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Clinical Practice

Special issue 'Infectious diseases, Part 3'

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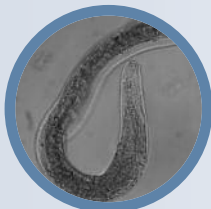
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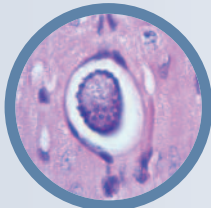
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Ten years' work on prevention of feline infectious disease

Infectious diseases may cause discomfort, pain and are often fatal – they, therefore, constitute an important welfare issue.

In 2005, the European Advisory Board on Cat Diseases (ABCD) held its first meeting in Lyon, France. The Board is an independent group of 17 veterinarians, from 11 European countries, with expertise in immunology, virology, vaccinology and/or feline clinical medicine. The ABCD's mission is to communicate scientific developments in feline infectious diseases and to establish a rational base for disease prevention and control. By the time the present issue of the *Journal of Feline Medicine and Surgery (JFMS)* is in your hands, the group will have met for the 25th time – improving existing guidelines, developing new ones and designing strategies to better protect cats from infectious disease.

This decade of concerted effort has produced 43 guidelines, 10 fact sheets and one brochure, which are intended to provide veterinarians with up-to-date knowledge on feline infectious diseases, and with recommendations for their management and prevention. The material has been published in Special Issues of the *JFMS* (July 2009, Volume 11, Issue 7 and July 2013, Volume 15, Issue 7).

The present Special Issue contains updates of the existing guidelines including the matrix vaccination guidelines, as well as articles on disinfection, on the risk of iatrogenic complications after blood transfusion and on feline injection-site sarcoma, which is frequently mentioned in the context of vaccination. These articles are followed by guidelines on some lesser-known infectious diseases. As some of the respective agents are emerging pathogens (eg, some streptococci) and/or carry a zoonotic potential (eg, some lungworms), these guidelines arm practitioners with the latest knowledge and make them aware of potential threats for cats (and humans). The articles were drafted during the meetings and are coauthored by all ABCD members, under the leadership of the respective first author.

The article about feline injection-site sarcoma was graciously coauthored by Michael Day, of the School of Veterinary Sciences, University of Bristol, UK.

The ABCD has included evidence-based medicine (EBM) qualifications where appropriate, to indicate the reliability of a statement or publication; Albert Lloret again carried this responsibility. Thanks go to Margaret J Hosie, who improved the manuscripts linguistically, and to Karin de Lange, the Board's secretary. Special thanks also go to the *JFMS* team for the great job of bringing the present Special Issue to completion.

The ABCD's work depends on the enthusiasm of the Board members and their unpaid time investment; however, it would

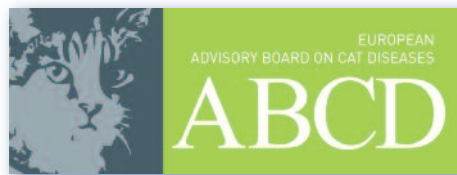
not have been possible without sponsorship.

In particular, Jean-Christophe Thibault, of Merial, must be thanked for his commitment to respect the Board members' independence. Florence Kahn-Ramos managed the Board's logistics with humour and

expertise. It is also Merial which sponsored the 'ABCD Merial Young Scientist Award (AMYSA)' for the eighth time this year.

Which leads us to thoughts about the future of the ABCD. It will be an ongoing task to keep all guidelines at the 'state of the art' level of knowledge and, where appropriate, to produce new ones. In the past, our recommendations were aimed at veterinarians. For the future it is our goal to reach out also to cat owners, to make them aware of the practical measures (especially vaccinations) which their veterinarians can offer. It will be important to obtain more information about the prevalence of feline infectious diseases in Europe, in order to apply the most effective and efficient preventive measures. The ABCD is prepared to give input, support and to carry out such projects.

And, finally, infectious diseases may cause discomfort, pain and are often fatal – they



For a decade, the ABCD has been communicating scientific developments concerning the prevention and control of feline infectious diseases

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- ❖ **Tim Gruffydd-Jones** Head, The Feline Centre, Professor in Feline Medicine, Bristol University, UK.
- ❖ **Katrin Hartmann** Professor and Head, Clinic of Small Animal Medicine, Ludwig Maximilians University, Munich, Germany.
- ❖ **Marian C Horzinek** Past Chairman ABCD, webmaster



