total body water is distributed between the intracellular fluid compartment (approximately 66%) and the extracellular fluid compartment (approximately 33%). These two spaces are separated by cell membranes. The extracellular fluid compartment is, in turn, further subdivided into an intravascular (8% TBW) and an interstitial space (25% TBW) [5], and these compartments are separated by the capillary wall (Figure 1).

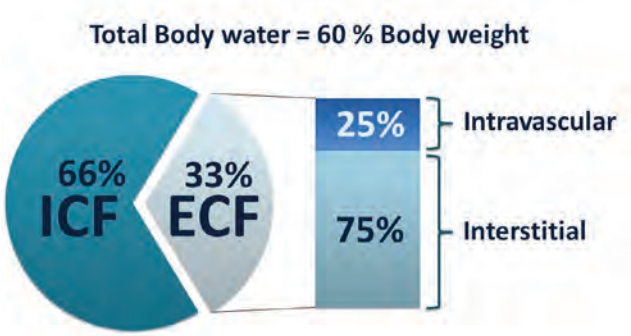

Figure 1. Distribution of total body water (TBW) within the body showing the proportion allocated into the intracellular and extracellular fluid compartments.

The barriers between fluid compartments have different permeability to different solutes based on size, charge and conformation. This selective permeability, along with hydrostatic and oncotic forces (i.e. Starling forces), determines the movement of fluids and electrolytes between compartments. Two other concepts that also play a role in the movement of fluid between compartments are osmolarity and oncotic pressure. Osmolarity is a measure of the number of particles present in a solution, independently of their size or weight. Water tends to distribute between compartments due to osmolarity gradients (osmotic pressure). Oncotic pressure, also known as colloid osmotic pressure, is a particular type of osmotic pressure that is generated by colloid molecules present in solutions.

Clinical signs associated with fluid deficits vary accordingly to the compartment affected (Table 1). Dehydration is defined as total body water deficit, while hypovolemia indicates a purely intravascular volume deficit. The total intravascular volume, including both plasma and cellular components, is estimated to be approximately 88 ml/kg in dogs and 66 ml/kg in cats [6]. The intravascular volume, despite containing only a small proportion of the TBW, is the main determinant of cardiac preload and, as such, plays a fundamental role in maintaining cardiovascular stability. Preload, along with cardiac contractility and afterload, determines cardiac output and blood flow to peripheral tissues (perfusion). As a consequence of this, during hypovolemia, oxygen delivery to peripheral tissues is affected: when the metabolic needs of the body are no longer matched by the blood flow provided by the cardiovascular system, circulatory shock ensues. If left untreated circulatory shock will result in organ dysfunction and eventually death. Therefore, rapidly restoring and maintaining an effective intravascular volume is essential to reverse the progression of the shock state. This therapeutic intervention is referred to as fluid resuscitation and will be the main focus on this review. Other common reasons to administer fluids include restoration of the interstitial and intracellular fluid balance (i.e. rehydration), compensation for on-going fluid losses and to induce diuresis and maintain acid-base and electrolyte homeostasis.

Types of fluids

Fluids used in veterinary patients can be classified into

Table 1. Differentiation of hypoperfusion from dehydration via clinical signs

Signs consistent with hypoperfusion	Signs consistent with dehydration
Increased heart rate	Dry mucous membranes
Hyperdynamic or hypodynamic pulses	Prolonged skin tenting
Hypothermia or cold distal limbs	Normal pulses
Prolonged capillary refill time	Sunken eyeballs
Pale mucous membranes	

4 basic types: crystalloids, colloids, haemoglobin-based oxygen carriers and blood products [7]. Crystalloids are solutions of water and electrolytes or glucose. Some formulations might also contain buffers (e.g. lactate, acetate or gluconate) that, once administered, are metabolised to bicarbonate and can influence acid-base balance. Crystalloids are classified into hypotonic, isotonic or hypertonic solutions based on their relative osmolarity compared to plasma. Isotonic fluids have an osmolarity that is similar to that of plasma (approximately 300 mOsm/L), while hypertonic and hypotonic fluids have an osmolarity that is, respectively, higher and lower than plasma. Isotonic solutions are the most commonly used type of crystalloids. Hypotonic solutions are contraindicated during fluid resuscitation and their use should be reserved to treat specific conditions (e.g. treatment of severe electrolyte imbalances). Hypertonic solutions, and in particular hypertonic saline, may be useful in certain cases that require fluid resuscitation; a dramatic increase in plasma osmolarity leads to a shift of water from the interstitium and intracellular fluid compartments to the intravascular space, resulting in a transient intravascular volume expansion. Isotonic crystalloids available in clinical practice include normal saline (0.9% NaCl) and balanced solutions (e.g. compound sodium lactate, Hartmann's, Ringer's lactate solution, Ringer's acetate solution, Plasma-Lyte). Balanced solutions differ from normal saline in the fact that they contain electrolytes in more physiological concentrations, closely resembling the electrolyte composition of human plasma.

Shortly after administration of crystalloids most of the infused volume will redistribute to the interstitium and intracellular space and by 1 hour only 20-25% of the infused volume will still be within the intravascular space [6]. With this in mind, one can see that if intravascular volume expansion is the main therapeutic target, crystalloid fluid therapy would seem to be an inefficient way of achieving this and their use might promote the formation of interstitial oedema (Figure 2).

Colloids are high-molecular-weight compounds (molecular weight higher than 30 KDa) that, in the normal physiological condition, do not readily leave the intravascular space and contribute to maintaining, or possibly improving, the patient's plasma oncotic pressure. Colloids are classified as synthetic (e.g. hydroxyethyl starches, gelatins, dextrans) or natural (e.g. plasma, albumin solutions, blood). The potential benefits of colloids include prolonged intravascular

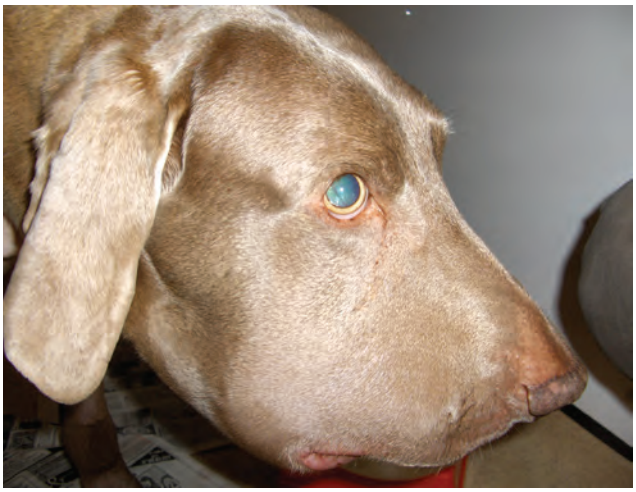

Figure 2. Dog with marked interstitial oedema where fluid has accumulated in the extracellular fluid compartment.

effect, smaller volume requirements and decreased risk of oedema formation when compared with crystalloids [8]. Therefore, if the goal of fluid administration is to address hypovolemia, colloids seem to present some obvious advantages over crystalloids. Ideal colloid solutions should be isotonic, iso-oncotic, rapidly degradable, inexpensive and have minimal side effects [9]. On first inspection, hydroxyethyl-starch solutions (HES) are the colloids that most closely match these criteria. For this reason they have become, up to recently, the most commonly used colloids in both human and veterinary medicine.

HES are synthesised from amylopectin, a highly branched polymer of glucose, and chemically modified by substitution of some hydroxyl- with hydroxyethyl-residues. These modifications provide some of the desired characteristics of colloidal fluid solutions. The various preparations of HES are classified based on their molecular weight, molar substitution (number of hydroxyethyl- residues per unit of glucose), pattern of substitution (C2:C6 ratio) and type of solutions in which they are suspended (e.g., balanced or unbalanced crystalloid solutions). Higher molecular weights and molar substitutions are associated with a longer half-life. Despite their widespread use and their perceived better safety profile compared to other synthetic colloids, HES use is associated with numerous side effects.

Undesirable side effects

Coagulopathy

Coagulopathy is one of the most common side effects associated with the use of synthetic colloids. The mechanism of this coagulopathy is not fully understood,

but some of the proposed pathways include decreased circulating factor VIII and von Willebrand factor concentration, impairment of platelet function and interference with fibrin polymerization [10]. These effects have been reported in dogs as well, although the clinical significance of the abnormalities reported is undetermined [11-15]. It should be noted that current veterinary dose recommendations for colloids (approximately 50 ml/kg/day for low molecular weight HES and 20 ml/kg/day for high molecular weight HES) are not based on efficacy data, but on human safety limits developed to minimise the risk of bleeding [8]. The coagulation disorder associated with HES administration appears to be proportional to the dose administered and the molecular weight and molar substitution of the molecule used. To reduce these risks, novel colloid solutions were developed with a lower molecular weight and lower degree of hydroxyethyl molar substitution [9].

Renal dysfunction

Renal dysfunction is another commonly discussed complication induced by colloid administration in people and this complication has become the focal point of the controversy surrounding the use of starch-based colloids in critically ill patients. All synthetic colloids undergo renal excretion and therefore have the potential to cause acute kidney injury (AKI). The first reports on colloid-induced renal failure were published in the late 1960s in association with the use of dextrans [16,17], but in more recent years, the potential for inducing kidney damage has been reported in other colloids classes [18]. Several hypotheses have been formulated to explain AKI following colloid administration [9]. The increase in the intra-glomerular COP determines a decrease in glomerular filtration rate. The filtration of colloid molecules in the glomerulus increases intra-tubular viscosity and decreases urine flow. In addition, some molecules are re-absorbed by the proximal tubular epithelial cells where they induce vacuolar lesions. This can result in cellular swelling, with an additional decrease in urine flow. Renal interstitial inflammation has also been reported [19].

Colloids vs. crystalloids

HES solution should be theoretically less nephrotoxic given their biochemical characteristics. However, tubular lesions were noticed with the use of high-molar substitution HES in brain dead kidney donors [20]. Studies regarding the renal safety of colloids started to appear in the early 1990s[21]. A trend similar to that observed with coagulopathy appeared: the use of solutions with higher concentrations, higher molecular weight and molar substitution colloids were associated with increased risk of renal toxicity. Low molecular weight, molar substitution and iso-oncotic HES appeared to have the best safety profile [22].

Despite their theoretical advantages HES were approved for medical use without adequate testing regarding safety and efficacy [21]. Up to very recently, limited evidence had been in support of colloids over crystalloids for fluid resuscitation. A Cochrane Collaboration meta-analysis published in 2007 concluded that "…as colloids are not associated with an improvement in survival, and as they are more expensive than crystalloids, their use should be limited to randomised clinical trials" [23].

In 2008 the first large randomised clinical trial (RCT) comparing HES versus crystalloids for fluid resuscitation was published [2]. This study revealed a dose related effect linking HES administration with higher incidence of AKI, need for renal replacement therapy (a form of haemodialysis) and number of blood transfusions. In 2011 numerous studies from a leading author regarding the safety and efficacy of starch-based colloids were retracted by a number of journals due to scientific misconduct and data fabrication [24], decreasing even further the level of evidence in support of HES use. The following year two other large high-quality RCT (6S and CHEST studies) investigating the use of HES versus crystalloids for fluid resuscitation in critically ill patients were published [3,4]. These studies confirmed the increased need for renal replacement therapy and blood transfusions associated with the use of HES and also showed a significant increase in mortality in the most severe population treated with HES. Based on these data the most recent Surviving Sepsis Guidelines [25] advised against the use of HES for the treatment of severe sepsis and septic shock. An updated Cochrane Collaboration meta-analysis published in 2013 failed to identify any benefit of the administration of colloids (any) versus crystalloids, and concluded that "… it is difficult to see how HES use can be justified in clinical practice."[26] Another meta-analysis focused on the effect of HES on kidney function and identified an increased risk of developing AKI and a higher need for renal replacement therapy [27]. These two meta-analyses revealed that the risk associated with HES administration is independent from the type of HES or the severity of the population treated. As a consequence, the European Medicines

Agency recommended the suspension of the marketing authorisation for all products containing HES [28] and the Food and Drug Administration of the United States issued a warning against the use of HES in critically ill patients [29]. At the time of writing HES-containing solutions have been withdrawn from the market in several European countries, although the European Union has ratified a recommendation allowing the use of HES in some selected situations such as hypovolaemia not associated with sepsis or burns [30]. Veterinary access to HES depends on local medical regulatory agencies authorising the trade of these products.

To date, there are no reports of nephrotoxicity induced by the use of HES in veterinary patients, but both renal lesions and renal dysfunction have been reported in experimental studies in dogs receiving dextran [17,31] and the pathophysiology of the nephrotoxicity appears to be similar across different classes of colloids. Although there is currently no evidence that HES-based colloids can worsen outcome in veterinary patients these fluids should be used cautiously, especially in patients predisposed to coagulopathy, AKI or with severe sepsis. Studies evaluating the incidence of AKI in veterinary patients treated with starch-based colloids are urgently needed.

It should be noted that the cost of colloids is significantly higher compared to crystalloids just as they are in human medicine, and this aspect should not be overlooked when deciding what type of solution to use for fluid resuscitation. In the context that resuscitation with colloids offers no real benefits in outcome, justification for their use can be problematic.

Other colloid solutions are available as alternatives to HES: gelatins, dextrans, haemoglobin-based oxygen carriers (HBOC), albumin and plasma. Gelatins are derived from bovine collagen and have a low molecular weight (30-35 KDa). Their immediate volume effect is similar to that of HES, but due to their low molecular weight they determine a much shorter duration of volume expansion. Compared to HES, the use of gelatins is associated with a higher risk of anaphylactic reactions [9]. There have also been concerns that the use of gelatins may pose a risk of inducing renal dysfunction, although this is not well described [9]. Dextrans are polysaccharides synthesised from sucrose by bacterial fermentation. Dextrans have the worst safety profile among colloids in terms of coagulopathy, renal dysfunction and for these reasons are not used commonly in human medicine. Oxyglobin® is a veterinary-specific stroma-free HBOC derived from bovine haemoglobin and licensed for the treatment of anaemia in the dog. Compared to other colloids, HBOC has the advantage of providing additional oxygen carrying capacity that can thereby improve tissue oxygenation. The use of HBOC has been demonstrated to enable more rapid achievement of resuscitation endpoints when compared with HES in both experimental and clinical veterinary studies [32,33,34]. However, it should be noted that HBOC has not been approved for human use due to safety concerns, and its use in veterinary patients is associated with significant side effects, especially in terms of volume overload [35]. Albumin has recently substituted HES as the most used colloid solution in human medicine, although there is no extensive evidence of a clinical benefit over crystalloids alone [25,36]. The use of human serum albumin in critically-ill veterinary patients can be associated with both immediate and delayed side effects [37,38]. Its use in healthy patients under experimental conditions was associated with sometimes fatal hypersensitivity reactions [39]. A canine-specific serum albumin had been commercially produced in North America, but it is no longer available. Plasma can also be used for fluid resuscitation and has a better safety profile compared to albumin solution, however, the risk of transfusion reactions remains. It is also important to note that plasma is not particularly effective as a plasma expander, nor is it practical as it is usually stored frozen and not cost effective given the need for large volumes in order to alter the COP of the patient.

When compared with crystalloid fluid solutions, colloids are considered more potent plasma expanders and this is partly due to their greater persistence within the intravascular space. This means that smaller volumes of colloids are required to expand the intravascular space compared with crystalloid solutions. The ratio of colloids:crystalloids that needs to be administered to achieve a similar volume effect is approximately 1:4, and this has been confirmed in several experimental studies [40,41]. An interesting finding that has emerged from several human RCT is that the actual volume effect of HES in clinical settings is less than previously thought. The ratio of HES to crystalloids administered to achieve similar resuscitation endpoints actually only ranged from 1:1 to 1:1.6, far below the predicted 1:4 ratio [2,3,4]. This raises questions on the validity of the assumption that colloids are more effective plasma expanders. This discrepancy in the volume effect could be explained through the concept of “context

sensitivity." This concept proposes that the volume effect of administered fluid is variable and depends on the cardiovascular context of the patient. The cardiovascular context takes into account derangements in vascular permeability, intravascular volume and hydration status. In other words, the highly desirable behaviour of colloids may only be apparent in subjects with normal vascular endothelium and these advantages may not be appreciated in critically ill patients [42,43].

Lending support to the aforementioned concept, a major advancement in the understanding of fluid homeostasis has been made possible by the recent characterisation of the endothelial glycocalyx layer (EGL) (Figure 3) [44]. This is an active interface between the blood and the capillary wall and appears to be the main determinant of vascular permeability. This structure can be damaged in several conditions, such as hypoalbuminaemia, sepsis, hypoxia, hyperglycaemia or hypervolemia. When the EGL is intact vascular permeability is preserved and colloids have a volume effect that exceeds that of crystalloids. However, damage to this structure leads to an increase in vascular permeability and consequently a loss of the "volume advantage" offered by the colloids.

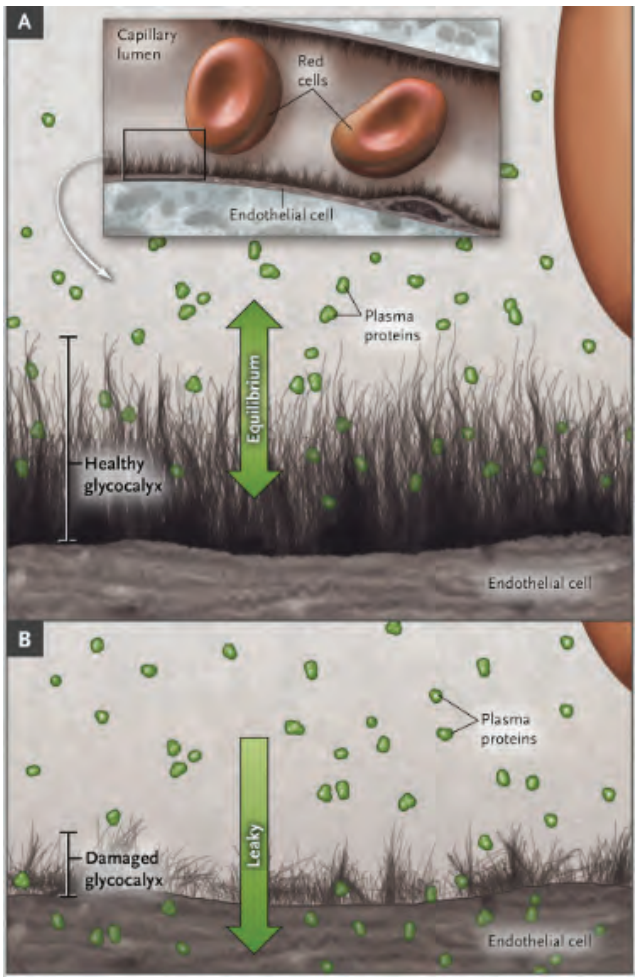

Figure 3: Endothelial glycocalyx layer in health (A) and damaged by disease (B). The integrity of the endothelial glycocalyx layer may dictate the permeability of membranes and may explain why there are differences in the response to colloid fluid therapy depending on the state of the animal. From N Engl J Med, Myburgh GA, Mythen MG. Resuscitation fluids. 369:1244. Copyright © (2013) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society

Are crystalloids safe?

Given the side effects associated with the use of colloids, crystalloids are expected to continue to play a major role in human fluid resuscitation despite the higher risk of interstitial oedema. Sodium chloride (0.9% Saline) has historically been the most used solution for volume expansion in human patients. However, due to its composition, the use of 0.9% saline is associated with the development of hyperchloraemic metabolic acidosis [45]. When compared to balanced crystalloids solutions (e.g. lactated Ringer, Plasma-Lyte, Compounded Sodium Lactate), 0.9% saline use is associated with a higher morbidity and mortality [46,47,48]. The role of chloride-load and hyperchloraemic metabolic acidosis in veterinary critical illness is unknown, but it seems prudent to prefer the use of balanced crystalloids over saline for fluid resuscitation.

An alternative to isotonic crystalloids for fluid resuscitation is hypertonic saline. These solutions contain a higher percentage of sodium chloride of 7.2% and 23.4%. As such they have a very high osmolarity and once infused produce an elevation in intravascular sodium. This in turn creates a concentration gradient that drives fluids from the interstitial and intracellular to the intravascular space with a short lived (20-30 minutes) intravascular volume expansion. The theoretical advantage is achieving fluid resuscitation whilst infusing a smaller volume of fluid. The displacement of fluid from the interstitium might also play a role in the treatment of traumatic brain injury. The use of hypertonic saline requires adequate intracellular and interstitial hydration and can cause bradycardia and hypernatremia as possible complications [5]. The safety and efficacy of hypertonic saline has not been established in human medicine and results of preliminary studies are contradictory [43].

Along this qualitative toxicity a quantitative toxicity has also been described. A positive fluid balance is associated

with the development of interstitial oedema and worse outcome [49]. In a trial comparing restrictive versus liberal fluid strategies, the latter has been associated with a reduced morbidity [50]. As we will see in the next paragraphs clinical research is focusing on methods to identify the adequate dose of fluid to administer.

How much fluid to give?

A variety of strategies have been developed to optimise tissue perfusion in critically ill patients and have been collectively defined as goal-directed therapies. The application of goal-directed therapy protocols has shown to improve both morbidity and mortality in people [51]. Restoring and maintaining an adequate circulating blood volume is considered the most important aspect in goal-directed therapy, however identifying the adequate fluid dose for each individual patient is not an easy task. Insufficient fluid resuscitation will be associated with inadequate tissue perfusion, but excessive fluid administration will also have negative consequences through the development of interstitial oedema, leading to organ dysfunction [52]. Therefore, identifying those patients that will benefit from fluid administration is essential. This concept is referred to as fluid responsiveness, and is defined as the ability of a patient's cardiac output to improve following the administration of intravenous fluid therapy. Historically fluid-responsiveness was assessed through "static" indexes of cardiac preload (e.g. central venous pressure), but such markers appear to be inadequate [53]. For this reason, a new approach using "dynamic" indexes has been developed and is based on evaluating the effect of changes in cardiac preload on the cardiac output [54]. An example of a dynamic index of fluid responsiveness evaluated in veterinary patients is pulse pressure variability (PPV) [54]. Arterial pulse pressure is used as a surrogate marker of stroke volume and its variation is assessed in patients undergoing mechanical ventilation. Due to lung-heart interactions, each respiratory cycle decreases preload in a predictable manner and this causes a decrease in cardiac output proportional to the patient "fluid-dependency." Therefore, patients that will benefit from fluid administration will have a proportionally higher PPV. This index has been validated in dogs [55,56]. The use of PPV in veterinary practice is limited by the need to place an arterial catheter, a procedure that can be technically challenging, especially in small or collapsed patients, and carries a risk of bleeding and infection. Moreover, only some monitors support algorithms that allow the measurement of PPV. The application of an early goal-directed haemodynamic optimisation protocol has been recently described in dogs undergoing surgery for pyometra [57]. Further studies are needed to identify the ideal end-points of resuscitation and if a goal-directed protocol would improve the outcome in veterinary patients.

Conclusions

Current evidence in human medicine suggest that the use of colloids over crystalloids carries very little benefit, with the potential of significant side effects associated with the use of synthetic colloids. Experimental veterinary studies show theoretical advantages of colloids, but these effects have not proven to be associated with an improvement in outcome. Moreover, although the nephrotoxic effects of HES have not yet been observed in veterinary patients, the mechanisms of toxicity seem to be similar across all synthetic colloid classes, independent of their composition, concentration and molecular size. Further studies will be needed to assess the safety profile of synthetic colloids in veterinary patients. In the meantime, we believe that a precautionary principle should be applied to colloid administration in veterinary patients, and their use should be limited to selected circumstances. Synthetic colloids should be used with particular caution in critically ill patients with sepsis or an established acute kidney injury. In conclusion, all fluids should be considered as drugs, and as such have the potential to cause toxicity if administered incorrectly. Context sensitivity appears essential in the selection process of fluid type, dose, and timing of administration. Fluid resuscitation protocols will therefore have to be tailored based on the clinical status of each individual patient. Veterinary research should focus on the identification of criteria for patients' stratification in order to develop individualised fluid resuscitation plans.

The authors declare no conflict of interests.

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COMMISSIONED PAPER

Plumage disorders in psittacine birds - part 2: feather damaging behaviour

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SUMMARY

Plumage disorders in parrots represent one of the more common, but also one of the more challenging and frustrating problems that veterinarians dealing with parrots in their daily practice face on a day-to-day basis.

This second part of the review will deal with diseases causing lack of feather growth and/or feather loss, including feather damaging behaviour. The latter certainly is one of the more difficult problems to address, as causes are numerous and may include (a combination of) medical, environmental as well as behavioural causes. In most cases, an extensive diagnostic work-up, including a thorough history, full physical examination and additional diagnostic tests, is therefore needed to identify the underlying disease or, in case of feather damaging behaviour, rule out the presence of a medical cause prior to being able to diagnose that the disorder is the resultant of a behavioural disorder.

Keywords: feathers; feather damaging behaviour, feather disorders; integument; parrot; plumage; dermatology

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Introduction

The first part of this review paper (EJCAP (2014) 24(1); Spring 2014, p34-p47) discussed the various feather abnormalities that may occur in psittacine birds. This second part will deal with lack of feather growth and feather loss – including feather-damaging behaviour (FDB).

Inactive feather follicles and lack of feather growth

Feather follicles are normally inactive between moults. Persistent generalized inactivity of the feather follicles should, however, be considered abnormal and may result in gradually progressive feather loss. In addition to PBFD, as discussed in the first part, endocrinopathies such as hypothyroidism should be considered in the differential diagnosis for feather follicle inactivity. Documented cases of hypothyroidism in companion birds are, however, rare[1-3], with only one well-documented, confirmed case of hypothyroidism available in the literature [4]. The clinical and laboratory findings in this bird, an adult male Scarlet macaw, consisted of (non-pruritic) progressive feather loss, obesity, hypercholesterolaemia, mild non-regenerative anaemia and low baseline T4 levels. A TSH-stimulation test using 1 IU of bovine TSH [5] yielded no response, thereby confirming the tentative diagnosis. Other tests that might have been useful to diagnose hypothyroidism but have to date not been used to confirm spontaneously occurring hypothyroidism in birds include scintigraphy and collection of thyroid biopsies [6,7].

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Similar to dogs and cats, treatment may be initiated with L-thyroxine (20 µg/kg q12h PO) which, in the case of the macaw, resulted in a resolution of the clinical signs a few months after initiation of the treatment [4].

Other endocrinopathies that may result in lack of feather growth include hyperadrenocorticism, hypoadrenocorticism and hyperoestrogenism. Thus far, however, no confirmed cases with clinical signs of feather loss have been documented, except for a single, confirmed case of hyperadrenocorticism due to an adrenal gland neoplasm [8]. When encountering a case of suspected endocrinopathy, appropriate testing, such as blood hormone analysis, stimulation and/or suppression tests, diagnostic imaging and histopathological examination of biopsies, will be necessary for confirmation of the tentative diagnosis.

Feather loss

In most parrots presented with feather loss, the loss of feathers is the result from the bird (or a cage mate) pulling out its feathers (Fig 1). This behaviour and its underlying medical and behavioural causes are considered in the next section ('feather damaging behaviour'). Feather loss may occasionally also occur in absence of this behaviour. In most of these cases, the underlying disease damages the feathers or follicles in such a way that they are easily lost or shed.

The differential diagnosis for feather loss without presence of feather damaging behaviour includes: a) normal apteria or normal moult (not recognized as such by the inexperienced parrot owner); b) excessive or irregular moult induced by malnutrition or irregular photoperiod; c) prior damage to the feather follicle resulting in cessation of feather growth; d) genetic conditions (e.g., baldness in lutino cockatiels); e) obesity, resulting in alopecia over the breast (particularly in budgerigars); f) ectoparasites (e.g., *Knemodikoptes spp.*, feather and quill mites, lice; see Part I, section 'poor feather quality'); g) clinical manifestation of PBFD or polyomavirus infection (see Part 1, section 'feather dystrophy'); h) mycotic infections, including candidiasis, *Malassezia* dermatitis and dermatophytosis; i) bacterial dermatitis and abscesses; j) neoplastic skin disease including xanthomas (Fig. 2); k) systemic disease including hepatopathies and nephropathies; and l) endocrinopathies such as hypothyroidism [9-14]. Often, the feather loss will remain localized (e.g. in case of obesity or neoplastic disease), but generalized feather loss may also occur (e.g. in case of PBFD). Based on the distribution of the feather loss, certain diseases may be ruled out. In addition, diseases may be ruled out based on the presence or absence of skin damage or skin lesions.
Diagnostic work-up of patients presented with feather loss should always begin with a thorough history and full physical and dermatologic examination. Additional diagnostic work-up may furthermore be warranted and largely depends on the type of diseases that remain on the differential diagnosis list. Likewise, the therapeutic plan will depend mainly on the presumptive or definite diagnosis that is made. In any case, additional contributing factors such as suboptimal diet, housing, environment and management may be corrected as well.

Figure 1. Feather loss is usually the result of self-inflicted damage. Typically, these birds have a normally feathered head. Occasionally, feather loss may also be inflicted by a cage mate, as was the case in this Green-winged macaw (Ara chloroptera), in which the baldness remained localized to the head.

Figure 2. Xanthoma on the wing tip of a budgerigar (Melopsittacus undulatus). Due to the presence of the mass, feathers appear to be missing in this area.

Feather damaging behaviour

Feather damaging behaviour (FDB), also referred to as feather destructive behaviour, feather plucking, feather picking or pterotillomania [15-17], is one of the most common and frustrating conditions to deal with in captive parrots. It has been estimated that approximately 10-15% of captive parrots chew, pluck, bite or pull their feathers [18,19], thereby inflicting serious damage to their plumage, particularly in areas that are easily accessible to the beak (i.e., neck, chest, flanks, inner thighs and wings) [15,20]. Although in many cases the consequences of this self-inflicted feather damage may be solely aesthetic, medical issues may also arise due to alterations to the birds' thermoregulatory abilities and metabolic demands, and/or the development of haemorrhage and/or (secondary) infections [20-22].