- www.abcdcatsvets.org, former Head, Department of Infectious Diseases, Division of Virology, Faculty of Veterinary Medicine; Director, Graduate School Animal Health; Director, Institute of Veterinary Research, Utrecht, the Netherlands.
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- ❖ **Albert Lloret** Clinical instructor, Veterinary Teaching Hospital, Universitat Autònoma de Barcelona, Spain.
- ❖ **Hans Lutz** Former Head, Clinical Laboratory, and Professor Emeritus, Faculty of Veterinary Medicine, University of Zurich, Switzerland.
- ❖ **Fulvio Marsilio** Professor of Infectious Diseases of Animals, Faculty of Veterinary Medicine, University of Teramo, Italy.
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- ❖ **Etienne Thiry** Full Professor and Head, Veterinary Virology and Animal Viral Diseases, Faculty of Veterinary Medicine, University of Liège, Belgium.
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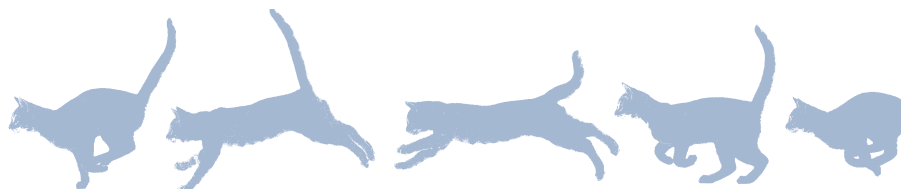
Some of the disease agents discussed in the current guidelines are emerging pathogens and/or have zoonotic potential.

therefore constitute an important welfare issue. This is why the ABCD has recently forged a partnership with a dedicated European-based cat welfare organisation CAROcat (www.carocat.eu). We realise that health is not the only issue in welfare and wellbeing, but it is certainly an important one. The ABCD is prepared to continue its work to protect and improve feline health and welfare.

Marian C Horzinek,
Past Chairman ABCD; Webmaster;
Editor in Chief Veterinary Sciences Tomorrow
Karin Möstl,
Chairwoman ABCD

Addendum

Karin Möstl writes: When reviewing the first 10 years of the ABCD, the founding chairman of the Board, Prof. Dr.Dr.h.c.mult. Marian C Horzinek, must be acknowledged. He brought the idea of the ABCD to life and managed it during its first 10 years. At his own request he recently stepped down and handed his position over to me, but he will stay on as Board member. It is a demanding task to follow Marian in this function. On behalf of the ABCD Board members and as new Chairwoman, I want to express my thanks to the past chairman and friend Marian.



SOMETHING OLD, SOMETHING NEW

Update of the 2009 and 2013 ABCD guidelines on prevention and management of feline infectious diseases



Karin Möstl, Diane Addie, Corine Boucraut-Baralon, Herman Egberink, Tadeusz Frymus, Tim Gruffydd-Jones, Katrin Hartmann, Margaret J Hosie, Albert Lloret, Hans Lutz, Fulvio Marsilio, Maria Grazia Pennisi, Alan D Radford, Etienne Thiry, Uwe Truyen and Marian C Horzinek

Overview: The ABCD has published 34 guidelines in two special issues of the *Journal of Feline Medicine and Surgery (JFMS)*: the first in July 2009 (Volume 11, Issue 7, pages 527–620) and the second in July 2013 (Volume 15, Issue 7, pages 528–652). The present article contains updates and new information on 18 of these (17 disease guidelines and one special article ‘Prevention of infectious diseases in cat shelters’). For detailed information, readers are referred to the guidelines published in the above-mentioned *JFMS* special issues.

European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest full versions of the disease guidelines updated in this article are available at www.abcdcatsvets.org and www.abcd-vets.org

Feline panleukopenia

Canine parvovirus type 2 (CPV-2), which is closely related to feline panleukopenia virus (FPV), was described in 1978 as a new parvovirus (cited in Carmichael¹). It evolved from FPV with the acquisition of five or six amino acid changes in the capsid protein² and does not infect cats. However, during further adaptation to the dog, which most likely occurred in the raccoon, the virus underwent amino acid changes that made the mutated virus bind more efficiently to the canine cellular receptor, while retaining the ability to infect cats.^{3,4} This led to the emergence of the new type, CPV-2a, which contains a series of further mutations including those at amino acid 426 of the VP2 that determine the antigenic types 2a, 2b and 2c. The parvoviruses currently circulating in dog populations worldwide (genetically and antigenically defined as types CPV-2a, -2b and -2c) can infect cats and may even cause disease.^{5–7}

However, CPV infections of cats are rare in Europe and the USA, and the virus has only sporadically been found in diagnostic material.⁶ CPV was isolated from feline peripheral blood lymphocytes after numerous blind passages, and viral DNA was demonstrated subsequently by PCR.⁸ Recently, however, a case of CPV-2c infection in a cat with severe clinical disease was described in Portugal.⁹

During the evolution of FPV to CPV-2 with its various antigenic types, neutralising epitopes have become modified such that cross-neutralisation by FPV antisera is markedly lower against the newer viruses.¹⁰

Persistent infections with viral shedding are rare; using PCR, healthy cats have been found positive for FPV in faeces over weeks,¹¹ and CPV-2 viruses could be isolated from the faeces of healthy cats in the UK in two shelters.¹² It is unknown whether these findings are of epidemiological significance.