Although FDB may be noted in all psittacine species, it appears to be most common in Grey parrots (*Psittacus erithacus*), cockatoos (*Cacatua spp.*) and Eclectus parrots (*Eclectus roratus*) and less common in Amazon parrots (*Amazona spp.*), cockatiels (*Nymphicus hollandicus*) and budgerigars (*Melopsittacus undulatus*) [23,24].

It is often difficult to identify whether the feather damage is self-inflicted or due to a medical or environmental-related condition that causes loss or damage to the bird's plumage irrespective of its behaviour. First, birds are often left unobserved throughout a specific portion of the day, which limits the owner's ability to properly observe the bird's behaviour. Second, it is often difficult for the owner to distinguish FDB from normal preening behaviour, especially since both may follow the same pattern [25]. Once it has been established that the feather damage or loss is self-inflicted, the next challenge is to identify whether the condition primarily originates from a medical condition, results from husbandry, management and/or nutritional related issues, or if it should be regarded as a behavioural problem (i.e. psychogenic FDB). Determining the exact cause often proves challenging, especially since multiple factors usually play a role, thereby also posing it a great challenge to effectively treat and eliminate the behaviour. In the following paragraphs the most common causes and risk factors associated with FDB, as well as the diagnostic and therapeutic approach to FDB, will be discussed.

Aetiological considerations for FDB

FDB is considered to be a multifactorial disease, in which various medical, genetic, neurobiologic and socio-environmental factors may play a role (Table 1) [26].
Numerous medical conditions have been associated with FDB, albeit without proper scientific documentation to determine a causal relationship. In short, any disease causing pain, discomfort, irritation and/or pruritus may result in development of FDB. This may both include primary feather and skin diseases as well as systemic diseases (Table 1) [12,24,26-34]. In cases of systemic disease, feather damage may either be diffuse and generalized or localized directly over the region of discomfort. Renal disease, for example, appears to induce FDB in the region of the synsacrum [35,36], whereas hepatic disease may either induce feather damage that is limited to the ventral portion of the body or follows a more diffuse, generalized pattern [37,38].

A commonly encountered risk factor for FDB also includes an inappropriate and/or suboptimal environment (Table 1). A small cage or poor cage design may cause damage to the feathers, in particular the primaries and tail feathers. As a result, the bird may remove the damaged feathers, which should actually be considered as normal behaviour [27]. Other environmental risk factors that have been implicated in FDB include nutritional deficiencies and dietary imbalances; airborne and topical toxins; low humidity levels and abnormal photoperiods [13,26,27].

If no medical or environmental basis can be found for FDB, behavioural and/or psychological factors should be considered. In general, two differential diagnoses should be taken into consideration when a behavioural disorder is suspected: 1) **maladaptive** behaviour resulting from attempts of the animal to behave normally in an abnormal or inadequate environment (either innate or learned); and 2) **malfunctional** behaviour resulting from an abnormal psychology, brain development, or neurochemistry introduced by features of the captive environment [39,40]. Although the two are not separate entities per se and may even represent consecutive phases of the same disorder, the ability to make a distinction between the two may be of particular importance when considering the success of future therapeutic interventions. Whereas maladaptive behaviour may benefit from changes to the environment that help to optimize the bird's living conditions, malfunctional behaviour may show a lack of response to these measures and will more likely require the use of psychopharmaceutic drugs to reduce the behaviour.

The list of factors that contribute to the development and maintenance of psychogenic FDB are numerous and

Table 1. Causes of feather damaging behaviour in parrots

Environmental	Medical	Behavioural
• Nutritional deficiencies (e.g. hypovitaminosis A) and/or dietary imbalances • Small cage or poor cage design with little space for the parrot to move around • Overcrowding • Airborne and/or topical toxins, including cigarette smoke, scented candles, air fresheners, hand lotions and creams • Low humidity levels, lack of bathing opportunities • Abnormal photoperiod • Poor wing trim • Trauma	• Ectoparasites (e.g. *Knemidokoptes*, feather or quill mites, lice) • Bacterial or fungal dermatitis and/or folliculitis (including *Staphylococcus, Aspergillus, Candida, Malassezia*) • Polyomavirus • Psittacine beak and feather disease • Skin neoplasia (e.g. xanthoma, lipoma, squamous cell carcinoma) • Hypersensitivity, skin allergy • Airsacculitis, pneumonia • Chlamydiosis • Proventricular dilatation disease (PDD) • Liver and/or renal disease • Hypocalcaemia • Endocrine disease (e.g. hypothyroidism, diabetes mellitus) • Reproductive disease (e.g. egg binding, cystic ovaries) • Heavy metal toxicosis (e.g. lead, zinc) • Gastrointestinal disorders such as colic, endoparasitism (particularly Giardiasis in cockatiels) • Obesity • Orthopaedic disorders (e.g. osteosarcoma, fracture, osteomyelitis)	• Hand-rearing and imprinting on humans • Social isolation • Overcrowding • Inability to perform species-specific behaviours, e.g. foraging • Boredom • (Sexual) frustration • Sleep deprivation • Stress • Anxiety • Sudden changes to the environment • Attention seeking behaviour; reinforced by actions of the owner • Abnormal repetitive behaviour resulting from neurotransmitter deficiencies and/or excesses (e.g. serotonin, dopamine, endorphins), similar to obsessive-compulsive or impulsive disorders

include socio-environmental factors (Table 1)[26,24,41-46]. In addition, the behaviour may be exacerbated by inappropriate responses by the owner (e.g. punishing or attempting to distract the bird while it is damaging its feathers) as the response may reinforce the behaviour [47,48]. Neurotransmitter deficiencies and/or excesses (e.g. dopamine, serotonin, endorphin) and a genetic background have also been proposed [26,49].

FDB has particularly been linked to the lack of foraging opportunities in a captive environment. As a result, time spent foraging is drastically decreased: whereas wild parrots spend up to 6 hours daily on searching, selecting and manipulating food [50], captive birds usually consume their daily food ration within 30-72 minutes [51,52]. The lack of appropriate target stimuli to engage in species-specific foraging behaviours may subsequently lead to onset of behavioural problems such as FDB [17,44]. Although the onset of this behaviour may in part be the result of the altered time budget of captive parrots, some studies have demonstrated that parrots are motivated to forage and will contrafreeload (i.e. work for food even when identical food is freely available), thereby suggesting that foraging may be a behavioural need [53-55].

For most of the other factors that have been implicated

in FDB, a causal relationship is less clear, thereby demonstrating the necessity for further epidemiologic and experimental studies into the aetiology and risk factors associated with FDB.

Diagnostic approach to FDB

In general, the diagnostic work-up of FDB is primarily aimed at identifying or ruling out any medical or environmental factors that may be involved. For this purpose, a thorough history and complete physical examination are deemed essential. During the physical examination, the self-inflicted nature of the feather damage and/or loss may be confirmed by the absence of feather abnormalities on the head, which is inaccessible to the bird's own beak (Fig. 3) [15]. In addition to the history taking and physical examination, a thorough dermatologic examination of the skin and feathers is warranted, after which diagnostic skin and feather samples may be collected (Table 2). If an underlying systemic cause is suspected, diagnostic work-up may be further expanded with e.g. a hematologic and/or biochemical blood panel, urinalysis, diagnostic imaging and/or endoscopy (Table 2) [27,56]. Intradermal skin testing for diagnosis of allergic skin disease has been described, but thus far found to be unreliable in part due to the bird's diminished reaction to histamine [57,58]. Definite diagnosis of allergic skin disease may therefore be difficult, although the collection of paired skin biopsies from affected and unaffected areas of the same patient may identify presence of inflammation consistent with delayed-type hypersensitivity reaction [59,60].

Figure 3. Grey parrot (Psittacus erithacus erithacus) with feather damaging behaviour. Note the normally feathered head, which is typical for a bird with self-inflicted damage to the feathers.

If the abovementioned tests fail to identify a medical problem, a psychologic or behavioural origin of the disorder becomes likely. It then becomes important to identify the potential underlying triggers (antecedents) and reinforcing factors (consequences) that may have contributed to the onset and maintenance of FDB [48]. The latter is, however, often difficult and time consuming and may be limited by reliability and accuracy of the owner's observations and his or her willingness to learn and commit to behavioural enrichment and modification techniques [27].

Therapeutic considerations for FDB

The therapeutic approach to FDB in the individual bird will largely depend on the findings of the history, physical examination and diagnostic tests. An initial therapeutic plan will often be aimed at correction of the diet and modification of the bird's housing and living conditions to address any environmental factors that may be involved. If any medical issues are encountered, these should be appropriately addressed, which may include the use of topical and/or systemic antibiotics, antifungals and antiparasitic drugs to treat any underlying parasitic or infectious disease. In case of suspected allergies, antihistamines (e.g. hydroxyzine hydrochloride, 2 mg/kg PO q8h) and/or corticosteroids may be considered in addition to dietary and/or environmental modifications that aim to decrease or eliminate exposure to the suspected allergen(s), although one should always be hesitant to use corticosteroids in birds because of the potential of profound immunosuppression and development of secondary infections [61].

Promoting a more stimulating environment by means of social contact, perches, chewing toys, puzzle feeders and other forms of environmental enrichment may be considered as an important part of the treatment regimen to alter the behaviour [3]. In particular foraging enrichment has been shown to effectively reduce FDB [17,25,44]. Providing such enrichment may be as simple as providing complicated food items such as corn on

Table 2. Diagnostic tests that may be performed in birds with feather abnormalities

Diagnostic test	Indications
CBC & Biochemistry	Hepatopathy, nephropathy, generalized infection or inflammatory process, diabetes mellitus, hypocalcemia
Toxicology	Suspected lead or zinc toxicosis. Collect heparinized whole blood (lead) or plasma/serum in non-rubber plastic or glass tubes
TSH stimulation test	Hypothyroidism
Fecal cytology (incl. wet mount and/or flotation)	Giardiasis (common in cockatiels), helminth infection, candidiasis, *Macrorhabdus ornithogaster* infection (avian gastric yeast), bacterial gastroenteritis
Radiology	Heavy metal intoxication, reproductive disorder (e.g. egg binding), hepato-, spleno- or renomegaly, proventricular dilatation disease, pneumonia, airsacculitis, neoplastic conditions, musculoskeletal disease (e.g. osteoarthritis, osteomyelitis, fractures, osteosarcoma)
Ultrasound	Hepatomegaly, reproductive disorders (e.g. egg peritonitis, cystic ovary), neoplastic conditions, cardiac disease, ascites
Endoscopy	Air sacculitis, hepato- or nephropathy, splenomegaly, pancreatic disorders, reproductive disease
Skin scrapings	Ectoparasites, in particular mites (e.g. *Knemidokoptes*)
Impression smear, swab cytology or tape strip	Bacterial or fungal dermatitis, dermatophytosis, *Malassezia, Candida,* ectoparasites (e.g. feather mites, lice), pox virus
Fine needle aspirate	Skin neoplasia, xanthomatosis, feather follicle cyst, hematoma, bacterial dermatitis or abcess
Feather digest (using potassium hydroxide)	Ectoparasites (quill mites)
Feather pulp cytology	Bacterial or fungal folliculitis, PBFD or polyomavirus infection, quill mites
Culture	Bacterial or fungal dermatitis, folliculitis
Skin and/or feather follicle biopsy (histopathology)	Various infectious, inflammatory and/or neoplastic skin diseases, e.g. PBFD, polyomavirus, bacterial and fungal folliculitis, quill mite infestation, xanthomatosis, squamous cell carcinoma, feather follicle cysts
Intradermal skin testing	Hypersensitivity reactions, allergic skin disease. Thus far not found to be reliable due to the bird's diminished reaction to histamine
Tests for specific causative agents	• PCR testing on whole blood, feather pulp or tissue for Psittacine beak and feather disease virus (PBFD) • PCR testing on faecal swab or tissue for presence of Polyomavirus • PCR on cloacal swab/faeces and/or serologic testing for Avian Bornavirus (ABV) • PCR on conjunctival/choanal/cloacal swab and/or serologic testing for *Chlamydia psittaci*

the cob, pineapples or pomegranates, providing food in larger chunks or pellets, using multiple feeding stations, scattering the food through the enclosure and/or mixing it with inedible items (Fig. 4a and b) [27,52,62]. Owners may also use paper bags, cardboard boxes, plastic bottles and other materials to create their own foraging toys, or, alternatively, buy one or more of the more complicated foraging devices and puzzle feeders that have become commercially available throughout the past years (Fig. 5). Although most of these foraging enrichments appear effective to significantly increase foraging time of captive parrots, they do not appear capable of naturalizing foraging times to levels comparable with those of wild conspecifics (i.e., 4-6 hours per day) [52]. New, more effective foraging enrichments may therefore need to be developed and tested for their efficacy to naturalize

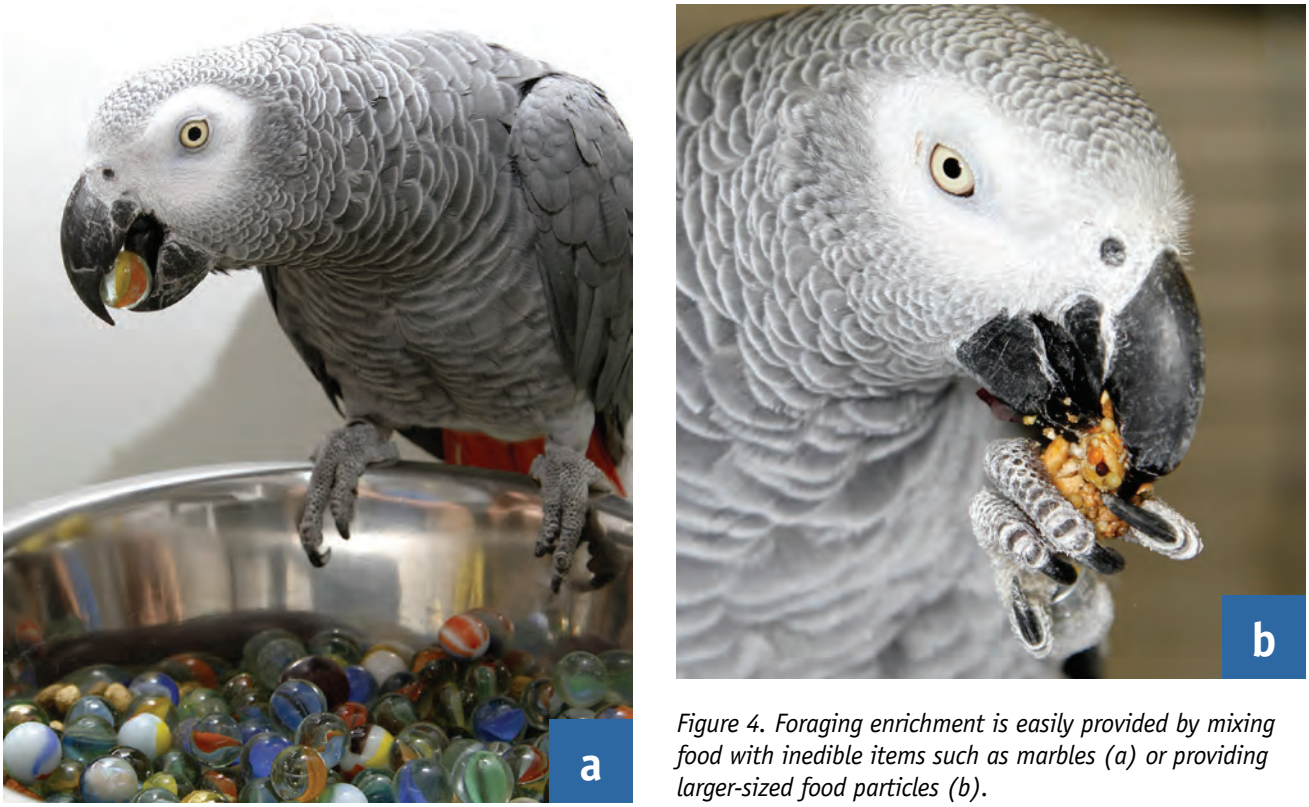

Figure 4. Foraging enrichment is easily provided by mixing food with inedible items such as marbles (a) or providing larger-sized food particles (b).

Figure 5. A variety of commercially available foraging enrichments (puzzle feeders), which may help to promote foraging activity and increase foraging time in captive parrots.

foraging behaviour and reduce abnormal behaviour.

In addition to providing environmental enrichment, behaviour modification techniques such as differential reinforcement of other behaviours may be employed to alter the behaviour of the bird [24,48,63]. Training may furthermore also help to provide the bird a mentally stimulating challenge or task, provided the owner is able and willing to employ the techniques in a proper and consequent manner.

Other treatments that have been used to treat psychogenic FDB include the use of Elizabethan collars and neck braces (Fig 6a-c), fabric "ponchos", "jackets" or "vests" (Fig 7a and b) and/or local application of foul tasting substances [27,41]. It should however, be remembered that these interventions are primarily aimed at preventing the symptoms rather than eliminating the underlying cause.

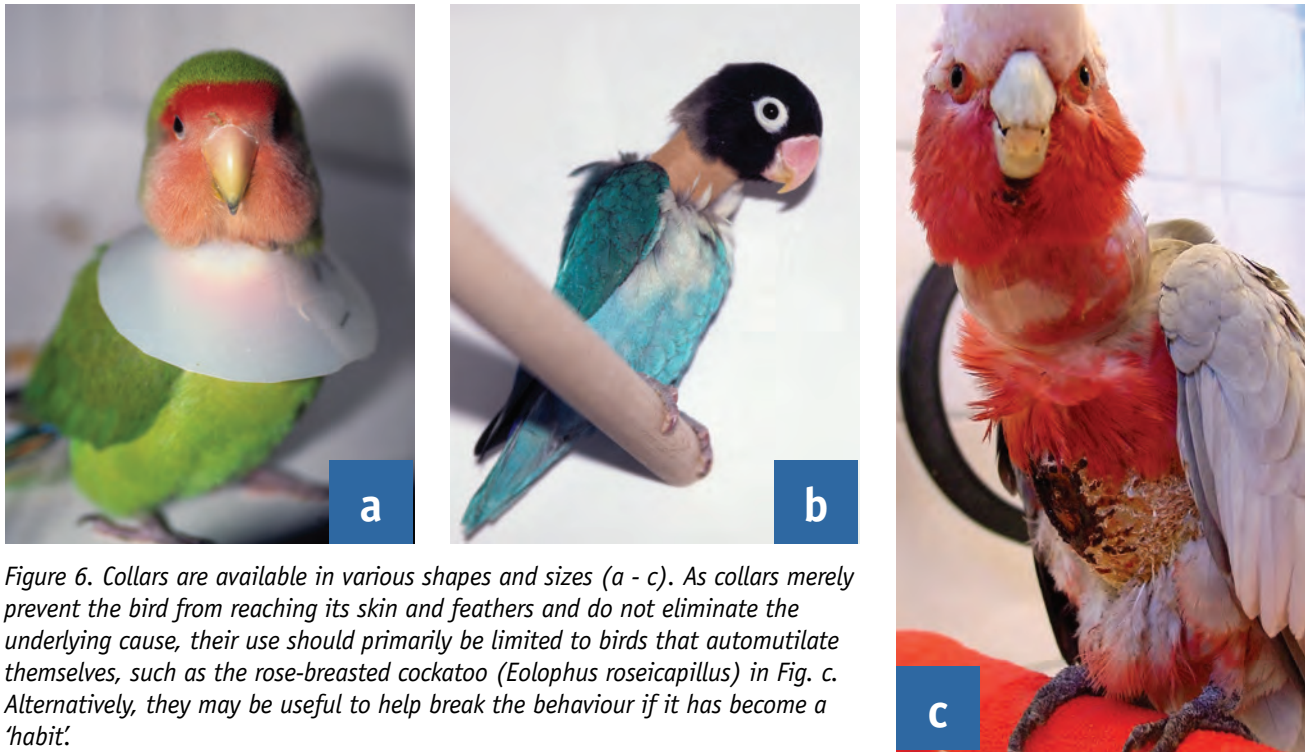

Figure 6. Collars are available in various shapes and sizes (a - c). As collars merely prevent the bird from reaching its skin and feathers and do not eliminate the underlying cause, their use should primarily be limited to birds that automutilate themselves, such as the rose-breasted cockatoo (Eolophus roseicapillus) in Fig. c. Alternatively, they may be useful to help break the behaviour if it has become a 'habit'.

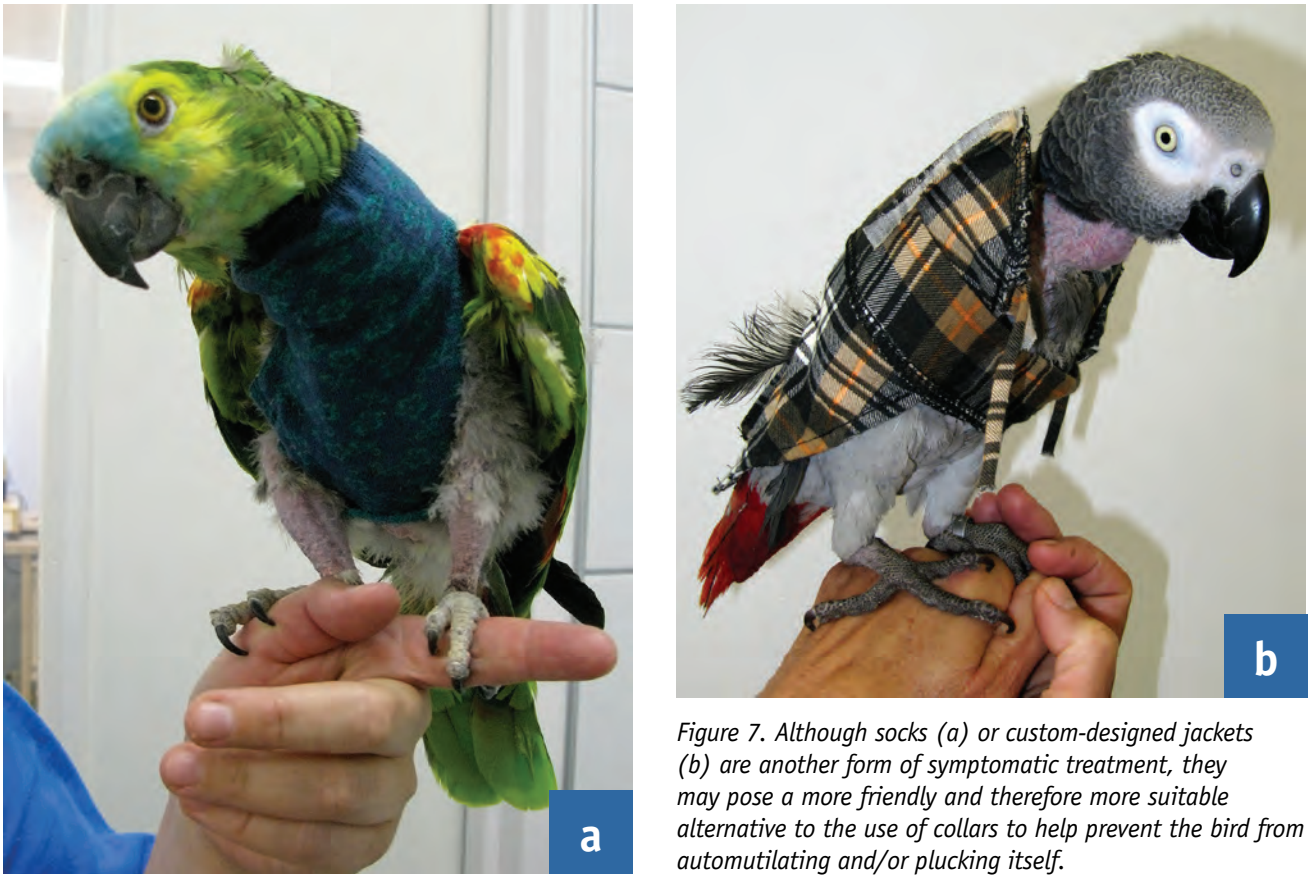

Figure 7. Although socks (a) or custom-designed jackets (b) are another form of symptomatic treatment, they may pose a more friendly and therefore more suitable alternative to the use of collars to help prevent the bird from automutilating and/or plucking itself.

They may, however, be helpful for a short period of time to stop birds from automutilating themselves and/or break the cycle of habitual FDB. When placing a collar, one should take into consideration that not all birds respond well to the placement; administration of tranquilizers such midazolam (0.3–0.5 mg/kg IM) may be considered helpful in those cases to facilitate acclimatization to the collar.

Pharmacologic intervention has also been proposed (for a review, see Seibert, 2007 [64]), particularly in those cases that appear refractory to treatment with behaviour modification therapy and environmental changes. Options include: a) anxiolytic drugs such as diazepam [64]; b) antipsychotic drugs such as the dopamine antagonist haloperidol [65,66]; c) tricyclic antidepressants such as

Table 3. List of psychotropic drugs that may be used in parrots with feather damaging behaviour

Drug	Mode of action	Suggested dose
Amitryptilline	Tricyclic antidepressant; antihistamine	1-5 mg/kg PO q12-24h
Buspirone	Anxiolytic drug, used in the treatment of anxiety disorders	0.5 mg/kg PO q12h
Clomipramine	Tricyclic antidepressant; antihistamine; used e.g. in the treatment of impulsive and obsessive-compulsive disorders (ICD/OCD), depression and/or anxiety disorders	0.5-1 mg/kg PO q12-24h
Diazepam	Benzodiazepine, tranquilizer, used in treatment of anxiety or panic disorders	0.5-0.6 mg/kg IM/IV q8-24h
Doxepin	Tricyclic antidepressant; antihistamine	0.5-1 mg/kg PO q12h
Fluoxetine	Selective serotonin reuptake inhibitor; antidepressant; used in the treatment of depression, post traumatic stress and panic disorders and ICD/OCD	0.4-4 mg/kg PO q12-24h
Haloperidol	Dopamine antagonist, antipsychotic drug	0.1-2 mg/kg PO q12-24h
Leuprolide acetate	Synthetic GnRH agonist depot drug; may be used in cases of FDB with suspected hormonal component	0.1 mg/kg IM q24h
Medroxyprogesterone acetate	Progesterone derivative; was used for reproductive-related FDB in the past, but not recommended nowadays due to severe side-effects!	5-50 mg/kg SC or IM
Naltrexone	Opiate receptor antagonist, used in the treatment of addictions	1.5 mg/kg PO q8-12h
Paroxetine	Selective serotonin reuptake inhibitor, antidepressant, used in the treatment of depression, post traumatic stress and panic disorders and ICD/OCD	2-4 mg/kg PO q12-24h

amitriptyline, clomipramine and doxepin [64,67,68]; d) serotoninergic reuptake inhibitors such as paroxetine and fluoxetine [64,69,70]; and e) opioid antagonists such as naltrexone [64,71] (Table 3). In cases of suspected sexual or hormonally related FDB (e.g. seasonal occurrence and presence of [hyper]sexual and nesting behaviours) treatment may be initiated with a depot gonadotropin-releasing hormone (GnRH) such as deslorelin or leuprolide acetate, or medroxyprogresterone acetate [64]. Of these aforementioned drugs, the tricyclic antidepressant clomipramine is best investigated, but yielded mixed results [67,68]. For most of the other drugs, placebo-controlled, double-blind, randomized and peer-reviewed studies concerning dosages, pharmacokinetics, toxicity and efficacy are currently unavailable, thereby limiting the ability to make recommendations at this stage.

Due to the inability to determine the antecedents and consequences that are associated with FDB, the chronicity and/or ritualization of the behaviour and the overall lack of scientific evidence regarding the efficacy of the various therapeutic interventions, management of the condition often proves to be challenging. To be able to assess changes in FDB over time resulting from specific preventive or therapeutic interventions undertaken, consistent and reliable scoring methods are needed. Although direct behavioural observations are possible [66], this method does not appear reliable since it is difficult to distinguish normal preening from abnormal FDB and bouts of FDB may be missed as these may occur during the night [25,44]. Feather scoring systems, which measure FDB indirectly by assessing plumage condition, pose a reliable and practical alternative and may be used for both scientific studies and individual patients (Table 4) [44,72].

Table 4. Feather scoring system of van Zeeland et al (2013).

(A) Score determination table for coverts and down feathers; used for chest/neck/flank, back, legs, dorsal and ventral surface of the wings

converts	Down feathers			
	No down removed	<50% of down removed	>50% of down removed	All down removed
All coverts intact	100	85	70	60
Fraying or breakage	95	80	65	55
<25% of coverts removed	90	75	60	50
25-50% of coverts removed	80	65	50	40
50-75% of coverts removed	70	55	40	30
75-90% of coverts removed	60	45	30	20
>90% of coverts removed	50	35	20	10

The percentage of damage to the covert and down feathers is assessed for each body part separately.
Deduct 10 points from the score if skin damage is present.

Total body plumage score (0-100) = 0.25 x chest/flank + 0.17 x back + 0.10 x legs + 0.28 x dorsal wings + 0.20 x (ventral wings)[1]

(B) Score determination for flight feathers; used for tail, primary and secondary feathers (wings)

Score	Description
0	Flight feather with signs of fraying and/or breakage over >50% of the original length
1	Flight feather with signs of fraying and/or breakage over <50% of the original length
2	Flight feather with little or no damage present

Damage to individual flight feathers is assessed.