Feline panleukopenia: ABCD guidelines on prevention and management

The feline panleukopenia guidelines that the present article is updating were published in *J Feline Med Surg* in 2009 (11: 538–546) and updated in 2013 (15: 530–531). This further update has been compiled by Uwe Truyen.



The parvovirus sequences encountered in wild carnivores (pumas, coyotes, raccoons, and others) revealed a wide genotypic variation. This finding suggests infection of predators by their parvovirus-infected prey, and hence a new route of infection.¹³

Following intrauterine infection, FPV antigen persists in the cerebellum of kittens for weeks.¹⁴

Detailed information on the prevention and management of parvovirus infection in cats is provided in the ABCD guidelines¹⁵ and a previous update.¹⁶

Feline herpesvirus infection

Feline herpesvirus (FHV), together with feline calicivirus, is involved in the feline upper respiratory tract syndrome. In addition, FHV has been recognised as the most important cause of corneal ulceration, both superficial and deep, and in particular of dendritic ulcers. The infection becomes latent, allowing lifelong persistence of the virus, which is sporadically interrupted by episodes of viral reactivation and re-excretion. Thiry et al¹⁷ and Horzinek et al¹⁶ presented a table summarising recommendations for treatment of acute FHV ocular disease.

The amino acid L-lysine has been proposed for systemic treatment, to be administered as a bolus, separate from food. No reports of side effects have been published, but findings on efficacy are conflicting.^{18–25} Cave et al²⁶ investigated the effects of physiological concentrations of L-lysine on the in vitro replication of FHV at L-arginine levels sufficient to maintain cell growth. FHV was not inhibited at any L-lysine concentration studied. The in vivo efficacy of L-lysine treatment on primary and recurrent FHV infection is unknown.

Feline leukaemia virus infection

Feline leukaemia virus (FeLV) is a gamma retrovirus affecting domestic cats worldwide. It also infects small wild cats including *Felis silvestris*, European and Iberian lynxes, Florida panthers and the Chilean wildcat (*Leopardus guigna*).^{27–30} The prevalence of FeLV infection in Europe and North America has greatly diminished. In individually kept cats it is low; often, but not everywhere, less than 1%.^{31–35}

After infection of bone marrow cells, viraemia develops within a few weeks. Mainly lymphocytes and monocytes are infected, whereas later infection involves mostly neutrophils.³⁶ Viraemia may be overcome by the immune system (transient viraemia) in some cats,³⁷ whereas others

develop a persistent viraemia. A smaller proportion (~5%) exhibits an atypical course of infection, displaying antigenaemia, but no or only low-level viraemia.^{38,39} A cat that has overcome viraemia remains latently infected. Reactivation may occur; it is not clear how often this happens under field conditions, but it is believed to be rare. Generally, up to 10% of all feline blood samples submitted to a laboratory prove to be provirus-positive and p27-negative; since FeLV may be reactivated in some of these cats, they should be considered latently infected.^{35,40,41} Probably no cat can clear an FeLV infection from all cells.

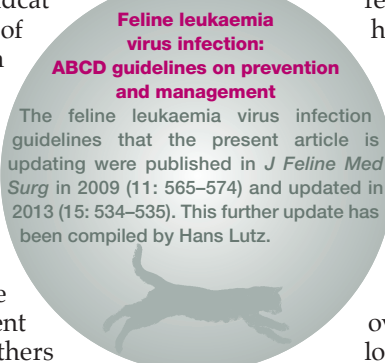
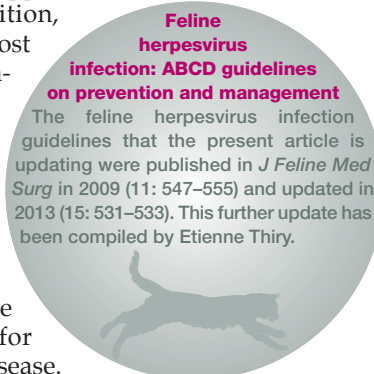
Experimentally, susceptible kittens can be protected from FeLV infection after passive immunisation with high-titred specific antisera.³⁸ This observation suggests that antibodies have a role in protection; however, once persistent viraemia has become established, treatment with neutralising monoclonal antibodies to FeLV has proven ineffective.⁴²

Recently, seroconversion was observed in cats as the sole evidence of FeLV infection.⁴³ These cats had been exposed once intranasally to low doses of FeLV (10,000 FFU). Since some of them seroconverted, it was concluded that the virus had replicated somewhere to sufficient levels to trigger antibody synthesis. The observation that PCR analysis of several organs was negative indicates that further replication must have been controlled by the immune system.

In most situations, individual cats are tested for FeLV infection. However, when the cost of testing is a limitation, pooled saliva samples can be used to detect FeLV RNA; the RNA PCR is sufficiently sensitive to detect a single infected cat in a pool of up to 30 samples. This approach may be chosen when screening multicat households.⁴⁴ While all viraemic cats are positive for FeLV RNA in saliva, a few may shed FeLV RNA in saliva, but are not (yet) viraemic or antigenaemic.⁴⁵

The observation that antibodies can develop as the sole parameter of exposure to FeLV⁴³ led to the examination of various FeLV antigens to assess their diagnostic potential to detect antibodies. In contrast to published results,⁴⁶ a recombinant preparation of FeLV p15(E) proved highly effective for the detection of antibodies induced by FeLV infection and thus for the diagnosis of a previous infection.⁴⁷

The HIV integrase inhibitor raltegravir was found to inhibit FeLV replication in vitro.⁴⁸ The drug is tolerated well by cats, and within 1 week leads to a marked reduction in viral loads. However, this is not sufficient for the immune system to control the viraemia, and treatment must be continued over long periods in order to maintain low viral loads and prevent disease [EBM grade III].⁴⁹



In many experiments it was shown that no FeLV vaccine provides complete protection nor prevents infection. Cats that overcome p27 antigenaemia without exception test provirus-positive in blood, and also test positive for viral RNA in plasma, although at much lower levels than persistently viraemic cats [EBM grade III].⁵⁰ These experiments confirm that FeLV vaccination does not induce sterilising immunity and does not protect cats from infection. However, cats vaccinated with conventional, adjuvanted, whole inactivated virus vaccines did not show p27, viral RNA or DNA after a low-dose challenge with the subgroup A virus FeLV A/61E.⁵¹ Various factors may have played a role: the challenge virus was used at a very low dose (10,000 TCID₅₀ injected once, intraperitoneally), the assays used were less sensitive than those used by Hofmann-Lehmann et al,⁵⁰ and the cats had a different genetic background. Testing for FeLV in internal organs would have resulted in observations as reported by Major et al.⁴³ Thus, the proposition remains valid that vaccination against FeLV protects cats from disease but not from infection.

Until recently, no data had been published to demonstrate that immunity lasts longer than 1 year after primary vaccination; most vaccine manufacturers therefore recommend annual boosters. However, the demonstration that one FeLV vaccine provided immunity for at least 2 years⁵² [EBM grade II] suggests that this may also apply to other vaccines. Combined with the lower susceptibility of adult cats to FeLV infection, the ABCD recommends that, in cats older than 3 years, a booster immunisation every 2–3 years is sufficient.

Wherever possible, cats entering a shelter should be kept in quarantine for at least 3 weeks, if not (re)homed sooner. All incoming cats (at least in shelters that allow contact between cats after the quarantine period) should be screened for FeLV antigen and feline immunodeficiency virus (FIV) antibody, and ideally also for FeLV antibody.⁴⁷ Antigen-negative but antibody-positive results suggest that the cat is not viraemic/antigenaemic, but may be latently infected. Therefore, PCR for FeLV DNA should additionally be performed. If the PCR shows a high FeLV DNA load, this cat should prudently be considered latently infected; those cats should best be placed in a home without other cats for several months. If only an FeLV antigen test is performed, cats testing negative should ideally be retested 6 weeks later (and kept in quarantine for this time period), as it may take 4–6 weeks after infection for the test to return positive results. To prevent (re)activation of other infections caused by the stress of entering the shelter, newcomer cats should be kept isolated

and observed for clinical signs. After quarantine, they can be introduced into small groups of healthy cats. FeLV antigen- and/or FIV antibody-positive cats should be kept separate, but may be housed together with other retrovirus-positive cats, and adopted out to suitable homes as soon as possible.