Total flight feather score (0-100) = (primary + secondary feathers left wing) + (primary + secondary feathers right wing) + (tail feathers)[2]

[1] To determine the total body plumage score, the scores for each body part are corrected for their relative body surface percentage, similar to scoring systems used in human burn victims. These percentages (expressed as % of the total body surface area excluding the surface area of the head and unfeathered parts of the legs) were determined in six grey parrots. Mean (± SD) values for the various body parts were 25 ± 1.2% (chest/neck/flank), 17 ± 1.5% (back), 10 ± 1.2% (legs), 28 ± 2.2% (dorsal wing surface, up to the level of the tertiaries) and 20 ± 1.9% (ventral wing surface, up to the level of the tertiaries).

[2] The maximum score is dependent on total number of flight feathers of the bird. In general, each wing has 10 primary feathers and 10 secondary feathers (remiges), whereas the tail has 10-12 flight feathers (rectrices). As each individual flight feather is awarded a score from 0-2, the score will range from 0-40 for each wing and from 0-20 (or 0-24 in the case of 12 tail feathers) for the tail, respectively.

Conclusions & future considerations

A variety of different conditions may affect the plumage of parrots resulting in various feather abnormalities. Of the various plumage disorders described in the two parts of this review, feather-damaging behaviour is by far the most complicated and frustrating problem to deal with for both owners and veterinarians. Various underlying aetiologies and associated risk factors exist and often the onset of the behaviour will result of a complex interplay between medical, environmental, nutritional, psychological and genetic factors. For all of the disorders a thorough history and medical work-up are needed to identify any underlying causes that should be treated accordingly. If psychogenic FDB is involved, a variety of enrichment and behavioural modification techniques may be used to create a more stimulating living environment and reduce the behaviour. Currently, however, there is a lack of scientific information on the efficacy of the available therapeutic options. Future research should thus focus on evaluating the efficacy of these therapeutic interventions using appropriate study design and evaluation techniques. Once a parrot displays FDB, it may become increasingly more difficult over time to break their habit, with treatments generally yielding disappointing results [78]. As a result, parrots with FDB frequently end up being euthanized or relinquished to a shelter or sanctuary [51,103]. Proper client education about the environmental and psychological needs of parrots as well as further studies into risk factors and the (behavioural) needs of parrots are thus warranted to optimize the parrot's living conditions and be able to effectively prevent and/or eliminate the disorder in the future.

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Commissioned paper

Feline injection site sarcoma: a Latvian piece to the puzzle

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SUMMARY

Injection site sarcomas (ISS) (previously designated vaccine-associated sarcomas) are rare, aggressive tumours that develop in cats as a side effect after administration of vaccines or less often other types of medication. Inflammation driven fibroblast proliferation is implicated in the pathogenesis of these tumours. There are variations in the ISS incidence among countries which may be attributable to differences in infectious disease prevalence, vaccination frequency, and regulations. Since 2007, ISS are commonly seen tumours in cats in Latvia, constituting 14% of all feline biopsies submitted to the veterinary pathology laboratories in the last 4 years. Mandatory annual rabies vaccination since 1991 and widespread vaccination of cats since 2003 may have contributed to the increase of these tumours in Latvia. Recommendations for the reduction of ISS occurrence include reduced frequency of vaccination without compromising prevention of infectious diseases, injections at recommended sites, and education of owners about potential local reactions. Analysis of data at veterinary pathology laboratories is essential for recognizing trends in disease incidence, which need then to be reported back to the veterinary community.

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Basic facts

Cats are genetically predisposed to development of malignant tumours at the site of a previous injury, e.g. posttraumatic sarcomas in the eye, sarcomas at fracture and osteosynthesis sites and sarcomas at injection sites [14, 18, 33, 35]. The key points highlighted about injection-site sarcomas (ISS) in the literature are:

- ISS are rare with various calculations about the incidence and variation in numbers depending on country [10, 42, 44].
- ISS occur at injection sites (Figure 1) [33].

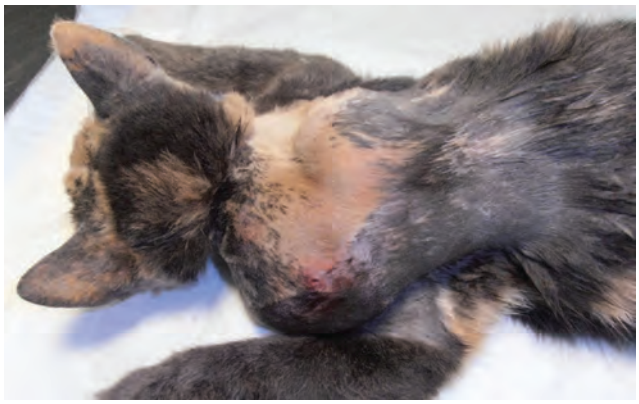

Figure 1. Cat with injection site sarcoma - large, multilobular tumour in the interscapular region.

- ISS are most commonly associated with inactivated adjuvanted vaccines. However, tumours have also occurred at injection sites of modified live, non-adjuvanted vaccines, long-acting steroids, penicillins,

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and recombinant (subunit) vaccines, therefore these tumours were renamed injection-site sarcomas [21, 34, 55].

- The incubation period is variable – most often between 3 months and 4 years but may be as long as 10 years [33, 40].
- The risk increases with repeated vaccine injections at the same site [33].
- The proposed pathogenesis is chronic inflammation-driven fibroblast proliferation with oxygen free radicals and growth factors such as platelet-derived growth factor (PDGF) playing a role in addition to mutations in the tumour suppressor gene p53 [22, 23, 28, 45].
- ISS are grossly well-delineated but show deep infiltration into surrounding tissues requiring wide surgical excision [6, 19]. Aggressive surgical excision is the treatment of choice with radiation and chemotherapy used as adjuvant therapies before or after surgery [44].
- Recurrence rates are high; they are reduced if radiation is used. Effectiveness of chemotherapy is not well proven [44, 58].
- Metastatic potential is variable but low (~20%) and may increase when adjuvant therapies are used [58, 60].

Link between vaccination policies and ISS incidence

Most of the epidemiologic data regarding ISS comes from research in the US [20, 25-27, 33, 34, 38, 44, 54]; there is substantially less incidence data on ISS in Europe, and most is limited to information about the UK [10, 56, 59]. Regional changes in vaccine usage and ISS incidence data in the US, UK and Latvia is summarised in Table 1. Not much data have been published about the incidence of ISS in other countries, although, there are case reports from Australia, New Zealand, Italy, France and many other countries [1, 4, 12, 43]. These publications and personal communications indicate that ISS are seen in many countries but are rare. Martano et al has proposed that incidence rates may vary considerably among countries [42]. This is likely influenced by regional vaccination guidelines, regulations regarding rabies control, and overall use of injectable medications.

USA

ISS was first recognised in the US in 1991 and linked to use of inactivated adjuvanted rabies vaccines [25]. This was based on circumstantial evidence: tumours were found at recorded rabies injection sites, and biopsies from these lesions contained adjuvant [26]. The emergence of ISS occurred approximately 5 years after adjuvanted rabies vaccines replaced modified live vaccines. Around the same time inactivated adjuvanted FeLV vaccines were introduced. Some research papers reported higher numbers of ISS being associated with FeLV than with rabies vaccines [38]. The ratio of injection-site to non–injection-site sarcomas increased from 0.54 in 1989 to 4.33 in 1994 [13]. Initial incidence was estimated to be 1-10/10 000 vaccine doses [16]. A later study by Kass (1993) reported 1.5 cases of ISS per 10 000 rabies vaccinations and a slightly lower rate - 1.2 cases of ISS per 10 000 leukaemia vaccinations in the US [33]. Other epidemiological studies and some case reports clearly showed that triggers for ISS were not limited to rabies and leukaemia vaccines, but also occurred at the sites of multivalent FVRCP+/-C vaccines, as well as long-acting penicillin and steroid injections [55].

In 1996, the Vaccine-Associated Feline Sarcoma Task Force (VAFSTF) was formed to develop guidelines for reduction of ISS and in 1997 recommendations were adopted by the American Association of Feline Practitioners (AAFP) [49, 52, 57]. One of VAFSTF's recommendations was to administer vaccines with the highest ISS risk in the rear legs. This allowed tracing of the tumours to a particular vaccine and permitted radical surgery, limb amputation, to ensure a greater probability of complete tumour removal. Since this recommendation was made, a drop in the number of sarcomas developing in the interscapular space was noted along with a more than doubling of the number of sarcomas in the rear legs [54]. This indicated that FeLV and rabies vaccines induce a higher risk than other vaccines. VAFSTF recommendations also called for the judicious use of all vaccines. Since tumours were rare to begin with, it is very difficult to determine if they have become even rarer after publication of the recommendations. Only 1 case of ISS was seen in a US clinic that adopted the VAFSTF guidelines and have administered more than 60 000 vaccine doses in 10 years indicating a sixfold lower rate than the 1/10 000 rate previously reported [46]. They reported using 53% non-adjuvanted vaccines (including 20 000 doses of recombinant rabies vaccine) and 47% adjuvanted vaccines (including 6 500 FeLV vaccines). The single ISS occurred at the site of injection of a recombinant rabies vaccine. By contrast, the proportion of skin masses diagnosed as ISS in the last 20 years at a Canadian private veterinary diagnostic laboratory has remained constant constituting 12-14% of all feline skin masses and 2% of all feline biopsy submissions [60].

Table 1. Timeline of injection-site sarcoma in the US, UK and Latvia: selected changes in vaccination and research results

Year	US	UK	Latvia
1985	Killed rabies vaccines replace modified live vaccines		
1987	Law in Pensylvania requiring mandatory cat vaccination against rabies		
1991	Increase in subcutaneous sarcomas reported by pathogists at University of Pennsylvania School of Medicine M. Hendrick, M. Goldschmidt [25]	Adjuvanted killed feline leukaemia vaccine introduced [59]	Mandatory vaccination against rabies required annually
1993	Causal relationship between vaccination and ISS; estimated incidence 3.6/10,000 cats [33]*		On average <10 000 cats vaccinated annually (Figure 2)
1996	VAFSTF[1] formed	First report of ISS [59]	
1997	VAFSTF recommendations adopted by AAFP[2]: recommended vaccine administration sites[3] RR for rabies, LR for leukaemia, RF for other vaccines		
2001	Updated VAFSTF report with research results; estimated incidence 1-10/10,000 vaccinated cats [57]	Veterinary product committee review – estimated ISS incidence 0.038/10,000 doses of vaccine [59]	
2002	Gobar and Kass study [20]: estimated incidence 0.63/10,000 cats vaccinated or 0.32/10,000 doses of all vaccines administered		
2003	Sarcomas associated with injections other than vaccines (long acting penicillin and methyl prednisolone acetate) [21, 55]		For the first time >30,000 cats vaccinated against rabies
2007		Estimated incidence 0.2-0.63 sarcomas/10,000 registered cats [10]	ISS seen by practitioners; pathology not done
2009	Shift in ISS location from interscapular region to rear legs [54]		ISS first confirmed by histopathology
2011			17 confirmed ISS cases; 33 353 cats vaccinated for rabies; incidence 5/10 000 vaccinated cats
2013			Legislation changed - rabies vaccination according to label

[1] Vaccine-associated feline sarcoma task force;
[2] American Association of Feline Practitioners;
[3] RR – right rear leg; LR – left rear leg; RF – right front leg;

The UK

Overall, ISS is regarded as a rare disease in the UK [10]. Beyond doubt, there are multiple differences between US and UK but most significant in relation to risk factors for ISS are rabies prevalence and associated control measures in these countries. UK is a rabies free country and animals are not vaccinated against rabies. However, feline leukaemia is a common disease and 84% of practitioners yearly vaccinate against leukaemia [9]. The first report of ISS from the UK was in 1996, 5 years after the introduction of adjuvanted killed leukaemia vaccines [59]. In the UK the estimated incidence in 2007 was 0.2-0.63/10 000 registered cats which is similarly low to the rate in the US[10]. However, it is nearly impossible to compare incidence data among studies and more so between countries because of variations in study methodology.

Table 2. Comparison of incidence of injection-site sarcomas in cats in pathology submissions in USA, Canada and Latvia

Country/Lab	Year	# feline submissions / year	# ISS / year	% ISS / feline submission	# cats vaccinated against rabies
ADDL, Indiana, USA[1]	1988-1994	ND	17	1.5%	ND
Histovet Surg. Path., Canada[2]	2005-2010	3684	87	2.4%	ND
Latvia	2010	48	4	8%	32 062
Latvia	2011	82	17	20%	33 353
Latvia	2012	123	16	13%	32 559
Latvia	2013	168	23	14%	31 060
Latvia	**Avg**	**105**	**15**	**13.8%**	**32 259**

ND – no data

[1] ADDL – Indiana Animal Disease Diagnostic Laboratory [51]

[2] Histovet Surgical Pathology, Guelph, Ontario

Latvia

Contrary to other countries, ISS in Latvia is not a rare tumour (Table 2). A disproportionately high number of ISS cases in cats have been seen in tumour biopsy submissions to both existing veterinary pathology laboratories in Latvia. ISS was diagnosed in 60/421 (13.8%) feline biopsy submissions in a 3-year time period (2010-2013) (Figure 2). This is a much higher percentage than the 2.4% reported by Wilcock et al. in the feline submissions to a private veterinary pathology laboratory in Canada [60]. In Latvia histopathology was not widely used prior to 2010 and data about the incidence of these tumours prior to this time is lacking. Personal communications with Latvian surgeons indicate that tumours in the interscapular region have been recognised for the past 6-7 years dating back to approximately 2007. ISS may have been present before this; however they were not recognised as a specific entity because histopathology was rarely performed and veterinarians in Latvia were not aware of them. There are several possible reasons for higher incidence of ISS in Latvia:

1. Since 1991, the state laws have mandated yearly cat vaccination against rabies.
2. Inactivated-adjuvanted rabies vaccines are used most commonly; recombinant subunit vaccines are available

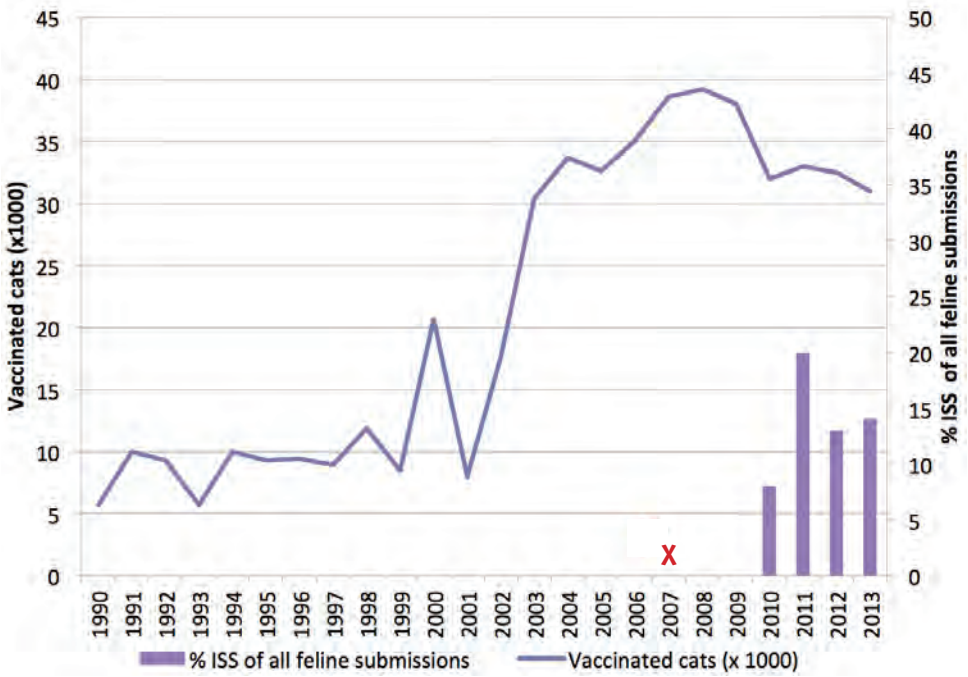

Figure 2. Temporal relationship between annual vaccination of cats and the number of diagnosed injection site sarcomas in veterinary pathology laboratories in Latvia (1990-2013). Tumours in the injection sites were first noted by some surgeons in 2007 (X).

but are infrequently used.

3. Until 2001, on average 9 800 cats were vaccinated yearly. After 2003 the number of vaccinated cats increased three fold to approximately 30 000 (Figure 2).
4. The total number of cats vaccinated for rabies is a fairly accurate estimate of all vaccinated cats in Latvia. Other vaccines may be given concurrently with rabies vaccination (herpes, calici and panleukopenia with or without chlamydia).
5. Cats are rarely vaccinated against FeLV because leukaemia is rare in the Latvian cat population (personal communication, Liene Dindone, DVM, Animal Medical Center, Riga, Latvia).

Data represented in Figure 2 show that tumours in the interscapular region were recognised about 4 years after rabies vaccinations became more widely used in Latvia. This lag time is similar to that seen in the US in early 1990 and in the UK after introduction of adjuvanted leukaemia vaccines. Yearly vaccination in combination with the large population of cats vaccinated for rabies may have caused the dramatic increase in ISS incidence, reaching approximately 14% of all feline biopsy submissions. Early research showed that ISS risk increased considerably with repeated vaccinations at the same site. This may have occurred in Latvia when yearly vaccination for rabies was mandated. There may be other contributing factors. Sick cats in Latvia are often treated with multiple daily injectable medications, receiving a large number of injections over the course of the illness. Additional analysis of ISS cases needs to be done to determine if cats with ISS have received multiple injectable medications over their lifetime. In 2013, due to improvements in rabies control in Latvia, the mandatory yearly vaccination for rabies was changed to mandatory vaccination according to the label. Veterinarians in Latvia need to adapt to the new regulations that permit decreased use of rabies vaccine.

Pathologic aspects of ISS

ISS have distinct gross and microscopic appearance that help to identify these as a specific subset of soft tissue sarcomas (Tables 3 and 4, Figures 3-7); however, neither gross nor histologic features are sufficiently specific for this diagnosis. The features that separate ISS from sporadic soft tissue sarcomas are: unusually large size of the cells and their nuclei, remarkable cellular pleomorphism, presence of multinucleated giants cells, and aggregates of lymphocytes and plasma cells at the periphery of the tumour [24, 27]. The presence of grey-blue material in the macrophages and extracellularly is noted in some tumours [26]. A pathologist can only suggest to the clinician that a sarcoma with these features may have been associated with an injection. The clinician must decide whether the location of the tumour and history of injections are compatible with ISS. ISS are deceivingly well-delineated but widely-infiltrative subcuticular or intramuscular masses and frequently contain multifocal fluid-filled cavities that form in the areas of necrosis. Microscopically these tumours have variable differentiation – most commonly being fibrosarcoma and less commonly osteosarcoma, chondrosarcoma, rhabdomyosarcoma, histiocytic sarcoma, and anaplastic sarcoma [6].

Table 3. Gross features of feline injection site sarcomas

Gross features of ISS
Located in subcutis
Well delineated
Infiltrative
Multilobular
Firm
Areas of necrosis
Cavitations filled with fluid
+/- cartilage or bone

Table 4. Microscopic features of feline injection site sarcomas

Microscopic features of ISS
Subtypes - fibrosarcoma (80%), chondrosarcoma, osteosarcoma and others
Tumour cells – large, pleomorphic spindle-shaped cells with large nuclei, multiple, large nucleoli
Variably high mitotic rate and frequent atypical mitoses
Presence of multinucleated giant cells
Aggregates of lymphocytes and plasma cells at the periphery of the tumor
Multifocal necrosis
Multifocal cavitations filled with fluid
Variable amount of fibrous tissue; may contain areas of cartilage or bone
Variable differentiation of tumour cells → tumour fibroblasts may be difficult to distinguish from mature tissue

Grading of ISS has not been shown to be highly useful; to date the grade of the tumour does not correlate with disease-free interval (DFI) or survival after surgery [50].

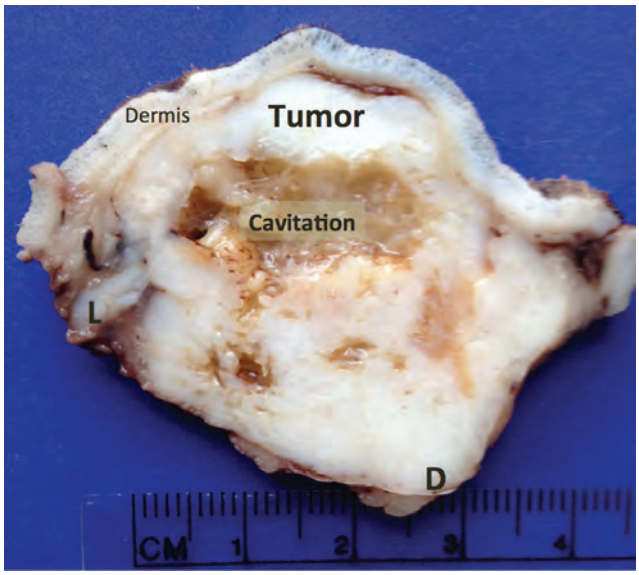

Figure 3. Biopsy of injection site sarcoma after fixation in formalin. Tumour is located in the subcutis. It is firm, light grey, multilobular and cavitated. The tumour is well delineated but it infiltrates a lateral (L) margin and extends to the deep (D) margin. Note that after fixation tissues lose contrast.

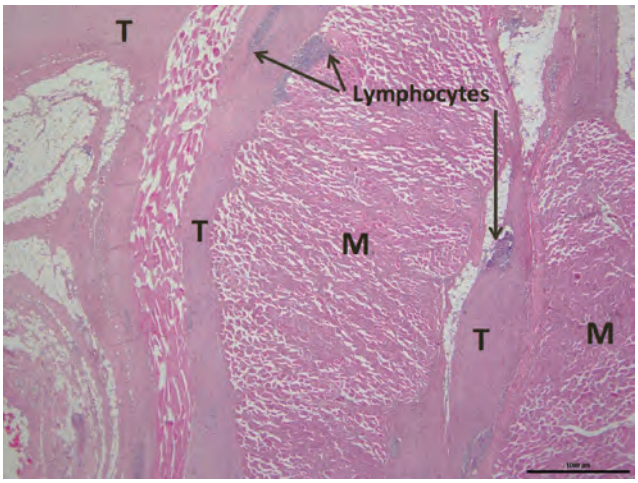

Figure 4. Injection site sarcoma – fibrosarcoma in a cat. Tumour cells (T) form long tentacles that infiltrate deep into muscle (M). Multifocally lymphocyte clusters are scattered (arrows). Haematoxylin and eosin stain; 20x.

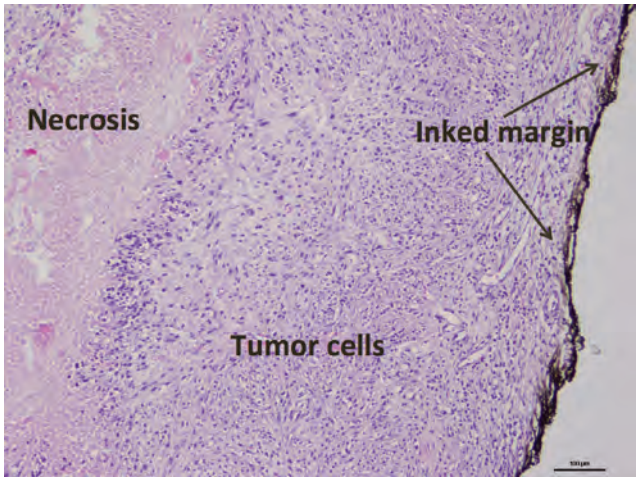

Figure 5. Injection site sarcoma – fibrosarcoma in a cat. Tumour contains area of necrosis. Tumour cells extend to inked margin indicating incomplete removal. Haematoxylin and eosin stain; 100x.

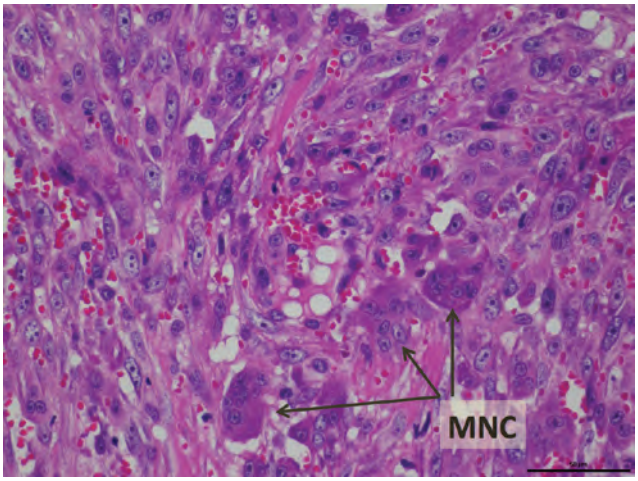

Figure 6. Cellular features of injection site sarcoma in a cat. Tumour cells are large spindle-shaped cells with large oval or irregular nuclei with 1-2 large nucleoli. Frequently, multinucleated cells are present (MNC). Haematoxylin and eosin stain; 400x.

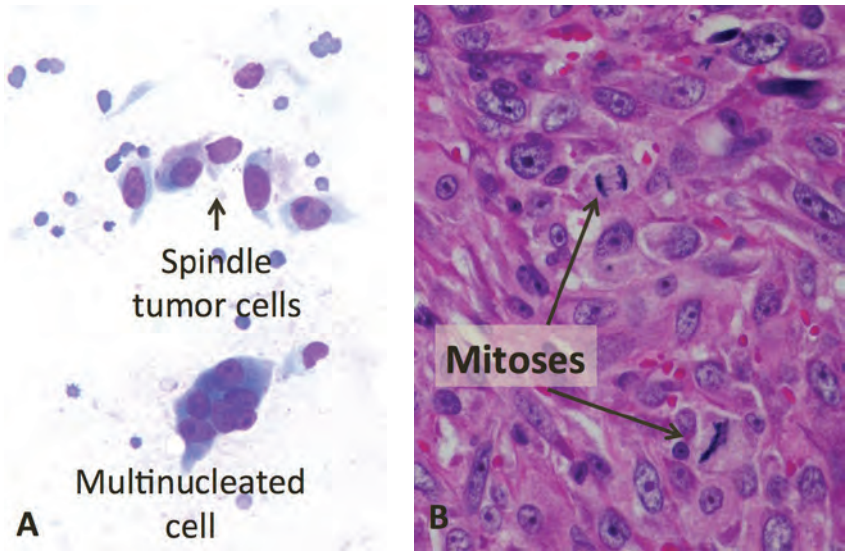

Figure 7. Cellular features of injection site sarcoma in cytologic (A) and histologic (B) preparations. On cytology spindle-shaped cells with large oval nuclei and large nucleoli are seen as well as multinucleated cells (1000x). In the histologic preparation several mitotic figures are seen (600x). Cytology image provided by Dori Borjesson.

However, this may simply reflect the relative scarcity of low grade tumours. The histologic sarcoma type of the tumour also is not a predictive factor. Grading is similar to that of other soft tissue sarcomas and based on: cellular differentiation, mitotic figure rate, and extent of necrosis [6]. It has been observed that grade 3 tumours have a higher likelihood of metastasis; however, some grade 1 tumours have also metastasised [58]. Overall, reported metastasis rate varies between 10 and 24%. Metastases most commonly are seen in the lungs and regional lymph nodes but other sites including liver, mediastinum, and the pericardium may be affected [3, 20].

Margin assessment

Microscopic margin assessment is done by the pathologist. There are several ways to assess margins. At minimum, the biopsy is sectioned across and lengthwise and 4 sides and a deep margin at these sites are evaluated. However, pathologists may also (or instead) choose to evaluate sites where tumour tissue grossly is the closest to the excisional margin. These methods allow determination of the distance from tumour to surgical margins. However, at the periphery of ISS, tumour cells may be well differentiated and indistinguishable from normal tissue fibroblasts thus complicating the margin assessment.

Current guidelines for margin assessment of soft tissue sarcomas suggest the following categories [11]:

- **incomplete margins** - neoplastic cells are continuous with at least one surgical margin in any plane;
- **close margins** - distance between a surgically created tissue edge and neoplastic cells is less than 3 mm, or surgical margins do not contain normal tissue outside the pseudocapsule;
- **clean margins** - distance between surgically created tissue edge and neoplastic cells is at least 3mm.

The surgeon should assist the pathologist by marking the sites of suspected incomplete removal of the tumour. These sites can be marked either with tissue dye or by placing a suture. Marking with tissue dye a portion or all surgical margins greatly aids the pathologist in margin assessment [32]. The downside is that tissue changes become masked by the tissue dye. A complete margin evaluation may be done by taking slices around the perimeter of the resected tumour [19]. Besides being labour-intensive and expensive, this procedure does not allow the evaluation of the proximity of the tumour to the margin or the visualization of the interface between tumour and surrounding tissues.

Diagnostic work-up of an injection site nodule

Early on, VAFSTF recommended that all nodules at injection sites require follow up and should be treated as potential malignancies, if they fall in one of the 3 categories (see Figure 8, rule 3-2-1). It is important to emphasise that most of the nodules that develop at injection sites are not malignancies and do not require wide excision [41].

If a "3-2-1" nodule is seen at the injection site, then incisional rather than excisional biopsy is recommended by VAFSTF. If sarcoma is diagnosed by the incisional biopsy, then a large excision can be planned in order to complete remove tumour tissues with wide margins. On the other hand, if just an inflammatory reaction is diagnosed (which is expected to be a more common event), then surgery may not be necessary. Fine needle aspirate (FNA) and core biopsies of injection site reactions may give false negative results for ISS since these are small samples in which it may be difficult to distinguish reactive fibroblasts of inflammation from malignant fibroblasts of neoplasia. However, FNA or punch biopsies may be excellent tests for confirming sarcoma if a diagnostic sample is obtained. To increase the likelihood of obtaining a diagnostic

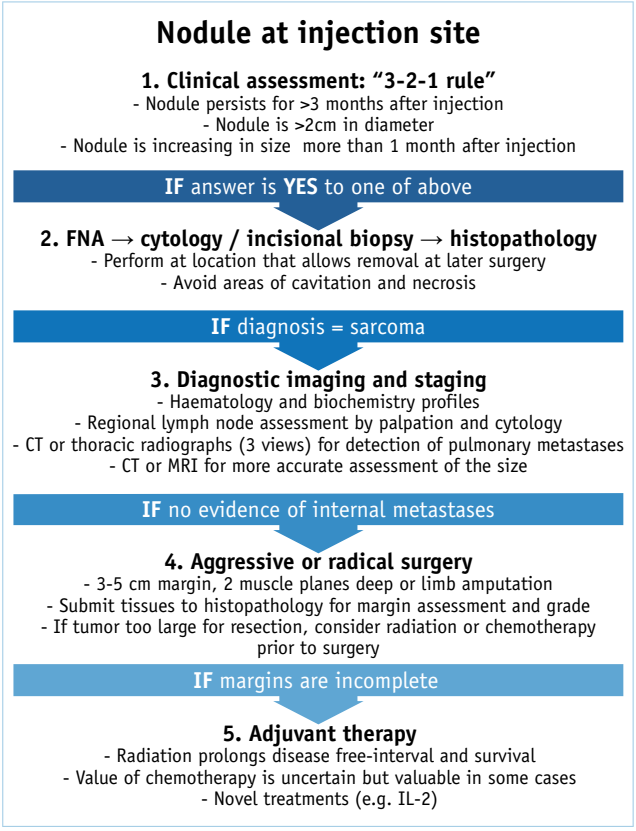

Figure 8. Flowchart for diagnostic work-up and treatment of injection site sarcoma.

Table 5. Guide for histopathology submission: BEFORE and AFTER

BEFORE submitting tumor to histopathology

• Indicate anatomic site, size, growth and gross features of the tumour
• Indicate type of biopsy - punch, incisional or excisional
• If excisional biopsy is done
  - Ink the margins for the margin check
  - Indicate on paper and the tumour if specific site needs to be checked for tumour cells
  - Indicate if only a portion of the tumour is submitted
• If a large tumour is submitted, some pathologists may prefer that you partially slice it with paralel cuts 1.5cm apart extending from apical portion of the tissue into the tumor, leaving the deepest layers intact
• Insert tissue in 10% neutral buffered formalin that is 10x more than tissue on a volume basis

What to expect from a pathologist's report

• Gross description of the tumour
• Microscopic description of the tumour
  - types of cells, mitotic figure rate, atypical mitoses, vascular invasion, necrosis
• Tumour type
• Tumour grade (1-3) – only for complete specimens
  - grading based on cellular differentiation, mitotic figure rate and extent of necrosis
• Margin assessment - only for complete specimens
  - clean, close or incomplete
  - may provide estimated size of the clean margin

AFTER histopathology – interpretation of the report

• Clean margins do not give 100% guarantee that the tumour was completely excised because
  - usually only limited number of sites are evaluated
  - tumour cells at the periphery may be well differentiated and indistinguishable from normal connective tissue fibroblasts
• Diagnosis of ISS may appear in the comment line as differential diagnosis because pathologists frequently do not know the precise location of the submitted tissues. The diagnosis of ISS requires 2 conditions: (1) histology is compatible with the tumour type, and (2) location is compatible with injection site.
• Punch biopsies may not be diagnostic if taken from necrotic areas or areas of inflammation.

sample, areas of tissue necrosis and cavitations should be avoided. Table 5 provides guidelines for ISS submission for histopathology along with what information to expect from the pathologist's report.

Treatment

A comprehensive ISS treatment review was recently published by Ladlow [37]. The recommended treatment scheme is included in Figure 8. This plan includes a CT or MRI scan prior to performing aggressive or radical surgery, followed by adjuvant therapy. However, good results may be achieved with surgery alone.

Surgery. A surgical procedure that achieves wide excision remains the cornerstone of treatment of ISS [39, 44]. The awareness of this disease in the veterinary community has ensured that surgeries performed by practitioners in the past ten years have become more aggressive providing better disease control rates. A recurrence rate of 14% with a median survival time of 901 days was reported in 91 cats with ISS treated with radical excision (3-5cm margins laterally and two muscle layers deep) [47]. In this study, clean margins were not predictive for recurrence of the tumour. This study underscores the direct link between the DFI and the aggressiveness of surgery. This was also demonstrated by a study 10 years earlier showing that aggressive surgeries performed at referral centres provided a fourfold longer DFI than conservative surgeries performed at general practices – 325 days vs. 79 days [29].

Radiation. Surgery in combination with radiation (prior to or after surgery) has been reported to consistently provide increased median survival time (600 -1300 days) [37]. Therefore, there is general agreement that radiation is beneficial in cases of ISS, especially, if surgical margins are incomplete. However, these recommendations need to be re-evaluated considering that present day aggressive surgeries and repeated surgeries for recurrent tumours may result in a similar median survival time for cats with ISS.

Chemotherapy. A variety of chemotherapeutic agents have been used as adjuvant treatment including doxorubicin, carboplatin, and cyclophosphamide, with controversial results. In a study done by Martano, doxorubicin used as

an adjuvant for surgical treatment did not affect local recurrence rate, DFI or survival time [43]. The recurrence and metastasis rates were 41% and 12% for doxorubicin + surgery group and 35% and 10%, respectively, for the surgery only group; however, this was not a randomized study and cats that received chemotherapy had larger tumors than cats treated with surgery alone. Lack of a positive effect of doxorubicin was also reported by Cohen and Bregazzi [3, 5]. There are several reports; however, in which there is evidence for some positive effects of chemotherapeutic agents with or without radiation: Poirier reported reduced metastatic rate (5.6%) with use of doxorubicin, Kobayashi reported on the additive value of carboplatin, and Eckstein reported prolonged survival in cats treated with chemotherapy [15, 36, 48]. More recently, epirubicin was shown to have promising results as adjuvant therapy for treatment of cats with ISS [2]. Three of 25 cats (14%) developed recurrence and more than 80% of cats remained alive after the median follow-up time of 1072 days.

Gene therapy. The first gene therapy treatment, a canarypox vector virus with a recombinant feline interleukin-2 gene (Oncept IL-2, Merial) is now available in Europe for ISS treatment [17]. Recombinant virus is injected locally at the tumour resection site (6 x 5 injection doses for a total of 30 injections over an 8-week period). The virus induces expression of the IL-2 gene at the inoculation site, but does not replicate in the cat. IL-2 expression in situ, stimulates anti-tumour immunity by increasing the tumour specific T lymphocytes and NK cells. This treatment is marketed as an adjuvant therapy to be used in addition to surgery and radiation. A randomised clinical trial showed that Oncept IL-2 treated cats with ISS showed a longer DFI (730 days) compared to control cats (287 days) which were treated with surgery and radiation alone [31]. ISS recurrence rates after 1 year were reduced from 61% in control group to 28% in the canarypox-vectored IL-2 treated group.

ISS treatment results in Latvia

In many instances there are variations in ISS treatments depending on regional circumstances. For example, in Latvia, radiation treatment is not available, CT or MRI scans are not routinely used and chemotherapy for ISS is not offered to clients because of high cost and uncertainty about its benefit. Treatment of ISS in Latvia deviates from

Table 6. Clinical outcome of 12 cats with ISS treated with 1-3 surgeries and followed for 1-3.25 years (on-going study).*

Descriptor	# of cats / total # of cats (%)	Median time	
		Days	Range
Death due to ISS	**4/12 (33%)**	**300[a]**	**135-355[a]**
Death unrelated to ISS	1/12 (8%)		
Tumour-free cats after S1	**2/12 (17%)**	**817[b]**	**735-899[b]**
Tumour-free cats after S2	**3/12 (25%)**	**708[b]**	**446-841[b]**
Tumour-free cats after S3	**2/12 (17%)**	**822[b]**	**452-1191[b]**
Local recurrence (R1)	**9/12 (75%)**	**170[c]**	**42-358[c]**
• Cats not treated with S2	3		
o Cats dead	3	261[a]	135-355[a]
o Time to R1		90[c]	60-150[c]
• Cats treated with S2	6		
o Cats dead	1	420[a]	
o Time to R1		210[c]	42-358[c]
o Cats living after S2	5		
• Cats that developed R2	2	191[d]	60-322[d]
• Cats that had S3	2		

R1 – first recurrence; R2 – second recurrence; S1 – first ISS surgery; S2 – second ISS surgery; S3 – third ISS surgery
*All but two cats had surgeries with incomplete margins. Of the 2 cats with clean margins 1 died of renal failure, the other cat developed R1, had S2 and remains alive.
[a] – survival time from S1 to death [b] – time from S1 untill current date; end point not reached; cats still enrolled in the study
[c] – time from S1 to R1 [d] – time from S2 to R2

the advised scheme in several ways: (1) most cats with ISS in Latvia are treated only with surgical resection; (2) tumour resection is usually not preceded by techniques to define a pathological diagnosis; (3) adjuvant therapy is not used and in cases of regrowth repeated surgery is performed. In recent years, due to increased awareness of ISS in Latvia, surgeries for ISS have become more aggressive. However, most of the tumours submitted for histopathologic assessment have incomplete surgical margins. The results from the ISS cases that have been followed since 2010 have shown incomplete margins in 83%, a recurrence rate of 75%, an ISS related mortality of 33%, and a mean survival time of 575 days for cats enrolled in the study for at least one year. Further details are shown in Table 6. None of these cats have developed metastasis during the follow up period (1-3.25 years). Although the number of cases is low, these results show that repeated surgeries may provide extended DFI.

Treatment failure – recurrence

One of the frustrations with ISS treatment is frequent recurrence despite aggressive surgeries and reported clean margins. This may occur because tumours appear grossly well-delineated when in fact, they form infiltrates that extend far beyond the main tumour location (Figure 4). However, substantial tumour recurrence (almost 20%) occurs even in cases when the entire tumour bed is evaluated and margins are reported clean. It is possible that some of the tumour tentacles contain well differentiated cells that are difficult to distinguish from normal fibrous tissues. Another hypothesis is that recurrence, in the cases with clean margins, represents a new malignant cell transformation event within the predisposed nidus of inflammation, rather than regrowth from tumour cells left behind after tumour resection [5, 19, 37, 43]

Risk factors for ISS development

Proposed risk and non-risk factors for ISS based on literature and the situation in Latvia are summarised in Table 7. Although any injection or trauma in a cat may cause ISS, it is clear that certain vaccines and repeated injections are most significant causes of ISS. There is consensus that the risk is considerably higher from injectable vaccines than from other injectable products and that no vaccine appears to be safe since ISS have been documented after injection of adjuvanted, modified live and subunit vaccines. Evidence shows that adjuvanted rabies and FeLV vaccines carry a higher risk than other vaccines or injectable medications. In the US after release of 1996 guidelines for rabies and FeLV vaccine administration in a rear leg, the shift in the location of ISS from the interscapular region to rear legs was seen [54]. This study estimated that if vaccines were administered as recommended and if ISS was not induced by other injectable products, then rabies, FeLV and FVRCP+/-C vaccinations accounted for 52%, 28%, and 20% of the ISS cases, respectively. Furthermore, a case-control study determined that cats with ISS in a rear leg were more likely to have received inactivated (adjuvanted) vaccine than recombinant (subunit) vaccine [55]. Adjuvanted vaccines have been shown to elicit more intense inflammation of longer duration than recombinant vaccines [8]. The

Table 7. Factors that DO and DO NOT influence development of ISS

Factors that have been shown to influence ISS incidence - ↑ risk
Injection of vaccine • Type of vaccine - Rabies > leukaemia > FVRCP+/-C - Adjuvanted vaccines > modified live/subunit vaccines • Number of repeated vaccinations at the same site - Increased number → increased risk - 3-4 vaccinations at the same site doubled the risk [33] • Temperature of administered vaccine - Cold vaccine → increased risk [34]
Injectable medications • Long-acting corticosteroids (methylprednisolone acetate, dexamethasone, triamcinolone) • Long-acting penicillin • Occasionally other medications – lufenuron, cisplatin
Genetic predisposition • Increased tumour incidence in closely related cats
Factors that HAVE NOT been shown to influence ISS incidence
Site of injection - subcutis or muscle • Nodular lesions can be detected earlier if vaccine is given in subcutis
Vaccine manufacturer
Type of adjuvant
Needle gauge
Mixing of vaccines in a single syringe
Re-use of disposable syringes
Shaking multidose vials
Massage of injection site
FeLV or FIV status

higher number of ISS seen in association with adjuvanted vaccines is in line with the hypothesis proposed by Hendrick and others that chronic inflammation may play a role in the development of ISS [26].

Decreasing the risk and recurrence of ISS

Reducing the frequency of vaccination while still achieving disease prevention is a pragmatic approach necessary to reduce the prevalence of ISS. Guidelines have been developed, published and updated – first by the VAFSTF in the US (adopted by American Association of Feline Practitioners, AAFP), then by WSAVA and the European Advisory Board on Cat Diseases (ABCD) [7, 30, 53]. Key points based on these guidelines for ISS reduction and management are as follows:

1) Vaccination frequency

a. Tailor to the patient – administer vaccines only as frequently as needed to provide protective immunity. Risk of ISS increases with increased number of vaccinations.

b. Rabies vaccination is regulated and state guidelines have to be followed. Veterinarians need to educate regulatory agencies about risks associated with vaccination more frequently than indicated on manufacturers' data sheets.

2) Type of vaccine

a. Choose vaccines that elicit least inflammation even though they may have to be administered more often. Modified-live and recombinant vaccines are expected to induce less inflammation than adjuvanted inactivated vaccines.

3) Vaccination location

a. Always administer vaccines subcutaneously because nodules are more readily detectable.

b. General guidelines in regards to the anatomic location is to vaccinate at a site that facilitates wide excision if a sarcoma arises and to keep a record about vaccination site and type of vaccine given. Specific guidelines differ between AAFP and WSAVA.

- *AAFP recommendation:* - vaccinate as distally on the limbs as possible In case ISS arises, the limb can be amputated facilitating radical surgery for ISS removal. Administer rabies vaccine in right rear (RR), leukemia vaccine – in left rear(LR) and FVRCP+/-C in right front leg (RF). [Authors' note: Consistent use of these sites allows tracing ISS to particular vaccine type but may increase the risk of ISS at the particular site.]
- *WSAVA recommendation:* avoid interscapular area; vaccinate over lateral abdomen; rotate vaccine administration sites because repeated vaccinations at the same site increase risk of ISS.

4) **Monitor for "post-vaccination nodules"** to facilitate early recognition of ISS

a. Inform the owner about nodule as a potential side effect

b. Biopsy nodule if it meets "3-2-1" criteria (Figure 8).

5) **Early intervention.** Remove tumour surgically as early as possible [with 3-5 cm wide lateral margins and two clean fascial planes in the deep aspect or limb amputation - authors' note]. Prior to the surgery, perform CT or MRI for optimal surgical planning. Consult with an oncology specialist for the use of radiation and / or chemotherapy to reduce risk of recurrence.

Conclusions

ISS are aggressive tumours that represent a serious iatrogenic problem associated with vaccination or injectable long-acting injections such as steroids and antibiotics. While generally ISS are rare, incidence in some countries is increased due to vaccination requirements, and infectious disease prevalence. For veterinary communities it is important to recognise this problem, develop, and adopt vaccination policies that provide judicious use of vaccines. This includes selection of vaccines that carry the least risk, giving vaccines only as necessary for providing protective levels of immunity, and administering injection at a site that can be removed with large excision or amputation. It is equally important to explain to cat owners the importance of vaccinations while providing information about potential side effects of vaccines and other injectable medications. Future challenges include identification of factors that predispose cats to the development of ISS.

Acknowledgments

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COMMISSIONED PAPER

Treating dogs with tibial shaft fractures using the transosseous osteosynthesis method according to Ilizarov

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SUMMARY

The authors present an anatomically substantiated technology for treating dogs with tibial fractures using the method of transosseous osteosynthesis according to Ilizarov; the technology provides recovery of the involved structures within relatively short periods of time.

Key words: fracture, tibia, circular external skeletal fixation, dog.

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Introduction

Canine tibial fractures make up 17.8% of all skeletal injuries. Their main causes are the following: household injuries in 67.0% of cases, traffic injuries – in 30% of cases [3,18].

Currently, both conservative (rigid external coaptation using plaster or polymer casts), and surgical methods (internal osteosynthesis using wire, plates, Kirschner wires, pins, screws and others) are used for the treatment of animals with such injuries [4,5,7,8,9,11,15,16]. However, these methods do not always provide stable fixation of fragments throughout the period of healing, and also complete recovery of limb function.

The method of transosseous osteosynthesis is increasingly used for this kind of injury along with the above-mentioned techniques of treatment [1,2,6,10,12,13,17]. However, available devices and techniques for fixation of fragments do not always take the anatomical features of the leg into consideration.

A technique of tibial osteosynthesis in domestic animals, as well as fixator designs that provide stable osteosynthesis of bone fragments throughout the treatment period for any fracture localization, together with the potential for defect filling by distraction have been developed and approved in the Federal State Budgetary Institution Russian Ilizarov Scientific Centre for Restorative Traumatology and Orthopaedics (RISC "RTO") on the basis of this method [13].

Material and Methods

The work is based on analysing the results of treatment of 78 dogs with tibial fractures. Clinical and X-ray examination of the animals was performed for diagnosis, assessing repair and to control repositioning and the dynamics of fragment fixation. Plain X-rays of the leg in AP and lateral views were made.

Results

The technology of treating dogs with tibial fractures by the transosseous osteosynthesis method according to Ilizarov includes several stages.

The Ilizarov fixator is prepared and mounted before surgery according to the fracture type and localization, taking into

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account the animal's age-related and individual features. It should provide sufficient rigidity of bone fragment fixation and free movements in the adjacent joints.

The Ilizarov fixator design comprises at least two supports interconnected by threaded rods, using nuts to give the potential for longitudinal movement (Fig. 1).

Supports for the Ilizarov fixator consist of either full-rings or open-rings (horseshoe shape). Proximally, open rings are used and the open ends should point caudally; distally, full ring or open rings can be used, the latter should have the open ends pointing cranially. This avoids compression of adjacent soft tissues near the stifle and hock joint. It should be taken into account that in adult animals the terminal supports should be located at the distance of at least 1 cm from the articular gap of the stifle and the tarsal joint, and in puppies and young animals (below seven months of age) at least 0.5 cm from the physes.

When selecting the configuration and size of the support a gap of 1.5 – 2cm should be allowed between the inner contour of the ring and the leg surface to facilitate wire insertion and to prevent soft tissue injury. However the use of supports of larger size reduces fixation rigidity.

Osteosynthesis is performed under sterile conditions using general anaesthesia and aseptic technique. The animal should be positioned in lateral recumbency with the affected limb uppermost.

In order to perform external fixation of bones and

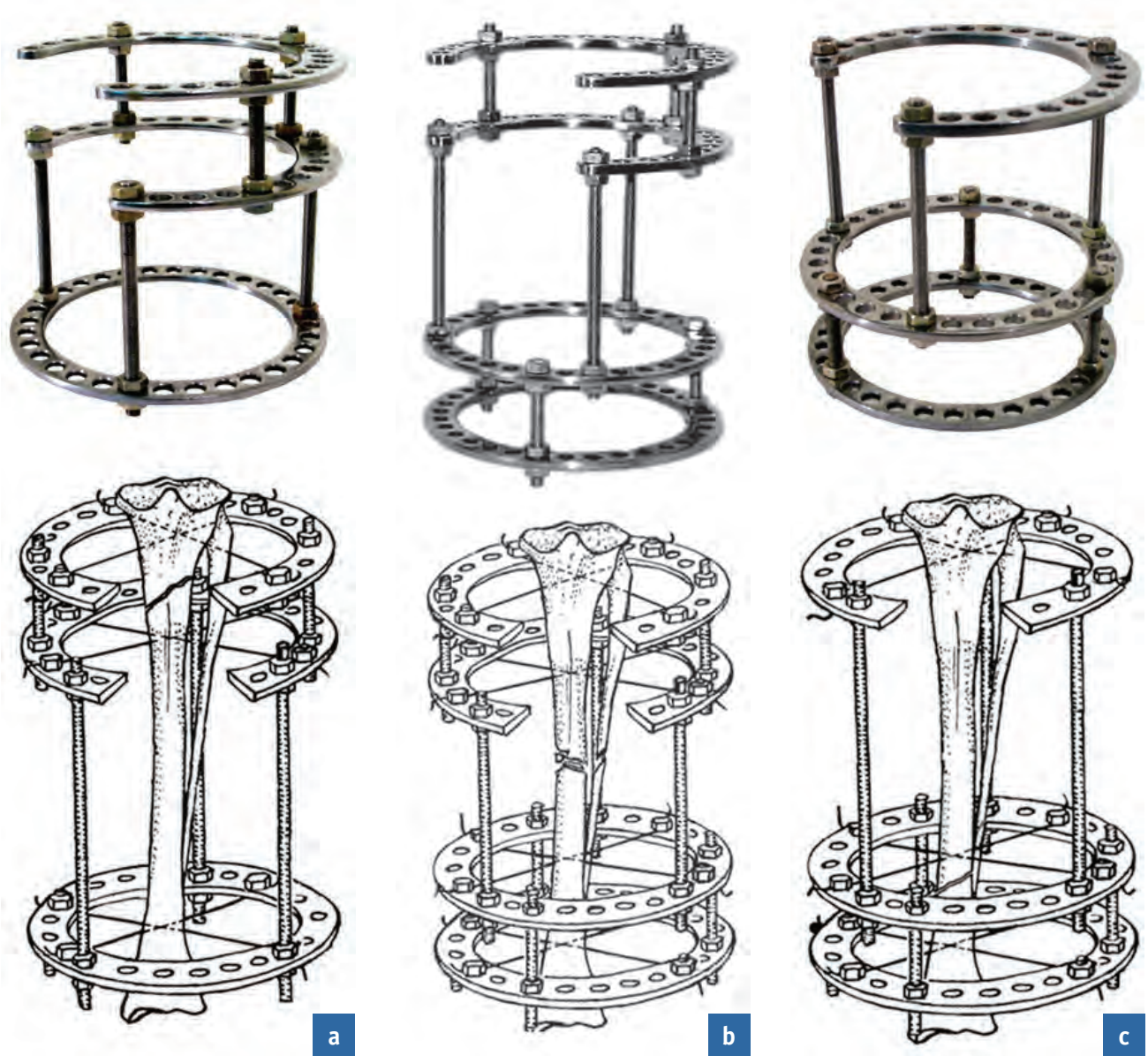

Figure 1. The Ilizarov fixator design, and the pattern of K-wire insertion through tibial fragments depending on fracture localization: a – in the proximal shaft part, b – in the middle shaft part, c – in the distal shaft part.

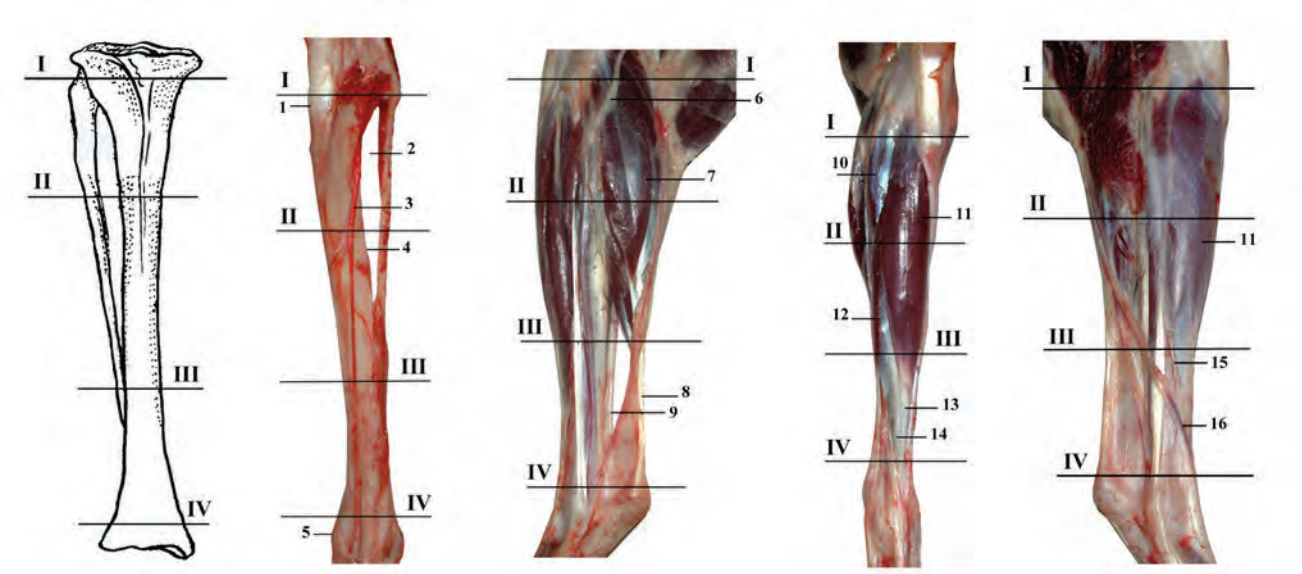

Fig. 2. The main anatomical structures of the canine tibia.
1 – tibial tuberosity, 2 – interosseous space, 3 – cranial tibial artery, 4 – lateral surface of the tibia, 5 – medial malleolus, 6 – common fibular nerve, 7 – m. gastrocnemius, 8 – Achilles tendon, 9 – tibial nerve, 10 – m. peroneus longus, 11 – m. tibialis cranialis, 12 – m. extensor digitorum longus, 13 – tendon of m. tibialis cranialis, 14 – tendon of m. extensor digitorum longus, 15 – superficial fibular nerve, 16 – lateral saphenous vein.

their fragments Kirschner wires (K-wires) are used with bayonet or trocar points. K-wires are inserted using a drill with adjustable speed. Wires are inserted slowly, with frequent stops, with drill rotation speeds of 50-500 rpm. It is important to avoid excessive axial pressure on the K-wire as this may result in arched deformation and, as a consequence, in deviating from the desired direction, as well as an increase in the K-wire tract diameter. Careful insertion as above will help to prevent additional injury (thermal and mechanical) of tissues and destabilisation of the bone fragment.

The K-wire insertion point will be dependent on the species and breed features of the tibia (Fig. 2).

K-wires should be inserted in the lateral (medial)-cranial and lateral (medial)-caudal directions, perpendicularly to the longitudinal axis of the fragment such that the wires cross inside the medullary cavity.

At the level of the tibial tuberosity, K- wires should be inserted at 75-90° crossing angle and it is important to consider the position of the common peroneal nerve, which runs along the lateral surface, directly near the caudal edge of the fibula head (Fig. 3 a), or 1-6 mm distal to it.

K-wires should be inserted into the distal edge of the tibial cranial border at a crossing angle of 45-65° due to the position of the anterior tibial artery which, when leaving the interosseous space, passes along the caudal edge of the lateral surface of the tibia and courses distally to the cranial surface of the tibia (Fig. 3 b).

Inevitably, K-wires of both pairs will, on the lateral surface,

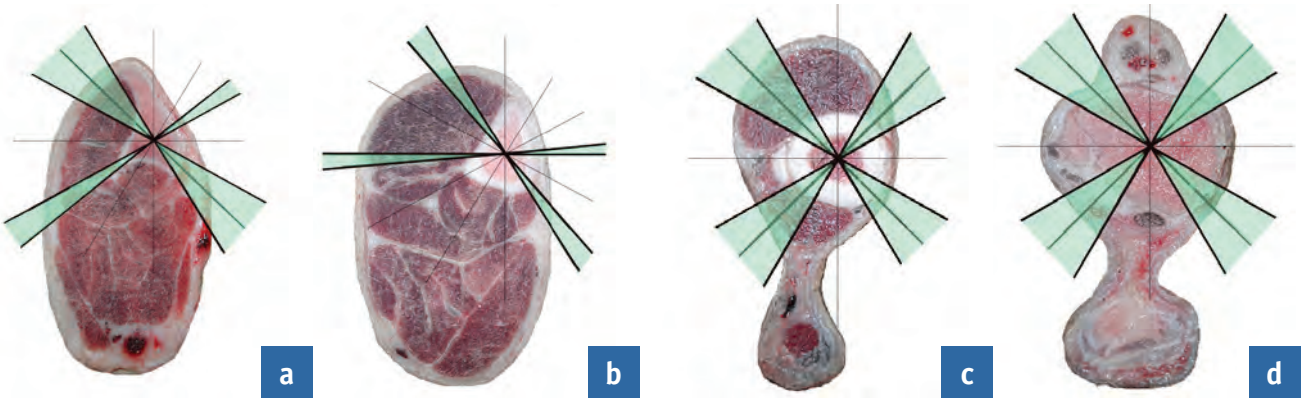

Fig. 3. The positions recommended to insert mutually crossed K-wires through tibia.

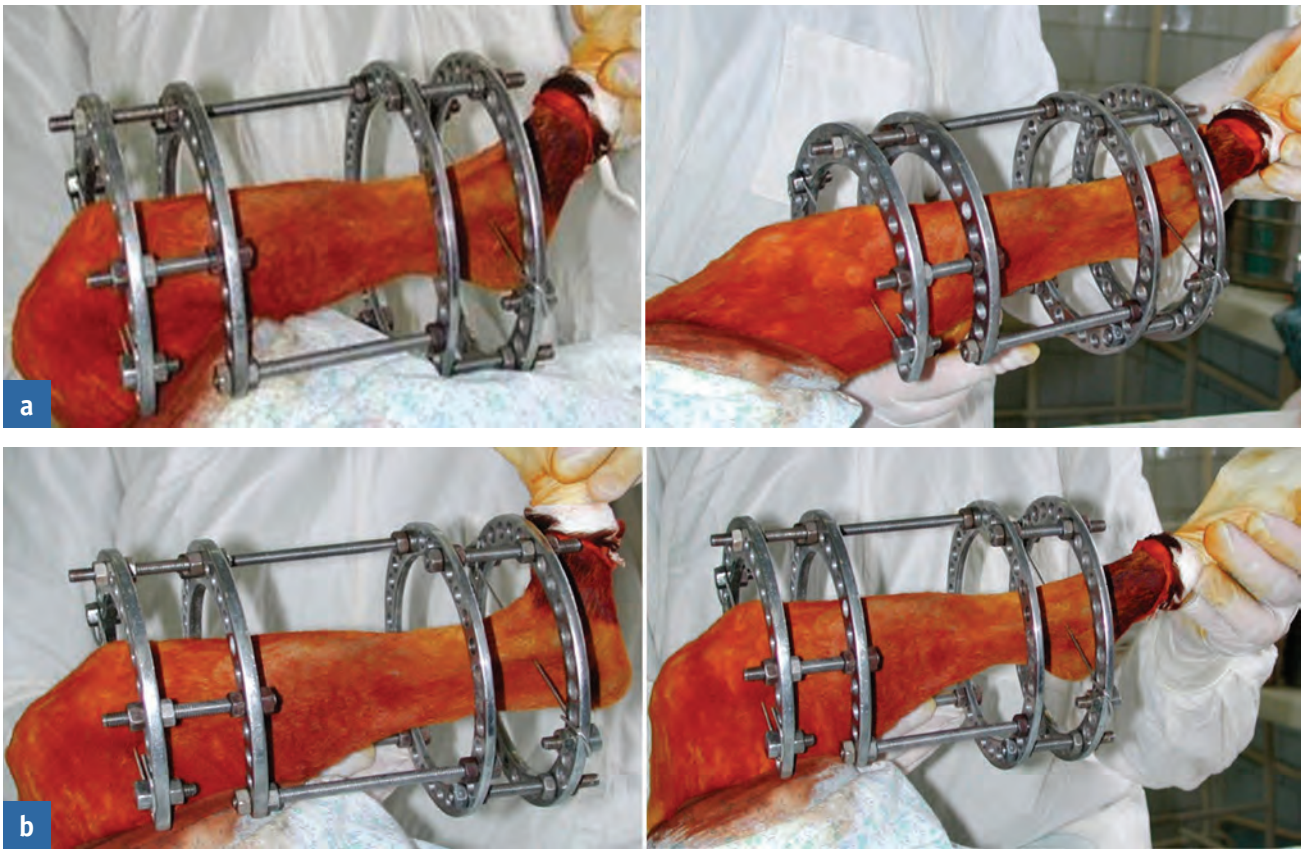

Fig. 4. The manipulations with the stifle (a) and the tarsal (b) joint: on the left – flexion, on the right – extension.

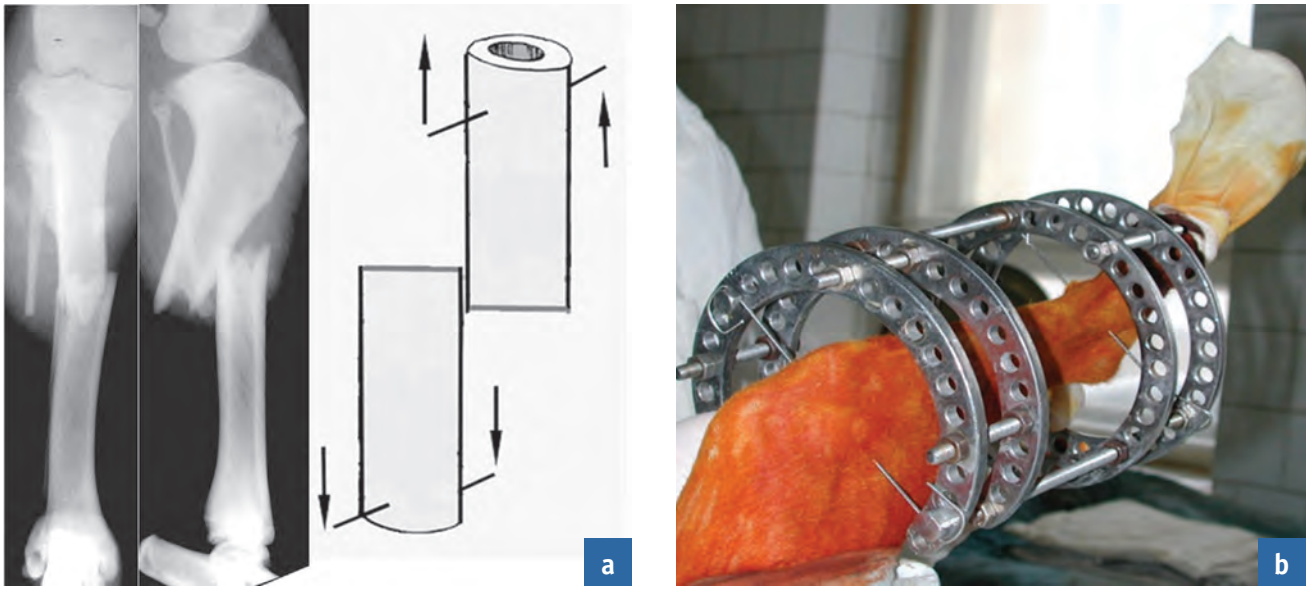

Fig. 5. Wire insertion through the proximal and distal fragments of tibia: a– X-ray AP and lateral views, b – postoperative appearance.

pass through the anterior tibial muscle, as well as through the long peroneal muscle, and the long digital extensor muscle. On the medial aspect they go from the zone of the semitendinosus muscle attachment, and rarely, from that of the popliteal muscle attachment.

K-wires should be inserted into the distal shaft of the tibia at a crossing angle of 70-90°. This should avoid damaging the anterior tibial artery and vein, the deep peroneal nerve, the tendons of the anterior tibial muscle and the long tibial extensor from the cranial surface (Fig. 3 c, d).

If the terminal pairs of K-wires are inserted properly, a full range of flexion and extension of the stifle and the tarsal joints is possible (Fig. 4). If it is apparent that range of motion is limited by intra-articular passage of a K-wire it should be removed and replaced according to the protocol described above.

If there is any doubt about the correct insertion of K-wires (especially with respect to damage to blood vessels or nerves), then they should be removed, and reinserted through a safe corridor.

If the tibial fracture is mid-diaphyseal, osteosynthesis is performed as follows. Manual repositioning of fragments is made first. One K-wire each is inserted through the proximal and distal tibial fragments, and the outer ends of these K-wires are attached to the fixator terminal supports. Distraction along the rods, which connect the fixator supports, will allow appropriate positioning to re-establish correct bone length and longitudinal displacement (Fig. 5).

One more K-wire should then be inserted through each of the proximal and distal tibial fragments closer to the fracture zone, and K-wires should be attached to the middle fixator supports, ensuring correct angulation of the fragments with respect to each other.

Once this repositioning is achieved, K-wires should be cross-inserted through fragments at each of the above-mentioned levels and attached to the appropriate supports. If fracture occurs proximally or distally, such that there is a short proximal or distal tibial fragment, K-wires should still be inserted in a similar way. However, in this case one pair of K-wires should be inserted through the short fragment, and two pairs of mutually crossed K-wires (attached to the appropriate supports) through the long fragment (Fig. 6).

K-wires with stoppers allow side-to-side compression and should be used to facilitate repositioning of comminuted, oblique and spiral fractures where there is considerable displacement of the fragments (Fig. 6).

The outer ends of K-wires should be attached (directly or indirectly via washers and/or cantilevers) to the outside fixator supports.

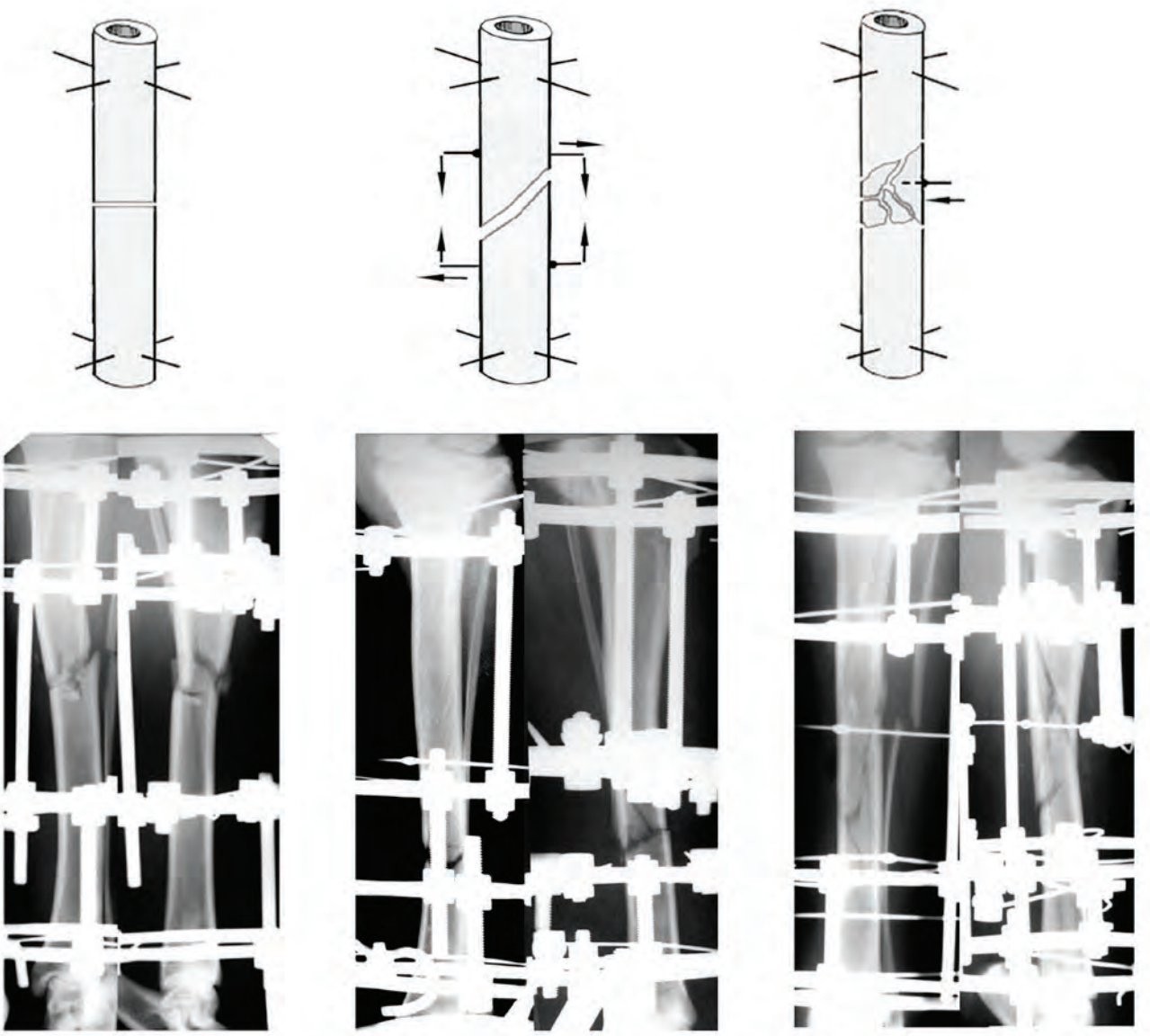

Fig. 6. Osteosynthesis options for different tibial fractures.

Table 1. The amount of K-wire tensioning depending on the support type and diameter

Type of support	Inside diameter of support	Tensioning of the first K-wire, kgf	Tensioning of the second K-wire, kgf
Ring-sector	60	70	60
	80	80	70
	90	90	80
	100	100	90
Ring	60	70	80
	80	80	90
	90	80	90
	100	90	100

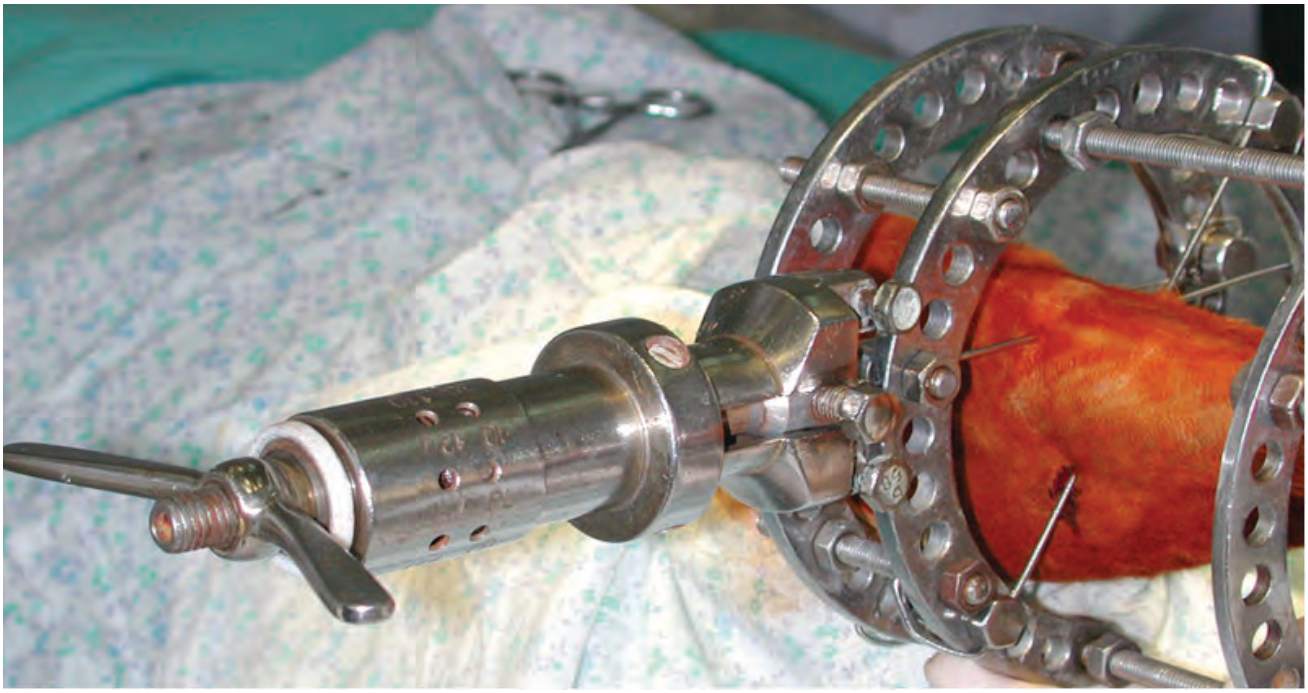

Fig. 7. The wire tensioner position after being mounted on the fixator support.

All K-wires should be tensioned in order to provide stable fixation of bone fragments. To achieve this, one of K-wire ends should be rigidly attached to the fixator support using a wire-fixation bolt, and a wire tensioner is mounted on the K-wire's opposite end (Fig. 7). Once the required amount of tensioning has been achieved (Table 1), the K-wire's second end should be rigidly attached to the support as well by tightening the nut on the wire-fixation bolt.

Most fractures can be repositioned with a closed technique, however, when closed repositioning of fragments is inadequate, as well as in cases of delayed fracture repair, repositioning is performed in an open way. Radiography is performed after implantation of all wires to confirm adequate repositioning.

Stable fixation of the tibia should be maintained throughout the treatment period until radiographic union is confirmed.

The length of time to achieve radiographic union will depend on many factors including the type of fracture (comminuted vs. simple transverse), the animal's age and the time between injury and surgery. In young dogs (under a year), fracture healing usually occurs within 14-30 days. In dogs above one year of age the union of oblique and spiral fractures occurs by 25-35 days of fixation, and that of transverse and comminuted ones – by 35-70 days of fixation.
The fixator may be removed if there are X-ray and clinical signs of union. The threaded rods, which interconnect the fixator supports, should be removed, and flexion and rotation of fragments should be made gently by hands. If

Table 2. The main complications in the treatment of animals with fractures using the transosseous osteosynthesis method, and measures for their elimination

Complication	Cause	Solution
Blood vessel damage during K-wire insertion	Lack of knowledge of topography of the anatomical area under fixation Non-observance of K-wire insertion technique	K-wire reinsertion Haemostasis
Nerve damage during K-wire insertion	Lack of knowledge of topography of the anatomical area under fixation Non-observance of K-wire insertion technique	K-wire reinsertion Neurological treatment
K-wire breakage	Technical defect Improper technique of K-wire insertion and/or wire attachment to the support K-wire tensioning above the permissible limit Non-conformance of K-wire diameter and animal's weight External mechanical impact on the fixator	K-wire reinsertion.
Bone fracture at the site of K-wire insertion	Non-conformance of bone size and K-wire diameter	Osteosynthesis of the site of injury
Inflammation of soft tissues around K-wire	Non-observance of asepsis regimen during surgery or in the early postoperative period	Antibacterial therapy and treatment of soft tissues around the K-wire If necessary – K-wire reinsertion
Destabilisation of the fixator or its modules	K-wire cutting-through bone due to K-wire edge insertion Bone lysis round K-wire Loosening of the fixator attachment elements K-wire breakage Improper mounting of the support No K-wire tensioning. Non-observance of the techniques and sequence of the fixator mounting.	K-wire reinsertion K-wire reinsertion Tightening of the fixator attachment elements K-wire reinsertion Mount the support according to the rules K-wire tensioning Remount the fixator
Secondary displacement of fragments	Non-observance of the techniques and sequence of the fixator mounting Destabilisation of the fixator or its modules due to untwisting of the attachment elements Destabilisation of the fixator modules due to K-wire breakage	Remount the fixator Tightening of the fixator attachment elements K-wire reinsertion
Delayed consolidation of fragments	Incorrect reposition Destabilisation of the fixator modules Disorder of metabolism	Re-reposition Stabilisation of the fixator modules Metabolism disorder correction
Refracture	Inadequate time of fixation with the device (fixator) Mechanical injury	Re-osteosynthesis Observance of the rules of postoperative management

there is no pathological mobility and pain at the fracture site the fixator should be dismantled; if there is still movement of fragments or pain on manipulation, the threaded rods should be set in the initial position, and fixation continued for 1-3 weeks.

Fixator removal is performed under sedation. Attachment elements should be untwisted and removed. The skin round K-wires, as well as the wires themselves, should be treated with antiseptic solutions. K-wires should be cut off at the skin surface and removed by pulling on the opposite end (K-wires with stoppers are removed from the stopper side). Wound tracts should be treated with antiseptic preparations.

When animals were treated using the proposed technique a positive clinical and morpho-functional result was obtained in all the cases, and the result persisted in the long-term period.

The retrospective analysis of case histories of animals with tibial fractures allowed us to analyse complications that developed both during the implantation of K-wires and postoperatively, as well as to develop measures for their elimination (Table 2).

Conclusion

This technique may be the method of choice in the treatment of dogs with tibial fractures of different locations because it provides stable fixation of fragments, as well as recovery of the morpho-functional relations of the injured structures within comparatively short periods.

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Commissioned paper*

Wellness plans in practice: what works and why

Pere Mercader [1]

SUMMARY

Veterinary practices have only a small percentage of highly engaged clients. A detailed analysis of the economic transactions involving 135,000 canine and feline patients of 100 Spanish veterinary practices during 2013 reveals that – on average – 20% of the patients account for 60% of the total revenue. The same research has also provided evidence that most clients never or hardly ever buy pet food or antiparasiticides in their practices. The current economic climate for veterinary practices (increasing costs, increasing competition, increased price sensitivity of clients and increasing reliance of clients on the internet) does not help either. In this context, wellness plans appear as a new and promising business model. The main economic rationale behind wellness plans is that all three parties involved (client, pet, and veterinary practice) obtain a benefit from the model. Clients benefit from lower prices as compared to purchasing the different veterinary services and products separately. The practice benefits from increased revenue and profit, because clients enrolled in the plan end up spending significantly more than before in their practice. And, last but not least, pets in wellness plans receive a better level of care. This article reviews the economics behind wellness plans and evaluates their possible financial impact on the veterinary practice.

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Part I: Patient economics in veterinary practices (empirical evidence)

How much does a good client spend every year in a veterinary practice? Is it true that a very small percentage of clients generate a large percentage of the practice revenue? What percentage of pet owners regularly buy their main pet care products – pet food, ectoparasiticides, endoparasiticides – from their veterinary practice? Clearly, there is not much research evidence available on these matters. With the goal of answering these and other related questions, Veterinary Management Studies (VMS) has carried out a large-scale quantitative research project to better understand the purchasing behaviour of veterinary practice clients in Spain.

Some key research design features were:

- 532,484 economic transactions related to 135,160 patients from 100 Spanish veterinary practices were analysed during 2013.
- The majority of transactions in these practices (90.4%) were linked to a specific patient id number, allowing the researchers to analyse service and product consumption patterns at individual patient level.
- The information was directly captured from the practice management information software (QVET, by QSOFT), in this way minimizing data transcription errors.

The key research findings were:

- The practices in the study averaged 1,351 patients and a yearly revenue of € 218,692 Euro.

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- The average spending per patient was € 190 (incl. VAT), and on average 3.5 economic transactions per pet were performed during 2013. On average, clients of these practices owned 1.2 pets.
- The pattern of veterinary practice clients' spending shows a large degree of variation, with a small percentage of clients being responsible for a large percentage of revenue. Table 1 shows the cumulative percentage of revenue generated by the different cumulative percentages of patients. For instance, the top 1% of patients with the highest expenditure in their practices generated 9% of the overall revenue of these practices (Table 1, first row).
- 20% of patients with higher spending in the practice generate 60% of the practice total revenue. Not only that: their average annual spend in the practice (€ 576 incl. VAT) triples the spending level of the total client base as a whole (€ 190, incl. VAT).
- Table 2 displays the pet food-purchasing pattern of these clients. Only a surprisingly low 15.5% of patients consume pet food purchased at their practice, and when they do, it is only an estimated 18.2% of their pet food needs. The calculation of pet food needs covered is estimated by comparing actual spending on pet food in the practice to a benchmark figure of € 390/year. This benchmark is calculated

Table 1: Patient / revenue concentration in veterinary practices (Spain)

# patients in an average practice	cumulative % of patients	cumulative % of practice revenue generated	average annual spending in practice (€)
14	1%	9%	1842
68	5%	28%	1078
135	10%	42%	808
203	15%	53%	668
270	20%	60%	576
338	25%	67%	509
405	30%	72%	457
675	50%	86%	329
1013	75%	96%	243
1350	100%	100%	190

Table 2: Pet food-purchasing patterns of patients in veterinary practices (Spain)

# patients in an average practice	cumulative % of patients	cumulative % of practice revenue generated	% of patients purchasing pet food	% coverage of pet food needs
14	1%	9%	65%	58%
68	5%	28%	55%	42%
135	10%	42%	48%	35%
203	15%	53%	43%	31%
270	20%	60%	40%	28%
338	25%	67%	37%	27%
405	30%	72%	34%	25%
675	50%	86%	26%	22%
1013	75%	96%	19%	20%
1350	100%	100%	15%	18%

with the following assumptions:
  - o 80% canine patients, eating an average of 250 grams per day of a dry pet food with an average cost of € 4.8/kg (incl. VAT)[1].
  - o 20% feline patients, eating an average of 60 grams per day of a dry pet food product with an average cost of € 9.0/kg (incl. VAT)[2].
- Even the top 20% of patients with higher spending in their practices show somewhat modest pet food purchasing patterns: only 39.6% of them consume pet food bought from their veterinarian, and when they do, that only cover 28.5% of their product needs.
- Similarly discouraging results were obtained when analysing other main product categories, such as ectoparasiticides and endoparasiticides.

Key research implications:
- There is no such thing as an "average client". Veterinary practice client bases combine a small percentage of high spending, highly engaged clients, with a large percentage of low spending, sporadic clients. In an average Spanish practice with 1,350 patients, the top 20% of patients (270 pets) provide 60% of the revenue, whilst the bottom 50% of patients (675 patients) generate only 14% of the practice revenue.
- Whilst from a strictly medical perspective all these patients deserve the same attention and standards of care from us, from a management perspective it makes no sense to approach their owners all in the same manner.
- Veterinary practices are capturing a very low share of pet owners' total spending on their pets. Most clients either never purchase or only occasionally purchase some key product groups such as pet food, ectoparasiticides and endoparasiticides from their practices.
- There appears to be a significant opportunity for both medical and economic improvement, if practices can replicate the high level of engagement of their top clients across their wider client base.

Part II: A challenging economic environment for veterinary practices

There are a number of factors impacting negatively on the business perspectives of small animal veterinary practices around the world. Figure 1 summarizes the most relevant ones:

Rising costs of veterinary care

The standard of pet care has evolved significantly in recent years, requiring highly skilled professionals and the intensive use of technology. Practices are seeing and caring for more senior pets than ever before. And more treatment options are available than ever before for pet-owners, but they are not free of cost.

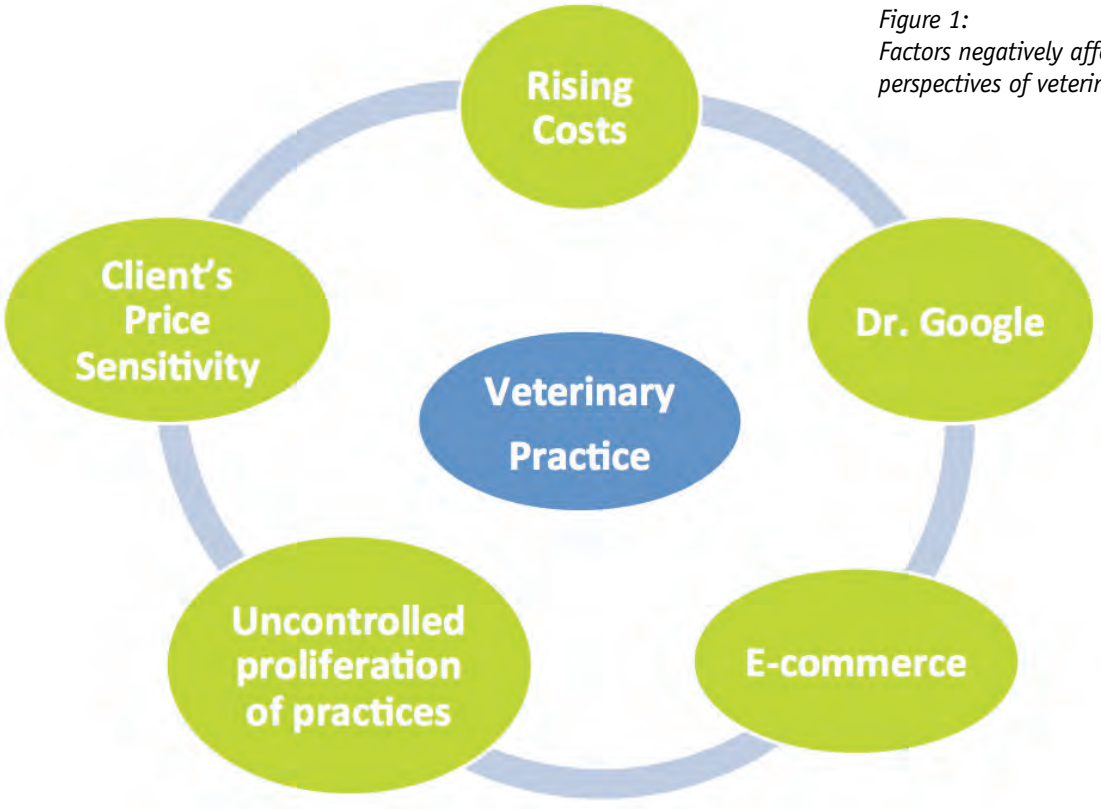

Figure 1:
Factors negatively affecting the business perspectives of veterinary practices

Increased client price awareness and sensitivity

Clients are increasingly concerned about the magnitude and unpredictability of veterinary costs. 53% of pet owners consider that "veterinary visits usually cost much higher than expected"[3]. When clients are asked what changes to their veterinarians' service offerings would most likely increase the number of visits to their clinic, 51% of dog owners and 43% of cat owners mentioned "competitive product prices"[4].

Increasing number of veterinarians; lack of differentiation and price competition.

Veterinary schools are blossoming all over the world, often at a faster pace than the increase in pet ownership and the related demand for pet care services. Veterinarians' inability to properly communicate and differentiate their services often generates a tough competitive climate in which price competition is the quick fix, easy shortcut.

Dr. Google

39% of pet owners admit "looking on-line first if my pet is sick or injured"[5]. There is plenty of anecdotal evidence of veterinarians reporting seeing patients "less, later or sicker" due to pet owners' increased reliance on internet based information combined with their perceived risk of incurring unpredictably high bills.

E-commerce

Pet care is no exception where e-commerce is concerned and it is making inroads rapidly and capturing an increasing share of pet care products (including medicines). E-commerce players often use aggressive marketing techniques - such as the "loss leader", in which limited quantities of a top-selling pet food or ectoparasiticide are aggressively priced and heavily promoted. This is shaping a worrying perception in some pet-owners' eyes about the overall price level of their usual, "off-line" veterinary clinic, which is now perceived as an expensive option.

Part III: Can wellness plans be the answer?

46% of dog owners and 44% of cat owners say that "wellness plans, billed monthly" are the addition to their veterinarian's service offering which would most likely increase their rate of visits to the clinic[6].

Having said this, what is - and what is not - a wellness plan? A wellness plan is a bundle (group) of preventive medicine services that are prepaid by the client in exchange for a service and price advantage. Some of the typical features of wellness plans include:

- They are normally designed, taking into account species, age, and specific health risks. Table 3

Table 3: Wellness plan offerings for adult cats (adapted with permission from Banfield)

Services	Essential Wellness	Active Prevention	Special Care
Comprehensive physical exam (x2 per year)	✓	✓	✓
Vaccinations	✓	✓	✓
Diagnostic testing	✓	✓	✓
Faecal Exams (x2)	✓	✓	✓
Deworming (x2)	✓	✓	✓
Dental cleaning		✓	✓
Urine testing		✓	✓
Additional diagnostic testing			✓
Electrocardiograms			✓
Unlimited office visits	✓	✓	✓
Discounts on other services and products	10%	15%	20%

presents some examples adapted from a leading provider of wellness plans in the US[6].

- They can range from a basic service level (vaccines, physical exams, deworming, faecal exams) to more inclusive options (analytics, dentals, reproductive surgeries).
- Wellness plans may or may not include free office consultations in addition to the predetermined physical exams. Proponents of including free visits in the plans argue that this is the single most attractive feature for pet-owners. They also claim that increased visit frequency – even if consultations are not charged for- ends up generating higher spending by these clients on additional services and products.
- Wellness plans often include discounts for additional services or products purchased outside the plan (e.g. pet food, ectoparasiticides, diagnostic tests, non-elective surgeries, etc.).
- The plan's costs can be paid up front, in total (usually with an additional discount) or in quarterly or monthly instalments.
- There is a relevant service component inherent to the plan, the idea being that "we will take care of everything". The practice is assumed to remind the pet-owner, on a continuous basis, about the different visits and procedures (already prepaid) that need to be performed.
- Wellness plans are different from insurance, because they cover a specific set of preventive medicine services that are mutually agreed by the practice and the pet-owner. They don't manage risk, since in wellness plans there is (at least there should not be) no room for surprises: it is clear from the start which services will be delivered, when and at what cost. Conversely, insurance does not include (normally) preventive medicine procedures and/or elective reproductive surgeries.
- It is possible to use wellness plans in combination with insurance, to offer "full spectrum" health coverage. Sometimes insurance is offered at a discount for patients enrolled in wellness plans, the logic being that they bear lower health risks due to their improved level of care.

The main economic rationale behind wellness plans is that all three parties involved (client, pet, and veterinary practice) obtain a benefit from the model. Clients benefit from lower prices as compared to purchasing the different veterinary services individually. They can also obtain relevant discounts when purchasing pet food and antiparasiticides. The practice benefits from increased revenue and profit, because clients enrolled in the plan end up spending significantly more than before in their practice. And last, but not least, pets in wellness plans receive a better level of care. Table 4 covers a more comprehensive list of benefits for the client and for the practice.

Table 4: advantages of wellness plans

Advantages for the client	Advantages for the practice
The most important: pet receives a higher level of care	**The most important:** pet receives a higher level of care
Peace of mind: free consultations allow them to visit the practice at the earliest sign of disease	**Improved relation with clients:** money discussions are minimized, clients and pets seen more often
Convenience: the practice staff manages all appointments, reminders, etc.	**Increased revenue:** enrolled patients consume more services and products in the practice
Personal financial planning: pet care costs under control	**Stable flow during the year:** wellness plans smooth seasonal fluctuations in workload and revenue
Cash flow: plan can be paid in convenient installment	**Increased profits:** if properly designed, wellness plans generate incremental margin for the same fixed cost structure
Value for money: excellent value in services and products	**Safety net against low cost competition:** clients in the plans minimize "shopping around" for bargains
Emotional benefit: pet owner feels proud ("I am a responsible owner") and smart ("This is a good deal")	

So far we have discussed the advantages of wellness plans, but how about the drawbacks? A common objection is the fear that only a small proportion of already high spending clients will enrol in the plans, meaning that the practice will end up basically selling the same services and products to the same clients but at a discounted price, therefore generating less profit. Let us perform some financial simulations to assess the different scenarios.

Part IV: Economic analysis of wellness plans

Table 5 shows the actual service and product purchasing behaviour of 135,000 patients of 100 Spanish veterinary practices during year 2013. Based on this current picture, we will analyse the impact (both from revenue and profit perspectives) of different wellness plan scenarios.

In our calculations, the following assumptions will be made:

- A menu of wellness plans will be offered to clients, valued on average at € 30 per month or the equivalent € 360 per year (all prices with VAT included).
- The services included in these plans will have a 20% discount on the regular price list of the practice.
- Clients enrolling in the plans will receive an additional 20% discount on other medical services outside the plan, plus a 15% discount on any products (pet food, antiparasiticides) purchased at the practice.
- Assuming a gross margin of 75% on medical services, a 20% discount on fees will reduce the gross margin on medical services from 75% to 68.75% of sales[7]. Gross margin defined as net sales minus variable cost (mainly supplies).
- Assuming a gross margin of 25% on pet food sales and 30% on antiparasiticide sales, a price reduction of 15% (coupled with a 5% improvement in purchasing costs, due to increased volume) will reduce the gross margin on pet food sales from 25% to 16.2%, and the gross margin on antiparasiticide sales from 30% to 21.8%[8]. Gross margin defined as net sales minus variable (purchasing) cost.
- The clients enrolled in the plans were in fact purchasing 50% of these medical services before the implementation of the plans, meaning that in fact only 50% of the revenue generated by the services included in the plans will be purely incremental.
- The average cost of feeding a pet with premium quality dry wellness food in Spain is estimated at € 390 per year; the average cost of a full year ectoparasiticide treatment is estimated at € 75; the average cost of a full year endoparasiticide treatment is estimated at € 52 Euro[9].
- The clients enrolled in the plan will purchase 50% of the annual pet food, ectoparasiticide and endoparasiticide needs of their pets.

Table 6 presents the economic impact (in revenue and gross margin) of different levels of patient enrolment in the wellness plans, for an average size Spanish practice with 1,350 patients.

The main conclusions of this initial simulation are:

- Wellness plans start generating revenue increases for the practice at modest levels of patient enrolment (5% of patients enrolled in the plan).

Table 5: Purchasing patterns of 135,000 patients of Spanish veterinary practices

# patients in an average practice	cumulative % of patients	cumulative % of practice revenue generated	average annual spend in practice (€)	annual spend on pet food (€)	annual spend on ectoparasiticide (€)	annual spend on endoparasiticide (€)
68	5%	28%	1078	163	58	34
135	10%	42%	808	136	54	30
203	15%	53%	668	121	51	28
270	20%	60%	576	111	49	26
338	25%	67%	509	104	47	25
405	30%	72%	457	98	45	24
675	50%	86%	329	85	41	21
1013	75%	96%	243	77	38	19
1350	100%	100%	190	71	36	17

Table 6: economic impact of different levels of patient enrolment in wellness plans (simulation)

# patients	% patients enrolling in Wellness Plans	practice revenue increase (in %)	gross margin increase (in %)	practice revenue increase (in €)	gross margin increase (in €)
14	1%	-1%	-2%	-1,708	-2,459
68	5%	2%	-3%	3,428	-3,942
135	10%	7%	-1%	15,648	-1,952
203	15%	14%	1%	30,518	1,712
270	20%	22%	4%	46,581	6,314
338	25%	30%	8%	63,916	11,605
405	30%	38%	12%	81,955	17,374
675	50%	74%	29%	158,180	43,133
1013	75%	121%	53%	258,942	78,987
1350	100%	170%	79%	362,884	117,045

- Due to discounting (and margin erosion), increases in a practice's gross margin require a higher level of patient enrolment (they start when at least 15% of practice patients enrol in the plan). Practice gross margin being defined as practice net revenue minus variable costs (mainly the purchase of supplies).
- If we assume that the practice can generate the incremental revenue without incurring additional fixed costs (i.e. new staff, new premises, new equipment, etc.) the incremental gross margin will result in incremental net profit. This means that for a typical Spanish practice (€ 250,000 of yearly net revenue, 10% EBITDA), a wellness plan programme achieving 30% enrolment among its patient base could generate a significant profit increase: from € 25,000 to € 42,374 (= 25,000 + 17,374), or a 68% increase.
- This simulation is conservative in the assumption that it is always the top spending clients who enrol first in the plans: the top 5% clients, the top 10% clients, the top 20% clients, etc. In a real life scenario, we can assume that if, let's say, 20% of clients end up enrolling in the plan, they will not necessarily be the current top 20%, meaning that the incremental sales due to enrolment in the plans will be higher in reality than the ones calculated in the simulation.

Part V: Implementation tips

Implementing a wellness plan strategy in a veterinary practice is not "one more marketing campaign". It is a radical concept that affects the whole organization, and as such it requires proportionate commitment and dedication. We will analyse implementation issues from 5 main perspectives: overall business strategy, human resources, marketing, administration and management.

Strategic perspective

Wellness plans make sense if the practice business model is built around first opinion medicine and a proximity clientele looking for convenience and "one stop shopping" solution for their pets. Conversely, it makes no sense to engage in a wellness plan strategy if you are a referral hospital (you will alienate referring veterinarians) or if a significant portion of your revenues comes from sophisticated diagnostic and surgical procedures.

Human resource perspective

Team involvement will be essential for successful implementation, just as in any other relevant project: veterinarians should participate in the medical design of the plans. Ideally veterinarians and support staff should have their own pets enrolled in these plans (probably as fringe benefits) so that they can experience directly their benefits and communicate them convincingly to clients.

Marketing perspective

Especially at the beginning, clients will not come "asking for" wellness plans. We will have to make an active effort to sell them, putting them at the core of any communication activity: website, e-mailing, Facebook, reception area displays, etc. But ultimately, there will be no shortcut as the most effective communication channel

will be one to one recommendation in the exam room. The specific nature of these plans requires a well-coordinated recommendation, initiated by the veterinarian (putting the emphasis on the medical benefits for the patient) and reinforced by the support staff (focusing on the economic and convenience benefits for the client).

Administrative perspective

Wellness plans can be quite demanding from an administrative perspective. Plans need to be sold, invoiced, collected… Enrolled clients must be charged (properly) at discounted prices for certain products and services. They must also be reminded efficiently about vaccines, deworming, tests, and other procedures that have been prepaid. It is not surprising, therefore, that some specialized companies have appeared in the last few years (mainly in the UK and in the US) offering different levels of outsourcing for wellness plan administration.

Managerial perspective

A successful implementation of wellness plans will also require close monitoring from the practice management team. Some suggested KPI's (key performance indicators) to watch include:

- Percentage of active patients enrolled in the plans (and breakdown by life stage and species).
- Total annual spending per patient enrolled in the plans versus non-enrolled patients.
- Total annual spending per patient enrolled in the plans, separating plan revenue from additional revenue coming from non-plan items.
- Plan renewal rate, again by life stage and species.
- Average annual number of transactions per patient (enrolled in plan versus non-enrolled).

Conclusion

In the current economic climate of small animal veterinary medicine, the "wellness plan business model" deserves at least some attention from the veterinary community. If properly implemented – in the right type of clinic, with the right service offerings, and with the right amount of dedication - it can help generate relevant revenue and profit increases. Veterinary clinics are predominantly fixed cost businesses (people, equipment and premises representing the high share of costs), and their economic performance is extremely sensitive to low caseload activity: once the investments are made and the structure is there, the practice needs to bring in people and pets!

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Abbreviations

VAT: value-added tax
EBITDA: earnings before interest, taxes, depreciation and amortisation

References

[1] VMS pet food report 2013 (unpublished) (average national price per kilo for canine wellness pet food sold in Spanish veterinary practices in 2013)

[2] VMS pet food report 2013 (unpublished), (average national price per kilo for feline wellness pet food sold in Spanish veterinary practices in 2013)

[3, 4, 5] Bayer Veterinary Care Usage Study (2011)

[6] Banfield the Pet Hospital (www.banfield.com)

[7] Initial medical services gross margin = (100-25)/100 =75%. After 20% discount, gross margin = (80-25)/80= 68.75%

[8] Initial pet food gross margin = (100-75)/100=25%. After 15% discount and 5% reduction in purchasing cost, gross margin = (85-71.25)/85=16.2%. Initial antiparasiticides gross margin = (100-70)/100=30%. After 15% discount and 5% reduction in purchasing cost, gross margin = (85-66.5)/85=21.8%

[9] VMS pet food report 2013, VMS ectoparasiticide report 2013, VMS endoparasiticide report 2013 (unpublished).

Reprint paper*

Use of porcine small intestinal submucosa for corneal reconstruction in dogs and cats: 106 cases

Frédéric Goulle[1]

SUMMARY

Objectives: To describe the efficacy of porcine small intestinal submucosa in corneal reconstructive surgery in dogs and cats through a large retrospective study.

Methods: A retrospective evaluation of 106 cases of surgical reconstruction of the cornea with small intestinal submucosa seen between May 2005 and January 2010 was carried out. The corneal defect was filled by microsurgical grafting of porcine small intestinal submucosa. The biomaterial implant was deposited in one or several layers depending on the depth of the defect. The animals were examined 3, 6 and 12 weeks after surgery.

Results: Vision was preserved in all eyes at three months post-surgery. In 74 cases (69.8%) the corneal scar was either transparent or discrete, whilst in 32 cases (30.2%) a mild or marked scar was observed. Minor complications occurred in 9 cases (8.5%) with partial integration of the small intestinal submucosa and in 24 cases (22.6%) with faint or mild corneal pigmentation, without impairing vision. In cases followed over a period longer than three months, major complications occurred in five dogs resulting in vision impairment because of pronounced pigmentation.

Clinical Significance: Corneal grafting of porcine small intestinal submucosa is an effective method for corneal reconstruction resulting in corneal transparency in most cases. It is an excellent alternative to conventional conjunctival grafts.

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Introduction

A severe corneal defect in dogs and cats can be defined as the loss of more than half of the corneal thickness, until corneal perforation in the most severe cases. These corneal defects have a variety of aetiologies including traumatic, infectious or surgical (keratectomy). Several surgical grafting techniques have been described for replacement of the lost corneal substance including lamellar corneal graft, penetrating keratoplasty, corneoscleral transposition, conjunctival graft (island, bulbar pedicle, tarsoconjunctival, bridge, advancement or complete bulbar graft) [Hakanson and Merideth 1987, Gelatt and Gelatt 1995, Hendrix 2007], as well as synthetic grafts [Wilkie and Dan Wolf 1991] and biomaterial grafts. Corneal graft surgery has proven efficacy [Hansen and Guandalini 1999], but the difficulty of obtaining corneal transplants (fresh or frozen) limits the availability of this option in veterinary surgery. The use of different biomaterials including amniotic membrane, renal capsule and equine pericardium for corneal reconstruction surgery in the dog and horse has been reported. However, these transplant materials can be difficult to obtain [Barros and others 1995, 1997,

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1998, 2005, Andrade and others 1999, Lassaline and others 2005, Ollivier and others 2006]. Small intestinal submucosa (SIS) is an alternative biomaterial that is easier to procure and preserve. It has been used extensively in veterinary ophthalmology [Lewin 1999, Featherstone and Sansom 2000, 2004, Featherstone and others 2001, Bussieres and others 2004, Vanore and others 2007]. SIS is a non-immunogenic biomaterial capable of acting as a scaffold for tissue regeneration in different organs [Featherstone and Sansom 2000] including the eye [Lewin 1999, Featherstone and Sansom 2000, Featherstone and others 2001, Bussieres and others 2004, Vanore and others 2007]. Its use has previously been described in surgery to repair the fascia lata [Dejardin and others 1999], the skull [Cobb and others 1999], loss of arterial substance [Badylak and others 1989] and for intra-articular grafts of the cruciate ligament [Aiken and others 1994]. This biomaterial is composed of an acellular collagen matrix that acts as a medium for corneal remodelling and reconstruction [Lewin 1999, Featherstone and Sansom 2000, Bussieres and others 2004, Vanore and others 2007]. A study in the rabbit showed that the grafted material merges with the corneal stroma in the healing process [Griguer and others 2001]. The biocompatible protein matrix is initially invaded by fibroblasts which are then replaced by corneal stromal cells [Vanore and others 2007]. In previous reports, SIS was used with a good outcome in the management of canine and feline corneal diseases [Lewin 1999, Featherstone and Sansom 2000, Featherstone and others 2001, Bussieres and others 2004, Vanore and others 2007]. SIS was used in conjunction with a conjunctival graft to repair a full-thickness corneoscleral defect resulting from the excision of a limbal melanoma in a German shepherd dog, with a good incorporation of the graft into the cornea and sclera [Lewin 1999]. In another report, two cases of feline ulcerative keratitis were treated with SIS, resulting in a good outcome [Featherstone and Sansom 2000]. In a study of 10 cases of feline corneal disease, eight eyes healed with minimal corneal scarring and with a very good cosmetic and visual result. One eye required a conjunctival pedicle graft whilst one required enucleation [Featherstone and others 2001]. Another article reported the use of SIS graft covered by a conjunctival flap for the repair of full-thickness corneal wounds in dogs, cats and horses [Bussieres and others 2004]. In their series, despite post-operative complications (aqueous leakage, conjunctival flap dehiscence, synechia, fibrin and cataract), 14 of the 15 cases were visual at the final re-evaluation. Finally, SIS grafts were used for the surgical repair of deep melting ulcers in five dogs and two cats. All eyes healed without complications and retained corneal transparency, even in the presence of corneal perforation in two cases [Vanore and others 2007].

This large retrospective study describes treatment of 106 eyes in 102 animals (60 dogs and 42 cats) by surgical reconstruction of the cornea with SIS between May 2005 and January 2010.

Materials and methods

This retrospective study includes 60 dogs and 42 cats (106 eyes) that underwent a microsurgical graft of SIS for reconstruction of corneas with severe corneal defects from May 2005 to January 2010.

Ophthalmologic examination

A thorough ophthalmologic examination was performed in each case, with bilateral evaluation of the menace response, palpebral reflex, dazzle reflex and pupillary light reflex. Schirmer tear test (Standardized Sterile Strips, Schering-Plough Animal Health Corp) and fluorescein test (Fluoresceine 0.5% collyre unidose, TVM) were performed. All corneas were examined by slit-lamp biomicroscopy (Hawk Eye, Dioptrix), before and after fluorescein testing (except in cases of corneal perforation), for accurate evaluation of the depth of the corneal lesion to be filled, except in the cases of surgically induced defects (deep keratectomy). In these cases, evaluation was performed during surgery under the operating microscope. All cases in this study displayed a severe corneal defect, i.e. the loss of more than the half of corneal thickness, with a descemetocoele or corneal perforation resulting in some cases. Applanation tonometry (Tono-Pen Vet, Reichert) was performed in all cases, except for those with collapse of the anterior chamber because of corneal perforation.

Surgery

In every case, surgery was performed using an operating microscope (Variflex, Möller-Wedel). General anaesthesia was induced by intravenous injection of 5 mg/kg tiletamine-zolazepam (Zoletil, Virbac). Pain management was by subcutaneous injection of 4 mg/kg carprofen (Rimadyl, Pfizer) or by intramuscular injection of 0.25 mg/kg morphine (Morphine 10 mg, Lavoisier). After endotracheal intubation, anaesthesia was then maintained with isoflurane (Isoflurane Belamont, Nicholas

Piramal Limited) and oxygen. Marbofloxacin (Marbocyl FD, Vetoquinol) was injected intravenously at a dose of 2 mg/kg. As recommended for all ophthalmic surgical procedures, careful disinfection of the periocular region and the globe was performed with a mild antiseptic solution of povidone-iodine (diluted at 1% for the eyelids and 0.2% for the cornea) [Gelatt and Gelatt 2003].
The cornea was surgically prepared before SIS grafting by excising all malacic tissues using a beaver blade. SIS was available in the form of fine sterile rectangular sheets (10 cm×7 cm) or circular discs (10 to 15 mm in diameter), with a smooth side and a rough side, and a thickness of about 100 µm (Vet Biosist, SurgiVet Veterinary Products, Smiths Medical Pm Inc). The rough side was the tunica muscularis mucosal surface whilst the smooth surface was the stratum compactum surface of the tunica mucosa [Bussieres and others 2004]. Only rectangular sheets were used in this series. For each case, the transplant was cut from the sheet, either with a trephine, if the corneal defect was circular, or with micro-scissors if the shape of the defect was irregular. The transplant was then systematically examined under the operating microscope to distinguish the smooth surface from the rough surface, which was not always visible with the naked eye. The SIS transplant was prepared so that its shape was identical to that of the corneal defect and its size was consistently 1 to 2 mm larger than the surface area of loss of substance. The transplant was then placed with its rough face directly against the surgical site. Several layers of biomaterial, depending on the depth of the defect to be filled, were laid into the corneal defect, by stacking one layer on top of the other. The multi-layered transplant was microsurgically sutured to the cornea using absorbable monofilament suture material (Vicryl, Ethicon sutures, polyglactin 910, size 9/0, Johnson & Johnson Int.). The transplant was positioned by four sutures placed at four cardinal points of the corneal defect, then completed with as many sutures as required depending on the size of the loss of substance, thereby fixing the transplant firmly to the cornea. Regular irrigation with isotonic solution was performed during the surgery to prevent any drying. A third eyelid flap (anchored to the upper eyelid) was then placed for a period of three weeks. Oral treatment with 2 mg/kg/day marbofloxacin (Marbocyl, Vetoquinol) was prescribed for three weeks, as was twice daily application of topical gentamicin eye drops (Soligental, Virbac) placed onto the eyelid flap.
The first re-examination of cases was made by the referring veterinarian 10 days post-operatively, with the aim of confirming that the third eyelid flap was in place and that no discomfort was present. All animals were examined three weeks post-operatively by the operating surgeon when the third eyelid flap was removed and corneal healing evaluated. At 6 and 12 weeks later, the patients were re-examined and a complete ophthalmological examination (including an examination of the cornea by slit-lamp biomicroscopy and fluorescein testing) carried out.

Results

Description

The 102 animals in the study included 60 dogs and 42 cats (4 of which had bilateral ocular disease) (Table 1). There were 23 breeds of dogs represented: Shih-tzu (n=11), French bulldog (10), English setter (5), Pekingese (4), Pug (4), Boxer (3), Yorkshire terrier (3), Beagle (3), Lhasa apso (2), Brittany spaniel (2), American Staffordshire terrier (1), Beauce shepherd (1), Braque d'Auvergne (1), Chihuahua (1), Chinese crested dog (1), crossbreed (1), Bordeaux mastiff (1), English bulldog (1), Great Dane (1), Gordon setter (1), Irish red setter (1), Japanese spaniel (1) and Siberian husky (1). The average age was 5.4 years (ranging from 3 months to 13 years). There were 31 males of which 2 had been neutered, and 29 females of which 7 had been neutered. Four breeds of cats were represented: Chartreux (1), domestic shorthair (11), exotic shorthair (1), Persian (29). The average age was 5.8 years (from 8 months to 13 years). There were 26 males of which 18 had been neutered and 16 females of which 12 had been neutered. Four Persian cats had bilateral ocular disease (corneal sequestrum).

Clinical examination

The three corneal insults identified were traumatic (corneal injury with severe loss of substance), infectious/keratomalacic (deep melting corneal ulcer) and surgical (deep keratectomy). All 60 dogs had unilateral lesions with the loss of corneal substance resulting from a melting corneal ulcer in 42 cases (Fig 1a), severe corneal injury in 17 cases and surgical excision by deep keratectomy of epibulbar melanocytoma invading the cornea in 1 case (Fig 2a and 2b). Six dogs were receiving medical treatment for keratoconjunctivitis sicca: five of these were in the group with melting corneal ulcers and one in the group with traumatic corneal injuries. Corneal perforations were present in 16 eyes (Fig 3a) and 7 had descemetocoeles (Fig 4a). In the cats the corneal defects

Table 1. Clinical findings and postoperative results of SIS graft surgery on dogs and cats with corneal defects

Corneal disease and animals	No. of eyes	Presurgical observations	Average number of SIS layers	Postsurgicalobservations (3 weeks after surgery)	Postsurgical observations (3 months after surgery)	Postsurgical observations (>3 months after surgery)
Melting ulcer (dogs)	42	corneal perforation (11 eyes) descemetocoele (7) kcs treatment (5)	4.0	additional topical treatment (5)	discrete or transparent scar (27) mild scar (11) marked scar (4) mild pigmentation (5) faint pigmentation (8)	discrete or transparent scar (9) mild pigmentation (2) pronounced pigmentation impairing vision (5)
Melting ulcerative keratitis (cats)	7	descemetocoele (2) suspicion of herpetic infection (5)	4.1		discrete or transparent scar (3) mild scar (3) marked scar (1) pigmentation: sequestrum formation (1)	corneal transparency (2) discrete scar (1)
Severe corneal injury (dogs)	17	corneal perforation (5) kcs treatment (1)	3.9	additional topical treatment (1)	discrete or transparent scar (13) mild scar (3) marked scar (1) discrete pigmentation (4) mild perilimbal pigmentation (2)	corneal transparency (5)
Severe corneal injury (cats)	5	corneal perforation (5)	3.6	additional topical treatment (1)	discrete scar (2) mild scar (2) marked scar (1)	faint scar (1)
Sequestrum* (cats)	34	corneal perforation (1)	2.9	additional topical treatment (2)	discrete or transparent scar (29) mild scar (4) marked scar (1) pigmentation: recurrence of sequestrum (4)	discrete or transparent scar (7) mild scar (1) discrete pigmentation (2)
Epibulbar melanocytoma (dog)	1	corneal invasion	4		marked scar (1)	mild scar (1)

Kcs: keratoconjunctivitis sicca *sequestrum with four bilateral cases

were the result of surgical excision by deep keratectomy of corneal sequestrum in 34 eyes (Figs 5 and 6), severe corneal injury in 5 eyes (Fig 7a) and melting ulcerative keratitis in 7 eyes (Fig 8a and 8b). Of the seven cases of melting ulcerative keratitis, five occurred in cats where there was a suspicion of herpetic keratitis. Six eyes had perforated corneas and two had descemetocoeles (Fig 9a). The 106 eyes included in this study were all affected with a severe loss of corneal substance, i.e. the loss of at least half of the thickness of the corneal stroma,

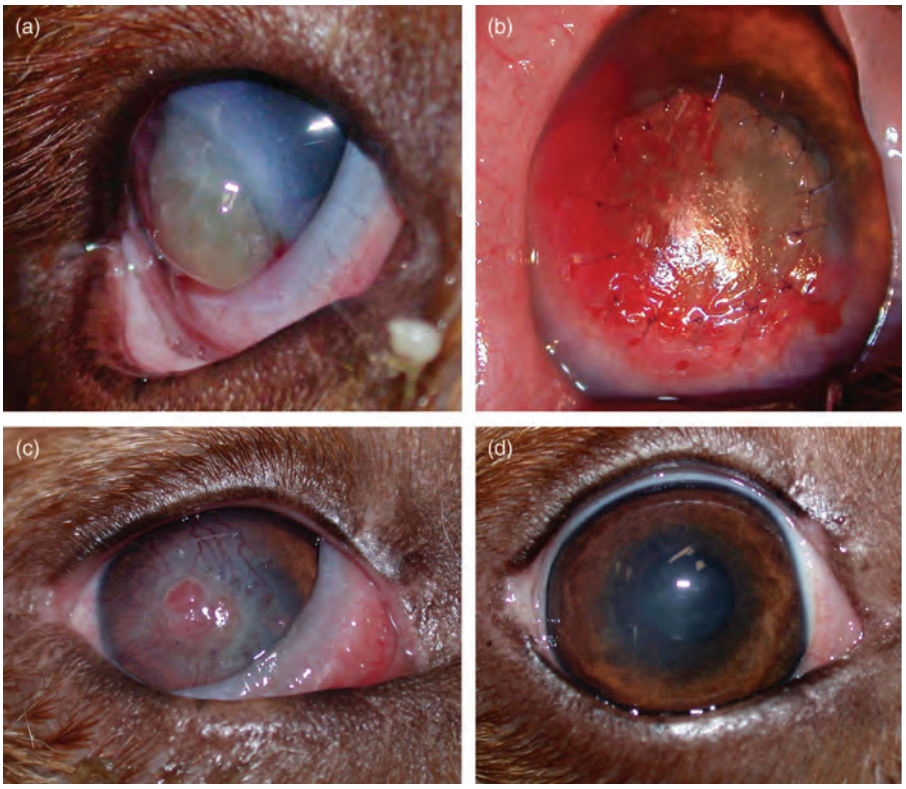


Fig 1.
(a) Right eye of a two-year-old Brittany spaniel before surgery. Note the deep melting ulcer and the large descemetocoele.
(b) Same dog as (a) after porcine small intestinal submucosa surgical graft and before covering with the third eyelid.
(c) Same dog as (a) three weeks after surgery. Note the acceptance of the transplant well integrated into the cornea, and the intense corneal neovascularisation.
(d) Same dog as (a) three months after surgery. Note that good corneal transparency resulted in restoration of vision.

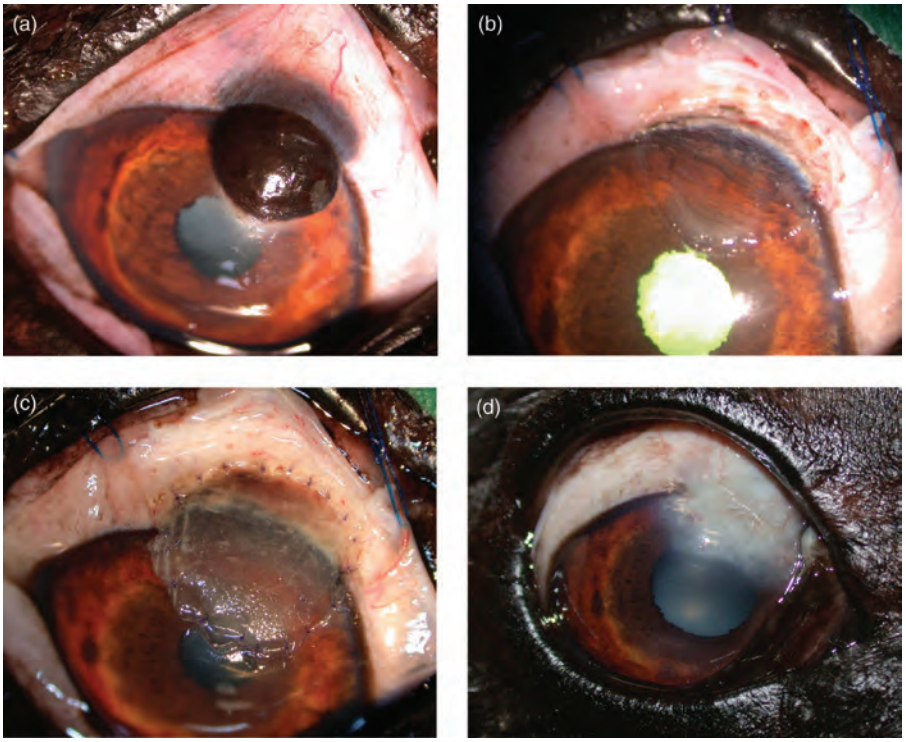
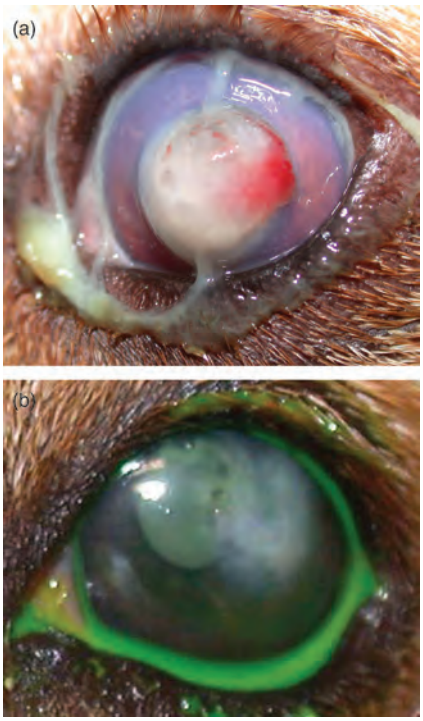


Fig 2.
(a) Right eye of a 13-year-old Braque d'Auvergne. Note the epibulbar melanocytoma invading the cornea. (b) Same dog as (a) after surgical excision by deep sclerokeratectomy.
(c) Same dog as (a) after SIS graft, before third eyelid flap.
(d) Same dog as (a) three months after surgery. Note the marked corneal scar with mild vascularisation, not impairing the visual axis.

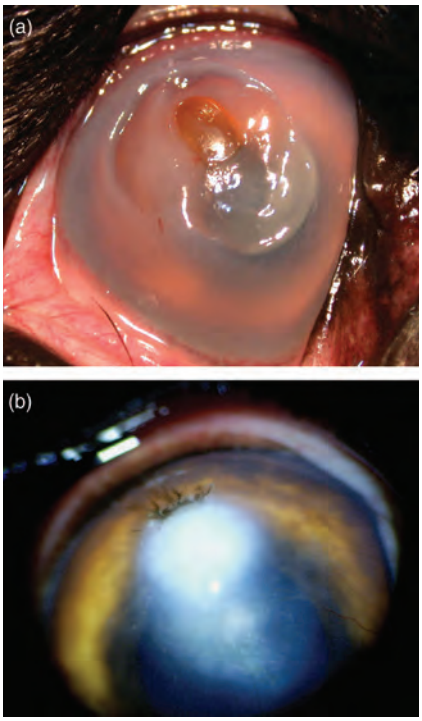
confirmed by examination with a slit-lamp biomicroscope or by an operating microscope for the 35 cases of surgically induced defect.

Treatment

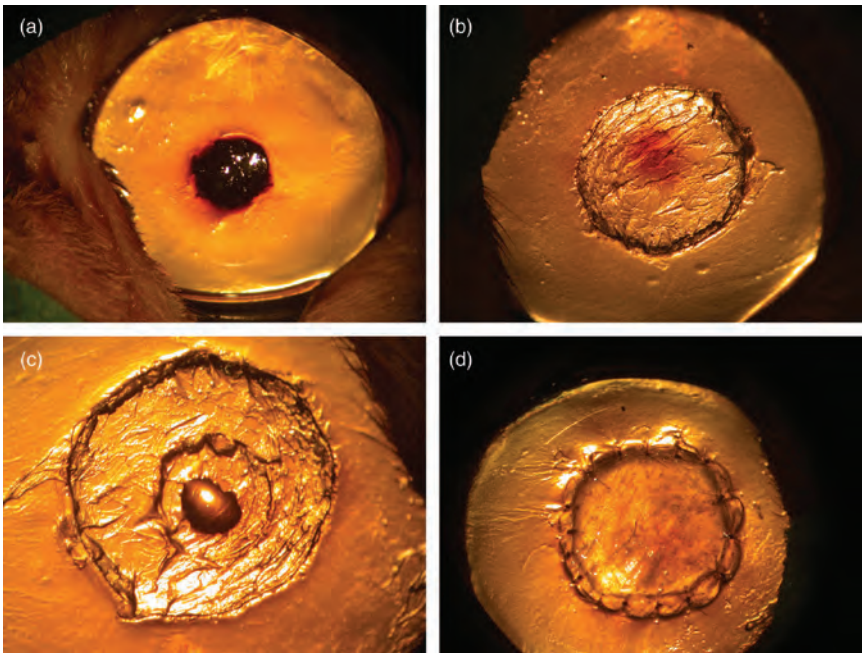
The number of layers of biomaterial used was dependent on the depth of the defect to be filled. The average number of layers for the 106 cases was 3.62 (ranging from one to six layers). In the canine group, the 60 cases were treated with an average of 3.98 layers (from one to six layers), with an average of 4.02 layers for melting corneal ulcers (Fig 1b), 3.88 layers for losses of substance through injury and four layers for the only case of epibulbar melanocytoma invading the cornea (Fig 2c). In the feline group, the 46 cases were treated with an average of 3.15 layers (from one to five layers), with an average of 2.88 layers for the cases of reconstruction following surgical excision of corneal sequestrum (Figs 5d



◀ *Fig 3. (a) Left eye of a four-year-old Beagle. Note the melting ulcer with large iris prolapse. (b) Same dog as (a) three months after surgery. Note the mildly vascularised corneal scar.*



► *Fig 4. (a) Right eye of a two-year-old Great Dane. Note the deep melting ulcer with descemetocoele. (b) Same dog as (a) three months after surgery. Note the marked corneal scar with faint pigmentation.*



◀ *Fig 5.*
(a) Left eye of a five-year-old Persian cat. Note the deep corneal sequestrum.
(b) Same cat as (a) after the first step of keratectomy. Note the persistence of pigmentation in the deep corneal stroma.
(c) Same cat as (a) after the second step of keratectomy. Note the total resection of sequestrum and the leakage of aqueous humour (keratectomy until perforation).
(d) Same cat as (a) after a porcine SIS surgical graft and before covering with the third eyelid.

and 6b), 4.14 layers for the cases of melting ulcerative keratitis (Fig 8c) and 3.6 layers for losses of substance through injury (Fig 7b).

Outcome

On removing the third eyelid flap three weeks post-surgery, centripetal corneal neovascularisation was systematically observed, with variable intensity for each case (Figs 1c and 8d). Examination of the cornea by slit-lamp biomicroscopy followed by fluorescein testing showed full integration of the biomaterial within the cornea, with total reconstitution of the corneal epithelium in 97 cases at the three-week assessment.

Minor complications occurred in nine cases, with partial integration of the biomaterial, estimated at about 75% integration (Fig 10). These nine cases were six dogs and three cats. Two of these six dogs were receiving treatment for keratoconjunctivitis sicca, and three others had been operated on for corneal perforations. Two of the three cats were being treated for herpetic keratitis, of which one had a perforated cornea before undergoing surgery. These nine cases of partial acceptance of the transplant finally recovered completely, with total integration of the biomaterial and resultant corneal healing after additional topical treatment over a one-to-three-week period following the third eyelid flap removal. This treatment

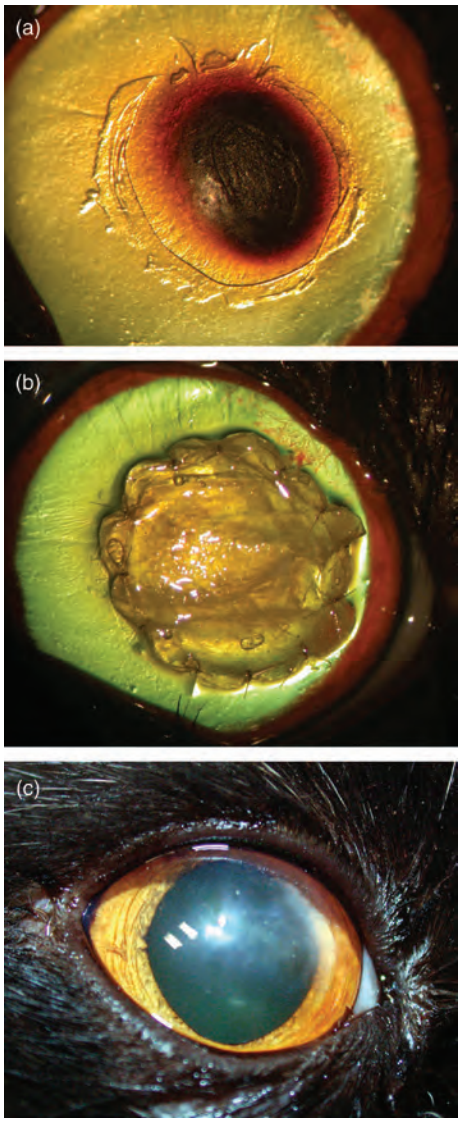


Fig 6. (a) Right eye of a fifteen-month-old Persian cat. Note the deep corneal sequestrum. (b) Same cat as (a) after a porcine SIS surgical graft and before covering with the third eyelid. (c) Same cat as (a) three months after surgery. Note the good corneal transparency resulting in restoration of vision.

included artificial tears and antibiotics three times a day: fusidic acid (Fucithalmic Vet, Dechra Veterinary Products) or gentamicin drops (Soligental, Virbac).

A topical suspension of dexamethasone 0.1% and tobramycin 0.3% (Tobradex, Alcon) was prescribed in a majority of cases with the aim of reducing corneal neovascularisation and scarring, except for cats with suspicion of herpetic infection. These cats were those presented with deep stromal keratitis and history of signs compatible with herpetic infection (initially spontaneous superficial corneal ulcers, complicated in secondary immune-mediated stromal keratitis). Topical dexamethasone was administered approximately one to two weeks after removing the third eyelid flap, except for the nine cases of partial transplant acceptance, for which the treatment was not started until the biomaterial was fully integrated (approximately three weeks after additional treatment). Topical dexamethasone was administered with caution, at first once a day or once every other day for approximately two weeks, then twice a day in some cases for a period depending on the intensity of scarring and corneal neovascularisation, often from one to four weeks. As for sequestrum cases, administration was only when necessary over a short period (less than a week). After 12 weeks, re-evaluation showed that corneal neovascularisation was present in 50 cases (47.2%), from discrete (34 cases, 32.1%) (Fig 3b) to mild (16 cases, 15.1%) (Figs 7c and 9b). Vision was preserved in all eyes without exception (positive menace response) at three-months post-procedure. In 74 cases (69.8%) the corneal

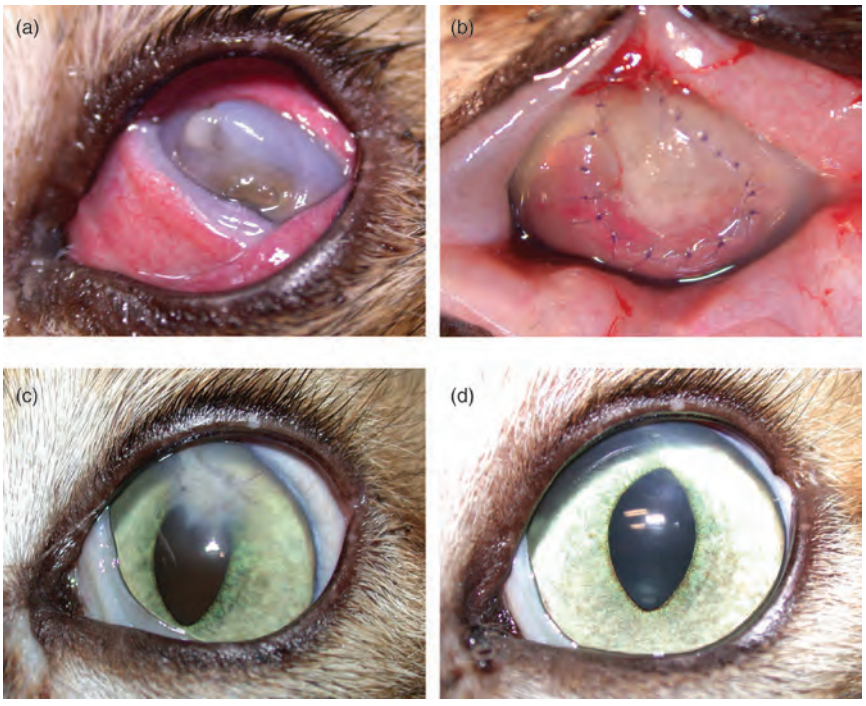


Fig 7. (a) Left eye of a six-year-old domestic shorthair cat. Note the severe traumatic corneal defect. (b) Same cat as (a) after porcine SIS graft. (c) Same cat as (a) three months after surgery. Note the marked corneal scar with mild vascular response. (d) Same cat as (a) seven months after surgery. Note the faint scar.

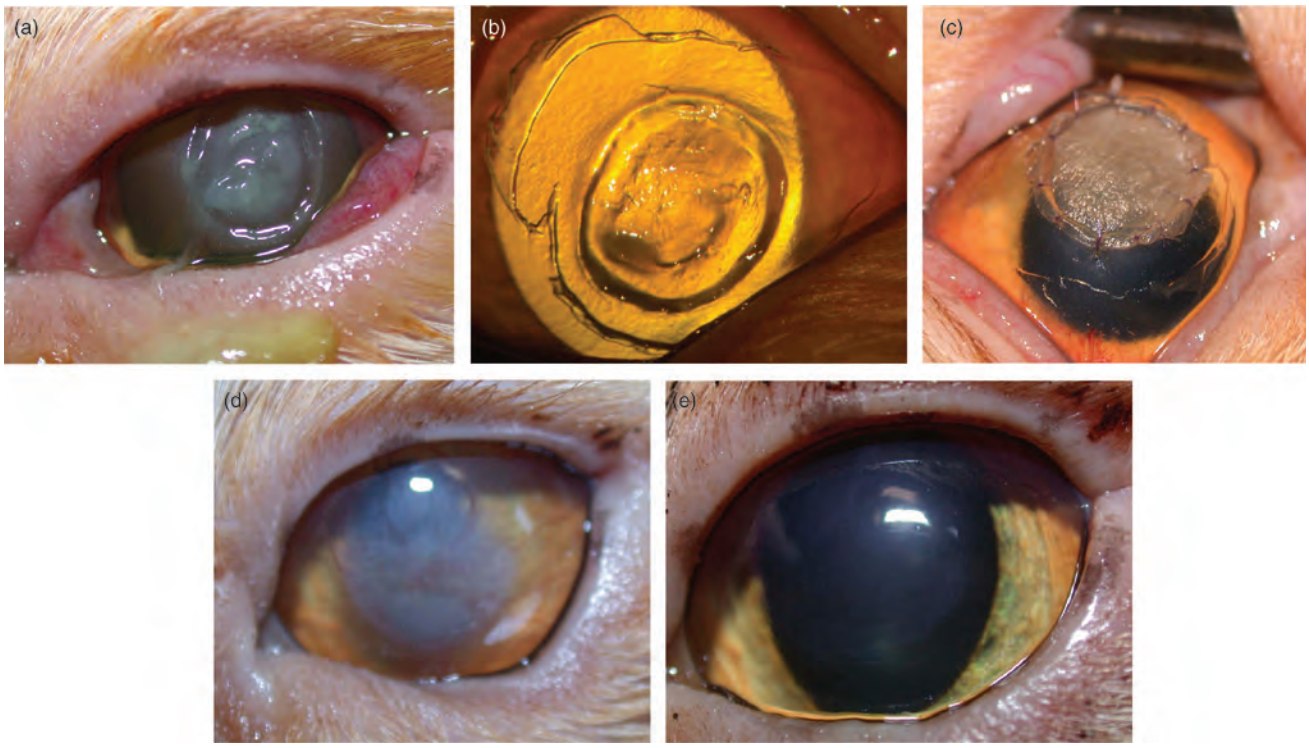


Fig 8.
(a) Left eye of a seven-year-old Persian cat. Note the melting ulcerative keratitis.
(b) Same cat as (a) before grafting. Note the deep melting ulcer.
(c) Same cat as (a) after a porcine SIS surgical graft and before covering with the third eyelid.
(d) Same cat as (a) three weeks after surgery. Note the acceptance of the transplant which has integrated into the cornea, and the slight corneal neovascularisation.
(e) Same cat as (a) three months after surgery. Note the good corneal transparency

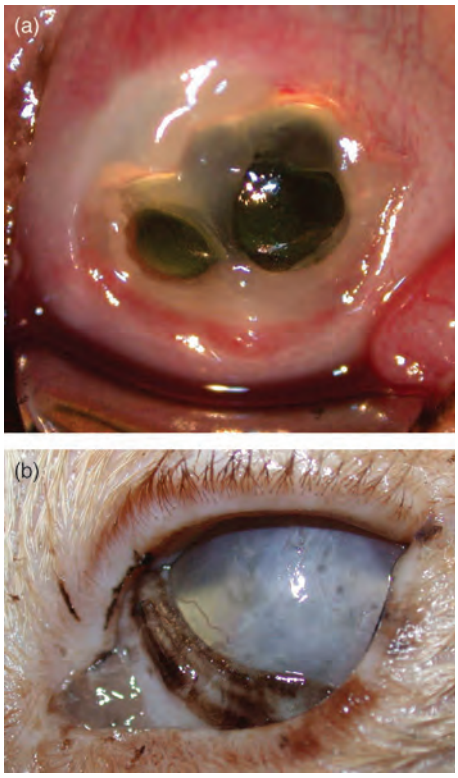


Fig 9. (a) Left eye of a eight-year-old domestic shorthair cat. Note the deep melting ulcer with a large descemetocoele. (b) Same cat as (a) three months after surgery. Note the marked corneal scar with mild vascular response.

scar was either transparent or discrete (Figs 1d, 6c and 8e), whilst in 32 cases (30.2%), a mild (23 cases, 21.7%) or marked (9 cases, 8.5%) scar (Figs 2d, 4b and 9b) was present but did not obstruct vision.

In seven canine cases (6.6%), a mild corneal pigmentation could be observed at three months, but did not impair vision. Of these seven dogs, all were brachycephalic: four were shih-tzus (including three that had perforated corneas), two were Pugs, whilst the last was a Pekingese with a perforated cornea. In five feline cases (4.7%), a mild stromal pigmentation (Fig 11) was present at the three-month check, and was diagnosed as the beginning of sequestrum formation. In two eyes, this resulted from sequestration in one Persian cat which was affected in both eyes. This cat was examined at nine months after surgery and the pigmentation had totally disappeared in both eyes. The three other feline cases (two sequestrations, one melting ulcerative keratitis) where onset of corneal pigmentation could be observed were not followed beyond three months. In 12 other canine cases (11.3%), a faint pigmentation was present. Thirty-four animals (36 eyes) were followed over a period longer than 12 weeks (from 4 to 11 months): major

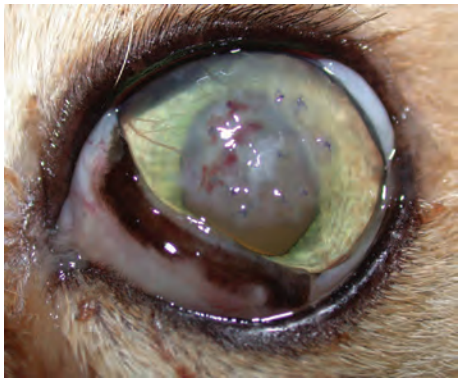


Fig 10. Left eye of a five-year-old Persian cat three weeks after surgery. Note the partial integration of the biomaterial.

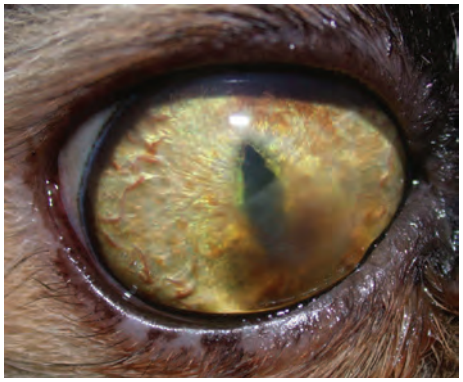


Fig 11. Right eye of a four-year-old Persian cat three months after surgery. Note the sequestrum reformation and the mild vascularisation

complications were encountered in five of these eyes, which became visually impaired because of pronounced pigmentation. Vision was preserved in 31 eyes (Fig 7d).

Discussion

SIS is a biomaterial composed of an acellular collagen matrix that is initially invaded by fibroblasts which are then replaced by corneal stromal cells [Vanore and others 2007]. In addition to collagen, this biomaterial is composed of fibronectin, hyaluronic acid, chondroitin sulphate, heparin sulphate and growth factors [Vanore and others 2007]. Besides promoting the proliferation and migration of stromal cells, these growth factors, particularly TGFbs (transforming growth factor-beta), also inhibit the synthesis of metalloproteinases [Roberts and others 1990). This biomaterial therefore appears particularly indicated for the surgical treatment of melting corneal ulcers in which the corneal stroma is quickly destroyed by a process of enzymatic lysis, caused by metalloproteinase activity. Melting ulcers (also termed keratomalacia) are characterised by gelatinisation and liquefaction of the corneal stroma, diagnosed by slit-lamp biomicroscopy. In such cases, an extensive debridement of necrotic and collagenolytic corneal tissue by keratectomy is necessary before SIS corneal grafting [Vanore and others 2007]. Although SIS appears resistant to protease activity, a surrounding healthy cornea is required to support the graft material [Bussieres and others 2004]. In cases with melting ulcer and severe traumatic lesions, the surgical preparation of the impaired site was performed in our series with micro-scissors to eliminate areas of lysed or devitalised stroma. Care was taken to preserve Descemet's membrane when it was visible. Regarding surgical defects (excision of sequestrum and the case of epibulbar melanocytoma invading the cornea), the keratectomy had to be continued until only healthy tissue remained at the base of the defect. In cases of corneal sequestra (Figs 5a and 6a), particular care was taken when performing the deep keratectomy, to eliminate the deep stromal brown pigmented tissue often visible after excision of the sequestrum (Fig 5b). In some cases keratectomy until perforation was required to ensure full excision of the sequestrum (Fig 5c). No samples for bacterial culture or cytology were collected in our series but topical gentamicin was used after surgery in all cases.

Previous studies have reported the use of soft bandage contact lenses [Featherstone and others 2001] and conjunctival graft [Lewin 1999, Bussieres and others 2004] in association with a SIS graft, but the use of a bandage contact lens can sometimes lead to the loss of the implanted material in lively patients [Whitley and Gilger 1999]. Conjunctival transplants may have the disadvantage of being opaque and impeding vision. Other grafting procedures such as corneoconjunctival or corneoscleral transposition may be used to decrease corneal scarring and allow a clearer post-operative cornea than that seen after conjunctival corneal graft [Gilger and Whitley 1999]. However, these grafting procedures damage normal and healthy corneal tissue [Gilger and Whitley 1999] and they are time-consuming techniques [Hendrix 2007]. Furthermore, they are not indicated in cases of melting ulcer, inasmuch as sufficient peripheral healthy cornea is needed for these grafting procedures [Gilger and Whitley 1999].

In the current series, following the SIS graft, a third eyelid flap was placed for a period of three weeks. The use of a third eyelid flap may be considered inappropriate for severe corneal disease. These flaps do obscure visualisation of the cornea, inhibit topical medications from reaching the cornea and they do not deliver an appropriate blood supply [Hendrix 2007]. However, in two previous cases which treated before this series (one

French Bulldog with melting ulcer and one Persian cat with sequestrum), the author had performed SIS grafting without a third eyelid flap protection, and observed significant desiccation of the transplant followed by graft failure. Therefore, it is proposed that the eyelid flap may help protect the biomaterial by keeping it hydrated, even if it prevents visualisation of the progress of the implant. Furthermore, the third eyelid flap has been used successfully in conjunction with frozen lamellar corneal grafts for two weeks after surgery, even in cases of melting ulcers, with the aim of protecting the graft from blinking movements and to help maintain pressure on the graft's surface [Hansen and Guandalini 1999]. In another report on seven cases with deep melting ulcers treated by surgical debridement and SIS grafting, a third eyelid flap was placed for a two-week period in six of seven cases to protect the SIS graft after surgery, and no complications occurred [Vanore and others 2007].

The period of three weeks before removing the third eyelid flap is one week longer than in the series by Vanore. The current author preferred to keep the implant protected under the eyelid flap for a further week, especially in severe cases (corneal perforation following trauma or keratomalacia). This period of three weeks may seem a long time for a severe corneal disease with potential post-operative complications, but the study of Vanore reported no complications in six cases with melting corneal ulcers treated surgically with surgical debridement, SIS grafting, and third eyelid flap placement [Vanore and others 2007]. In the current series, only nine cases exhibited minor healing complications (partial integration of the biomaterial).

A topical suspension of dexamethasone was cautiously administered in most cases to reduce corneal neovascularisation and scarring, except for cats with suspicion of herpetic infection. These cats were those with deep stromal keratitis and history of signs compatible with herpetic infection (initially spontaneous superficial corneal ulcers, complicated in secondary immune-mediated stromal keratitis). The administration was very careful, at first once a day or once every other day for approximately two weeks, then twice a day for some cases, for a period depending on the intensity of scarring and corneal neovascularisation. The use of topical corticosteroids three times a day for six weeks after lamellar keratoplasty was recommended in a series of four Persian cats (six eyes) with corneal sequestra to lessen the probability of corneal vascularisation and subsequent graft rejection [Pena Gimenez and Morales Farina 1998]. However, cats treated with topical corticosteroids may be at risk of developing corneal ulcers from activation of latent herpesvirus, inasmuch as up to 50% of clinically normal cats have FHV1-DNA in the cornea [Stiles and others 1997, Townsend and others 2004]. Despite the temptation to use topical steroids to keep the cornea clear, topical corticosteroids must be very cautiously administered and their use delayed if possible [Hansen and Guandalini 1999], as in this series. Recrudescence of any FHV-1-associated disease in this series was not encountered, except in five cases for which marked or faint stromal pigmentation was present at the three-month check, that is to say recurrence of sequestrum.

The use of topical corticoids is normally contraindicated in cases of melting ulcers, because they increase the proteolytic activity of corneal collagenase. However in the current series, keratomalacia was totally resolved in every case of melting ulcer (total integration of SIS and corneal healing), when topical dexamethasone was commenced.

A faint to mild pigmentation without vision impairment was observed at the three-month post-procedure examination in 22.6% of cases. Some of these cases had corneal pigmentation before surgery, and similar findings were described in a study describing the use of frozen lamellar corneal grafts [Hansen and Guandalini 1999]. In our series, only 36 eyes were followed over a period longer than 3 months (from 4 to 11 months), among them five became visually impaired because of pronounced pigmentation. These five cases were all brachycephalic dogs with melting ulcers, which initially had very severe corneal lesions (perforation or descemetocoele) and presented with corneal pigmentation before surgery. In brachycephalic breeds, the pigmentary keratitis syndrome is one of the disorders in which corneal pigmentation occurs [Gilger 2007]. In these breeds, chronic exposure (because of prominent eyes with large palpebral fissures, also known as euryblepharon) causes chronic corneal irritation, resulting in corneal pigmentation due to migration of melanocytic cells from the limbal and perilimbal tissues [Whitley and Gilger 1999]. Melanin pigment within macrophages and fibroblasts may also develop [Whitley and Gilger 1999]. In our series, it seems that brachycephalic dogs with

pigmentary keratitis syndrome and initially pigmented cornea may be predisposed to a worsening of the pigmentation after grafting surgery, especially in cases of severe corneal lesions (descemetocoele or perforation). In summary, this large retrospective study presenting data from a larger number of cases than previously published shows that SIS is a very efficient biomaterial for use in corneal reconstruction surgery. Furthermore, this grafting technique allows preservation of vision by restoring corneal transparency in the vast majority of cases. However, brachycephalic dogs with initially pigmented cornea and severe corneal defect may be predisposed to a worsening of the pigmentation after grafting surgery.

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*

Neurological complications in 4 critically ill patients with haematological emergencies: bleeding disorders, hypercoagulation and thrombosis

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SUMMARY

Objective: To describe unusual neurological complications secondary to haemostatic disorders in critically ill dogs and to discuss the actual controversies in the clinical diagnosis and management of these patients.

Series Summary: Cases for the study were identified from the population of dogs examined at the Veterinary Teaching Hospital (VTH) between May and November 2006. Patients were included if they fitted two selection criteria: presence of a haemostatic disorder documented by CBC and coagulation profile, and neurological complications secondary to the haemostatic disorder documented by means of clinical signs, necropsy and other complementary examinations. Four dogs satisfied the inclusion criteria, one of these was referred for evaluation of neurological disease and the remaining cases developed neurological signs in the intensive care unit (ICU). Three of the patients evaluated showed neurological complications associated with bleeding, while the fourth had signs secondary to arterial thrombosis. Three dogs died or were euthanised due to poor prognosis, and the outcome was excellent in the surviving dog with a multifocal (spinal cord and forebrain) vascular problem secondary to DIC. The post-mortem examination performed in 2/3 patients confirmed the presence of haemorrhages, fibrinous thrombi, and ischemia-related lesions in the central nervous system (CNS).

New or unique information provided: Our observations suggest that coagulation disorders should be considered when neurological complications develop in critically ill patients. In addition, there is a high variability between detectable alterations in coagulation profiles and real haemostatic status in critically ill patients. An early and complete in vitro haemostatic status evaluation, as well as treatment of the underlying disorder are essential to be able to anticipate these severe and unpredictable complications in intensive care patients.

Key words: bleeding disorders, hypercoagulation, thrombosis, neurological signs, dog.

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Introduction

Haemostatic abnormalities have been well documented in veterinary medicine for many years, and they have most consistently been associated with bleeding problems. Disseminated intravascular coagulation (DIC) is one of the most common haemostatic disorders that manifests often in the form of haemorrhagic diathesis and has been reported as an important complication in critically ill veterinary patients.[1]

The clinical presentation can range from sub-clinical prolongation of clotting times to fulminant disease with widespread and simultaneous microvascular thrombosis and profuse bleeding. It is now accepted that DIC is always secondary to an underlying disorder and it can be associated with several systemic diseases.[2,3,4,5] DIC is not always characterised by a haemorrhagic syndrome, but also by multiple organ failure, depending on the intensity of the haemostatic activation and the amount of microvascular fibrin formation.[2-5,6,7] To the authors' knowledge, there are no specific reports in the veterinary literature about neurological complications associated with hypocoagulable or hypercoagulable states in critically ill patients.

The purpose of this case series is to describe unusual neurological complications secondary to haemostatic disorders in critically ill dogs with a high impact on outcome, in terms of financial concerns and prognosis.

Case 1

A 13-year-old spayed female German pointer was admitted by the Internal Medicine Service of the VTH for evaluation of mammary tumours in the right M2, M3, M4, and M5. Complete blood count (CBC), serum biochemistry, urinalysis, abdominal ultrasound and thoracic radiographs were performed prior to surgical excision (Table 1). Survey thoracic radiographs revealed no significant anomalies. Abdominal ultrasound examination showed slight enlargement of the left adrenal gland and a lesion in the spleen consistent with nodular hyperplasia. The day of the planned surgery the patient suddenly developed an acute tetraparesis. The neurological examination localised a lesion between the C6-T2 spinal cord segments (Table 2). The main differential diagnoses included: acute disc extrusion (Hansen I), fibrocartilaginous embolism, a vascular problem (infarct, haemorrhage), neoplasia, or an inflammatory or infectious process affecting the spinal cord. Repeated complete blood work revealed a mild normocytic, normochromic anaemia and thrombocytopenia. A coagulation profile displayed severely increased prothrombin time and activated partial thromboplastin time, and low fibrinogen concentration. The result of the haemostatic evaluation thus confirmed the presence of an overt disseminated intravascular coagulation and the patient's condition progressively worsened despite intensive care management. A few hours later the patient developed refractory hypovolemic and distributive shock, which was manifested by a very depressed mental status, normothermia (39°C), tachycardia (185 bpm), tachypnoea (56 bpm), hypotension (SAP: 40 mmHg), pale mucous membranes and a prolonged capillary refill time (> 2 seconds). Medical management included oxygen therapy and low volume resuscitation techniques. Intravenous fluid therapy consisted of lactated Ringers® solution (5-50ml/kg/h), and hetastarch (a bolus of 2.5 ml/kg, followed by a CRI at 1-2 ml/kg/h). In addition, inotropic support was provided by administering dobutamine (5 µg/kg/min) in order to restore the effective circulation blood volume. Due to the worsening of the patient's condition, therapy with blood products was initiated as soon as the coagulation profile was obtained. A transfusion of one unit of fresh frozen plasma (FFP [10 ml/kg] in 2 hours) and therapy with low-molecular-weight heparin (dalteparin [70 UI/kg q24h SQ]) were initially administered, followed by a packed red blood cell (PRBC) transfusion due to the evidence of continuous bleeding and a lowering PCV. Despite treatment, the animal's condition progressively worsened and death occurred a few hours later in the ICU.

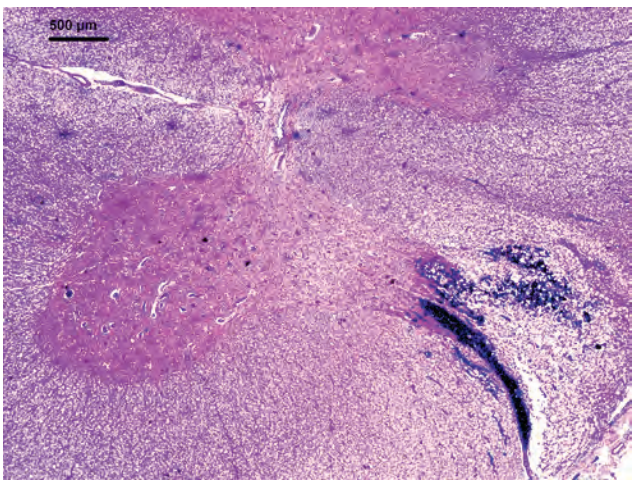

Fig. 1 Microscopic image of the spinal cord (case 1). Histopathological lesions compatible with acute myelomalacia.

Table 1. Results of analytical profiles performed in the patients submitted to study

	Units	Reference interval	Patient 1	Patient 2	Patient 3	Patient 4
Haematology						
Red blood cells	cells 10^6/µl	(5.5-8.5)	5.48	7.64	4.85	5.11
Haemoglobin	g/dl	(12-18)	13.5	18.5	12.2	12.7
PCV	%	(37-55)	37	53	35	34
MCV	fl	(62-77)	67.5	69.4	72.2	66.5
MHCH	g/dl	(33-37)	36.5	34.9	34.9	37.4
MHC	pg	(21.5-26.5)	24.6	24.2	25.2	24.9
Total WBC	cells 10^3/µl	(6,000-17,000)	10,860	26,450	17,290	17,300
Seg Neutrofils	cells 10^3/µl	(3,000-11,500)	8,145	23,276	15,042	14,878
Bands	cells 10^3/µl	(0-300)	0	0	0	0
Lymphocytes	cells 10^3/µl	(1,000-4,800)	977	794	865	1,730
Monocytes	cells 10^3/µl	(150-1,350)	869	2381	1,383	692
Eosinophils	cells 10^3/µl	(100-1,500)	760	0	0	0
Platelets	cells 10^3/µl	(200-500)	38	120	96	387
Coagulation						
TP	seconds	(6-8)	180	12.6	24.1	5.7
ATTP	seconds	(9-16)	180	25.4	21.4	11.3
Fibrinogen	mg/dl	(200-400)	25	130	96	580
TT	seconds	(12-18)	-	42.5	-	-
D-dimers	ng/ml	(<250)	-	>1,000	-	-
AT-III	%	(>75%)	-	-	-	56
Biochemistry						
Sodium	mEq/l	(141-152)	156	151.2	144	144.7
Potassium	mEq/l	(4.37-5.35)	3.9	4.04	4	4.13
Chloride	mEq/l	(105-115)	115.3	107	106.9	108.6
Phosphorus	mg/dl	(2.6-6.2)	4.8	6.0	4.8	5.5
Calcium	mg/dl	(9-11.3)	12.4	9.4	8.7	10.7
BUN	mg/dl	(6-25)	8	12.2	15.4	8
Creatinine	mg/dl	(0.5-1.5)	0.7	1.19	0.75	0.70
Glucose	mg/dl	(65-118)	114.5	75	140.3	105.8
Total proteins	g/dl	(5.6-7.5)	6.8	6.0	5.5	6.6
Albumin	g/dl	(2.6-3.3)	2.8	1.7	2.6	2.8
Globulins	g/dl	(1.7-4.0)	4.0	4.3	2.9	3.8
CK	IU/l	(10-150)	211.2	89	280	25,112
AST	IU/l	(23-66)	48	68	63	-
ALT	IU/l	(21-102)	67	120	59	120
Alk Phos	IU/l	(20-156)	1,042.65	114.3	166.57	204.06
GGT	IU/l	(1.2-6.4)	6	5	1	3
T Bili	mg/dl	(0.1-0.5)	0.27	0.4	0.36	0.32
Chol	mg/dl	(135-270)	413.3	379.0	313.8	170.4

Table 1. continued ...

	Units	Reference interval	Patient 1	Patient 2	Patient 3	Patient 4
Urinalysis						
Sample method			Cystocentesis	Cystocentesis	Cystocentesis	Cystocentesis
Specific gravity			1,036	1,023	1,050	1,027
pH			7.5	8	7	7
Glucose	mg/dl		-	-	-	-
Ketones	mg/dl		-	-	-	-
Bilirubin	mg/dl		-	-	-	-
Proteins	mg/dl		+	+	+++	+
Blood			+++	+++	+++	+
Leucocytes			-	-	++	-
Sediment			Amorphous phosphate crystals	Cocci++	Tubular casts, erythrocytes	Epithelial cells

PCV: packed cell volume; MCV: mean corpuscular volume; MHC: mean corpuscular hemoglobin; MHCH: mean corpuscular haemoglobin concentration; WBC: white blood cells; TP: prothrombin time; ATTP: activated partial thromboplastin time; TT: thrombin time; AT-III: antithrombin III; Phosp: phosporus; BUN: blood urea nitrogen; CK: creatine kinase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Alk Phos: alkaline phosphatase; GGT: γ glutamyl transferase; T Bili: Total bilirubin; Chol: Cholesterol.

Post-mortem histopathological examination confirmed the presence of acute spinal cord myelomalacia affecting the cervico-thoracic intumescence, and caused by a haemorrhagic and ischemic process (DIC). In addition, multiple haemorrhagic foci and fibrin deposits were found in many other organs, but were more severe in the liver and lungs. A mammary carcinoma was detected in a mammary gland, intravascular metastatic carcinomatosis in the lungs and multiple adenomas in both adrenals and thyroid glands.

Case 2

A 10-year-old female Belgian shepherd dog was admitted by the Internal Medicine Service for evaluation of a possible pyometra. The patient had a history of depression, anorexia, and polyuria/polydypsia. On physical examination, a distended and mildly painful abdomen and a purulent vaginal discharge were found. CBC, serum biochemical panel, urinalysis, abdominal ultrasound and thoracic radiographs confirmed the presence of a non-perforated pyometra with no other systemic complications (Table 1). The patient was transferred to the surgery service and a routine ovariohysterectomy was performed. The patient's condition deteriorated over the 24 hours after surgery and it was transferred to the Intensive Care Unit. On admission the patient was very depressed and had hyperthermia (39.6°C; N=38-39°C), tachypnoea (45 rpm; N=6-20 bpm), tachycardia (156 bpm; N=80-120 bpm) with hyperdynamic femoral pulses and mild hypertension (SAP: 170). Mucous membranes were congested and capillary refill time was shortened (<1s; N=1-2 seconds). Haematological and coagulation profile abnormalities included a mature neutrophilia, normal prothrombin time, normal activated partial thromboplastin time, low fibrinogen concentration, increased d-dimers and prolonged thrombin time. Thus the results of the coagulation profile confirmed the presence of acute intravascular disseminated coagulation, probably due to an on-going septic status.

Therapy with fresh frozen plasma (FFP: 10 ml/kg q8h IV) and dalteparin (70 UI/kg q24h SQ) was initiated immediately. Medical management included also crystalloid fluid therapy with lactated Ringer's solution (1-5 ml/kg/h) supplemented with potassium chloride (30 mEq/L), dextrose supplementation according to the monitored glucose level, hetastarch (20 ml/kg/day), clindamycin phosphate (11 mg/kg q12h IV), enrofloxacin (5 mg/kg q24h IV), and buprenorphine (20 µg/kg q6h IV). Despite medical treatment, two days after surgery the patient suddenly developed acute ambulatory difficulties and seizures. Neurological examination was consistent with the presence of a forebrain and a T3-L3 spinal

Table 2 Neurological findings of the patients submitted to study

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Neurological exam				
Mental status	Alert	Coma	Severe depression	Alert
Posture	Lateral recumbency	Lateral recumbency	Lateral recumbency	Normal
Gait	Non ambulatory tetraparesis (right side)	Non ambulatory tetraparesis	Non ambulatory paraparesis	Monoplegia LTL
Postural reactions (conscious propioception)	LTL: delayed RTL: absent LPL: delayed RPL: absent	LTL: delayed RTL: absent LPL: absent RPL: absent	LTL: normal RTL: normal LPL: depressed RPL: depressed	LTL: absent RTL: normal LPL: normal RPL: normal
Spinal reflexes	Decreased RTL	Normal	Normal	Absent LTL
Deep pain sensation	Normal	Not evaluated	Normal	Absent LTL
Cranial nerve function	Normal	Bilateral miosis Reactive pupils	Absent menace response OU Decreased PLRs OU	Normal
Other	Horner's Syndrome OD	Cheyne-Stokes respiration	Seizures	Non- detectable blood flow in the affected limb
Neuroanatomic localisation	Spinal cord C6 -T2 (right side)	Cerebral hemispheres Thalamus	Spinal cord T3 - L3 Cerebral hemispheres	Peripheral left radial and median nerves
Differential Diagnosis				
	IVD protusion/extrusion FCE Neoplasia Vascular/Ischemic process Inflammatory process Infectious process Inflammatory process Infectious process	Traumatic process Metabolic process Toxic process Neoplasia Vascular/Ischemic process Inflammatory process Infectious process	Vascular/Ischemic process Inflammatory process Infectious process Neoplasia	Traumatic process Vascular/Ischemic process Inflammatory process Infectious process Neoplasia
Work-up				
	CBC Biochemistry Urinalysis Thoracic radiographs Abdominal ultrasonography Anatomopathology	CBC Biochemistry Urinalysis Thoracic radiographs Abdominal ultrasonography Thoracic ultrasonography Histopathology	CBC Biochemistry Urinalysis Thoracic radiographs Abdominal ultrasonography Myelography CSF analysis	CBC Biochemistry Urinalysis Thoracic radiographs Abdominal ultrasonography Echocardiography Axillary doppler ultrasound
Diagnosis				
	Focal and unilateral cervical spinal cord necrosis (myelomalacia) due to acute ischemic and haemorrhagic process	Severe haemorrhages in the forebrain. Multiple fibrin and neutrophils deposits and lymphoplasmocytic infiltrates in the leptomeninges	Suspected multifocal vascular/Ischemic process in the spinal cord and forebrain.	Ischemic neuromyopathy LFL arterial thrombosis

LTL: left thoracic limb; LPL: left pelvic limb; RTL: right thoracic limb; LTL: left thoracic limb; CBC: complete blood count; CSF: cerebrospinal fluid; PLR: pupillary reflex; ID: intervertebral disc.

cord lesion (Table 2). An ophthalmologic exam was also performed and revealed severe bilateral papillary oedema, which was consistent with increased intracranial pressure (ICP). In order to decrease ICP, therapy with mannitol was initiated (1 g/kg of 20% solution over 30 min IV). Phenobarbital (4 mg/kg q12h IV) and diazepam (1 mg/kg IV as needed) were also added to the therapeutic regimen to control the seizures.

After initial stabilisation, a cerebrospinal fluid sample was obtained via lumbar tap and myelography was performed. Cerebrospinal fluid analysis displayed increased protein concentration (58.2 mg/dL), and myelography revealed absence of extradural compressive lesions, supporting the clinical suspicion of a CNS vascular process as the most likely explanation for the neurological signs.

The patient's condition improved progressively and it was discharged from the ICU 12 days after admission. Upon discharge from the hospital, the patient was ambulatory, and did not show any analytical abnormality.

Case 3

A 9-year-old female mixed-breed dog was admitted by the Emergency Service for a history of seizures, altered mentation and suspicion of a traumatic event by the referring veterinarian. On presentation the patient was stuporous and recumbent, and showed signs of hypoperfusion: pale mucous membranes, prolonged capillary refill time (>2"), hypotension (SAP: 70 mmHg) and hypothermia (36.5°C). Muffled cardiac sounds were found on auscultation, as well as exophthalmia of the left eye and a large hematoma in the orbitary region and sclera of the same eye. A neurological exam was also performed on presentation, and revealed multifocal neurologic injury (Table 2). Thoracic ultrasound confirmed the presence of moderate pericardial and pleural effusion, but abdominal ultrasound did not detect any serious abnormality. CBC results showed a regenerative anaemia and abnormalities in the differential leucocyte and platelet counts (Table 1). Schistocytes and acanthocytes were detected on blood smear examination. A coagulation profile revealed abnormalities in prothrombin time, activated partial thromboplastin time and fibrinogen concentration. The haemostatic and haematologic results were considered consistent with the presence of an on-going disseminated intravascular coagulation process.

Despite initial resuscitation therapy with intravenous fluids, oxygen, blood products, and pericardiocentesis to relief the pericardial effusion, the patient died a few hours later.

Post-mortem histopathological examination confirmed the presence of severe haemorrhages and fibrin deposits in the CNS (more profuse in the left temporal lobe and cerebral cortex), subcutaneous tissue, intestines, lungs, liver, heart, pericardium, pleural cavity and kidneys, with signs of massive acute tubular necrosis. No signs of an underlying clinical condition that could have predisposed the patient to the development of disseminated intravascular coagulation were found on necropsy.

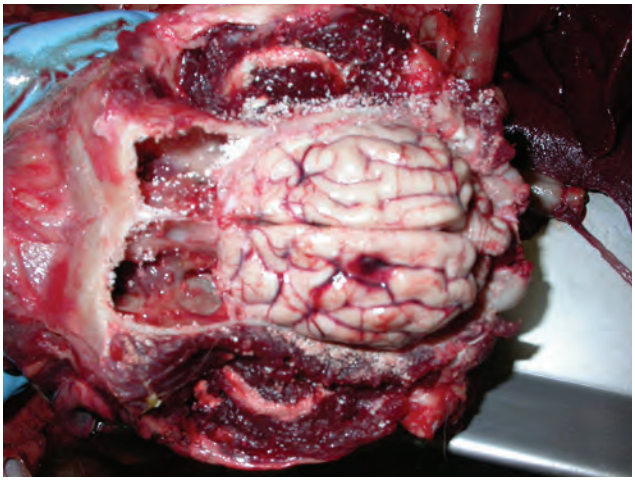

Fig. 2 The brain at necropsy (case 3).
Note the macroscopic haemorrhage of the cortex.

Case 4

A 10-year-old female German pointer was admitted by the Emergency Service for an acute, severely painful, left thoracic limb lameness. Previous history included a mammary carcinoma that had been surgically treated one year before. On physical examination, the patient showed a left thoracic limb lameness, but no other significant abnormalities except for the presence of small mammary nodules in the right M4 and M5. The affected limb was extremely painful on palpation, cold and without detectable blood flow on Doppler examination. A neurological examination confirmed the presence of a left thoracic limb monoplegia with absent spinal reflexes and decreased pain sensation. Lesion localisation was left median and radial nerves (spinal segments C7-T2) (Table 2).

CBC showed a mature neutrophilia. Serum biochemistry revealed increased creatine kinase concentration,

increased β-proteins, increased fibrinogen concentration and low antithrombin III activity values, consistent with a hypercoagulable state (Table 1). Thoracic radiographs displayed a nodular pattern in both lungs consistent with the presence of primary or metastatic neoplasia, and ultrasonographic examination of the axillary region could not detect any mass.

The patient was treated with lactated Ringer's solution (2ml/kg/h) supplemented with potassium chloride (20mEq/L), fentanyl (3-6µg/kg/h IV), ketamine (0.5mg/kg/h IV), aspirin (10 mg/kg PO q48 h), low-molecular-weight heparin (dalteparin 50 IU/kg q24h) and acepromazine maleate (0.01-0.05mg/kg IV, as needed) for sedation. Since the patient's condition did not improve despite treatment with analgesic drugs, the owner decided to euthanise the animal three days after admission. Post-mortem examination was not allowed.

Discussion

In this retrospective case series clinical conditions associated with disseminated intravascular coagulation were found in each case. Sepsis, malignancies, and trauma have been reported in the human and veterinary literature as potential causes of systemic activation of coagulation. [4,5,8,9,10] Nevertheless, there are specific variations in the pathogenesis of DIC caused by different underlying disorders.

In case 1 the presence of malignancies and clinical signs of hyperadrenocorticism due to the presence of adrenal tumours, could be the main factors explaining the overt DIC detected in the patient. Probably endothelial layer disruption, systemic inflammatory mediators and renal losses of antithrombotic factors (protein C, S, and ATIII) could explain the fulminant systemic bleeding tendency (hyocoagulation phase) detected in the patient and the subsequent MODS.

In case 2 the pyometra was thought to be the origin of the septic status of the patient. Bacterial infections, in particular septicaemia, are also a common clinical condition associated with DIC. The generalised activation of coagulation occurring in these cases is mediated by cell membrane components of micro-organisms (lipopolysaccharides or endotoxins) or bacterial exotoxins which cause a generalised inflammatory response through the activation of pro-inflammatory cytokines. Probably interactions between the inflammatory and coagulation pathways lead to a vicious circle that amplified the responses further and ended in a non-regulated activation of the systemic coagulation, causing increased thrombin generation, impaired function of the physiological anticoagulant pathway (reduction in ATIII levels, depression of protein C system, insufficient tissue factor pathway inhibitor-TAFI), impaired fibrinolysis (increased levels of plasminogen inhibitor-PAI-1, inhibited activity of TAFI) and redundant activation of the inflammatory pathway.[11]

In case 3 a traumatic incident was suspected, but the origin of the haemostatic disorder could not be elucidated due to the sudden death of the patient. Neurotrauma has been reported in the literature as a clinical condition associated with disseminated intravascular coagulation. [12] Thromboplastic substances are released from traumatised tissue into the circulation, precipitating the development of DIC.[13,14] In addition, the decreased blood flow induced by hypovolemia is a contributing factor. Clinical and laboratory evidence indicate that the passage of blood through traumatised tissue causes erythrocyte lysis, which triggers coagulation. Traumatic injuries are conditions associated with a systemic inflammatory response syndrome (SIRS) and cytokine activation, and are commonly associated with DIC in the dog. The release of cellular enzymes in combination with the presence of vasoactive substances in the circulation are thought to contribute to the induction and perpetuation of DIC. Since the fibrinolytic system is also activated both microthrombi and haemorrhage may be observed.

In case 4 the presence of pulmonary metastases from mammary tumours could explain, in conjunction with the coagulation profile, a hypercoagulable state but not an established DIC. In this patient, acute arterial thrombosis in forelimb or spinal cord vessels could explain the monoparesis, although this presentation is rarely detected in patients at risk because location of thrombi in the forelimbs is infrequent in dogs. Malignancies can potentially contribute to the development of thrombosis through biochemical effects as well as by mechanical effects on the venous blood flow.[15,16] However, the exact aetiology of thrombosis in association with cancer is unknown. Thromboembolism is thought to result from the interaction of various factors, such as the effects of synthesis of molecules with procoagulant activity by tumour cells (constitutive tissue factor expression), the

release of inflammatory cytokines (IL-1, IL-6; TNF-α), the interactions between tumour cells and monocytes, macrophages, platelets and endothelial cells, and associated complications (i.e. infections, dehydration) or acquired and/or inherited prothrombotic defects.

Definitive diagnosis of DIC remains controversial in both, people and animals. [1,5,17] Since no single laboratory tests or set of tests have been shown to be sensitive or specific enough to make a definitive diagnosis, a sensitive approach should be based on the combination of various laboratory findings in a patient with a clinical condition associated with DIC. Based on the current understanding of DIC, a proper diagnosis requires identification of active coagulation (thrombin production, fibrinogen consumption), on-going fibrinolysis (usually via detection of fibrin degradation products), endogenous anticoagulant depletion, and end-organ failure in a patient with an underlying disease associated with DIC. In human beings CNS dysfunction has been recognised as an end organ failure phenomenon and the pathogenesis of this dysfunction may involve decreased perfusion from hypotension, hypovolemia or microthrombosis.[18] In fact, any brain abnormality resulting from a pathologic process affecting its blood supply is defined as a cerebrovascular accident or stroke. Strokes, either ischemic or haemorrhagic, have been reported to be uncommon in small animals, but the actual true prevalence of strokes in dogs is unknown. On the other hand, the incidence of strokes in the human population is high and up to 15% of them have been reported as in-hospital strokes (embolic in a large proportion).

Many diseases have been associated with in-hospital strokes, and haematological factors can be an important mechanism for these. Higher than normal concentrations of procoagulant proteins have been measured in critically ill human patients, and changes in clotting factors have been noted in some post-operative settings.[19] Infections are increasingly implicated as precipitants of vascular events; therefore patients admitted for infections could be at increased risk of developing in-hospital strokes. Hypercoagulable states have become increasingly recognised for their role in thrombogenesis and may account for an important proportion of unexplained strokes in humans. Recent studies in the veterinary literature have suggested a possible relationship between haemostatic disorders and ischemic strokes in dogs.[20] Acute strokes occur associated to a variety of tumour-related conditions in human cancer patients, and embolic focal cerebral ischemia is the most frequent type of stroke.

Moreover hypercoagulability is more frequent in the adult cancer population than in the non-oncological population, and this situation could also play a role in these processes, and probably in small animals with cerebrovascular disease. There is controversy about the relevance of altered coagulation in cancer patients, but some studies report that intravascular coagulation is the second most common cause of stroke in human cancer patients.[21]

Intracerebral haemorrhage (ICH) is a subtype of stroke with high morbidity and mortality, accounting for about 15% of all deaths from stroke in human beings. [22] ICH can be classified based on its presumed cause. Primary ICH describes spontaneous bleeding without a precipitating event, and it is typically attributed to small vessel disease from hypertension in humans, whereas secondary ICH describes intracerebral bleeding from causes including trauma, haematological disorders, sympatomimethic drugs, structural lesions, thrombolytic therapy and anticoagulant use (aspirin, warfarin). The ICH location depends on the cause of bleeding. The most common sites of ICH are in the deep cerebral grey matter, affecting the putamen, thalamus, and caudate nuclei, although lobar haemorrhages are also frequent in humans.[22]

In addition, the development of coagulation abnormalities following head trauma is well recognised in the human literature,[12,13,14] with reports of incidences of coagulopathy in up to 64% of patients after head trauma. [23] Recent animal studies have suggested roles for clot-derived factors, as well as the initial physical trauma and mass effect from the haemorrhage for the neurological signs observed in these patients. The coagulation cascade (especially thrombin), haemoglobin breakdown products, and inflammation all play a role in ICH-induced injury. On the other hand, the incidence of spinal cord infarction in humans is very low compared with that of cerebral strokes (1.2% of all strokes),[24] but outcomes may be even more disabling. Spinal cord infarction can be ischemic or haemorrhagic. The aetiologies, with some exceptions, are similar to those for cerebral infarction. In humans, atherosclerosis and hypertension occur in approximately 50% of cases of spinal cord infarction,[24]

but many other disorders such as diabetes mellitus, hyperlipidaemia, cardiac arrest, vasculitis, infections, embolic events, and consequences of surgery or trauma have been associated with vascular events affecting the spinal cord. Causes of acute spinal cord infarction include thromboembolism, hypercoagulable states, vasculopathies, parasitic and septic embolisation. To the authors knowledge and in contrast to what it is described for human beings, there are few reports in the veterinary literature reporting coagulopathies as the cause of acute spinal cord ischemia.[25,26]

Finally, many hypercoagulation syndromes due to decreased AT-III, S-protein and C-protein levels, or increased fibrinogen, globulin or platelet concentrations have been associated with acute ischemic (thrombotic origin) limb events.[27]

The prognosis of cerebrovascular and spinal cord vascular accidents in humans is poor. The mortality for in-hospital strokes can be up to 54%,[19] reflecting the high morbidity of these and related processes in the human population. Specifically, in cancer patients the reported median survival time is only 4.5 months.[19] Hence 25% of patients die within 30 days after the stroke, and survival is strongly correlated with the initial neurologic disability.[19] A variety of factors influence the outcome of patients with cerebrovascular disease in these cases: severity of malignancy, metastatic disease, type of cancer, stroke aetiology, and degree of disability following stroke. Similar to what it is reported for ischemic strokes, ICH outcome is related to the size and location of the haemorrhage. In fact, haemorrhage volume is the strongest predictor of 30-day mortality, and coma presentation seems to be associated to mortality rates of 50 to 100% in human patients.[22]

In cases of acute limb ischemia, the neurological signs are also an indicator of severity of disease. In human patients, the prognosis is directly related to the presence of pre-existing collateral circulation, the aetiology of the occlusion, the duration of ischemia, the topography of the occlusion, and other causes of morbidity (renal failure, reperfusion injuries).[27]

Current diagnostic imaging techniques, such as diffusion-weighted magnetic resonance imaging are used routinely in human patients and demonstrate high sensitivity and sensibility for the diagnosis of stroke.[28] In veterinary medicine, MRI is the only diagnostic technique available to many clinicians, and the characteristics of most MR machines do not allow performance of diffusion techniques. In the authors opinion, early recognition of hypercoagulable states, use of antiplatelet agents and management of high risk patients with anticoagulants could decrease the rate of vascular accidents in critically ill patients and prevent some of the neurologic complications.

Whole blood assays (thromboelastography)[29] and new laboratory tests to detect early activation of haemostasis and prothrombotic states have been recently developed in human medicine.[5,30,31] In addition, there are new sensitive and specific markers for the activation of coagulation (thrombin-antithrombin III complex, soluble fibrin monomer, fibrinopeptides A and B, prothrombin fragments 1+2,) and the activation of fibrinolysis (D-dimer, plasmin-plasmin inhibitor complex, tissue-plasminogen activator, plasminogen activator inhibitor). The major drawback of these tests is proper interpretation and accessibility in order to obtain results early enough to be able to administer proper and early treatment and to prevent complications.

To conclude, an early and extensive evaluation of the haemostatic status in the 4 cases reported in this paper could have identified more effectively the actual and progressive phase of the haemostatic disorder (hypocoagulability and hypercoagulability), as well as the thrombotic tendencies of every particular case, thereby improving the prognoses in some of the cases.

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