The ABCD does not recommend euthanasia of healthy FeLV-positive cats. However, if no adequate home can be found, if separation from the rest of the population is impossible, or if the cat is sick, euthanasia should be considered. Detailed recommendations are provided in the ABCD guidelines 'Prevention of infectious diseases in cat shelters'.⁵³

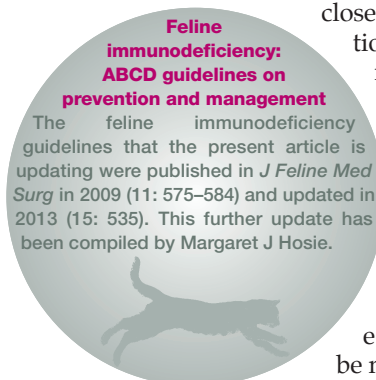
Detailed information on the prevention and management of feline leukaemia is provided in the ABCD guidelines⁵⁴ and a previous update.¹⁶

Feline immunodeficiency virus infection

It is generally accepted that feline immunodeficiency virus (FIV) infection can induce clinical signs of immunodeficiency, leading to opportunistic infections or lymphomas, and clinical signs consistent with immunodeficiency in natural infection have been documented.⁵⁵ However, in some cats the clinical signs are mild, which likely reflects both heterogeneity among circulating field isolates as well as host factors, and it has been reported that many FIV-infected cats have a normal life expectancy.^{56–58} Therefore, surrogate markers are required to provide an objective assessment of FIV progression in individual cats. Recently it was shown that viruses dominating in early infection display a distinct receptor usage phenotype and that the emergence of viruses with an altered receptor usage phenotype coincides with the onset of immunodeficiency.⁵⁹ Accordingly, viral phenotyping might assist in the clinical staging of individual cats diagnosed with FIV infection.

FIV infection was found to be prevalent in a survey of four large-scale hoarding situations;⁶⁰ this high prevalence was probably related to the fact that the cats were living in close confinement under stressful conditions, and exhibiting aggressive behaviour. Therefore, it is recommended that cats should be tested for FIV infection at the time of seizure during hoarding investigations, as the results will influence housing decisions, medical care and adoption options.

FIV infection is also common in rescue shelters and it is recommended that all cats in rescue centres should be neutered and kept indoors, in order to



reduce the risk of territorial aggression, which can result in penetrating bite wounds and consequently FIV transmission. This recommendation is supported by studies linking cat bite wounds and abscesses with FIV infection.^{61,62} A recent survey of cats in a rescue shelter, in which FIV-infected cats were housed together with uninfected cats, found no evidence of FIV transmission, in spite of the cats having unrestricted access, and sharing food and water bowls, litter trays and bedding for several years.⁶³ However, it is possibly significant that the cats had been neutered before entering this shelter and the median age of the uninfected cats was 4 months; kittens are a low risk group for FIV infection⁶⁴ because territorial aggression has not yet developed. Similarly, neutered cats are less likely to display territorial aggression than intact cats and, therefore, FIV transmission might be more likely to occur in rescue centres housing older cats, especially if those cats exhibit aggressive behaviour.

Detailed information on the prevention and management of feline immunodeficiency virus infection is provided in the ABCD guidelines⁶⁵ and a previous update.¹⁶

Rabies

Rabies is caused by a *Lyssavirus*, a member of the *Rhabdoviridae* family. The genus *Lyssavirus* contains 12 species: rabies virus, Mokola virus, Lagos bat virus and Duvenhage virus from Africa, European bat lyssaviruses (EBLV) 1 and 2, Australian bat lyssavirus, and five recently recognised species (International Committee on Taxonomy of Viruses, 2012). Each of these viruses is considered capable of causing a rabies-like disease in animals and humans.

Various control measures (eg, vaccination of wildlife, immunisation of dogs and cats, diagnostic measures, control of pet movements) eliminated rabies from large regions of Europe, especially its western and northern parts. In rabies-free countries, however, though sporadic, the illegal importation of pets from regions where this disease is endemic poses an increasing risk.⁶⁶ Rabies was recently recognised in a kitten imported into France from Morocco,⁶⁷ and a few cases in dogs were documented in Europe recently.

As a result of the mass vaccination of dogs in many areas affected by wildlife rabies, cats have become the companion animal species most commonly reported as rabid, as is the case in many states of the USA.⁶⁸ In a recent report from Pennsylvania, among 2755 rabid animals with reported human exposure, as

many as 799 (29.0%) were free-ranging cats, whereas only 57 (2.1%) were dogs.⁶⁹

Because of the public health risk associated with susceptible domestic cats becoming infected following exposure to rabid wild or domestic animals, all cats with outdoor access in endemic areas should be vaccinated. The vaccine should be administered in accordance with local or state regulations. In countries where rabies is absent, rabies vaccination is indicated when a cat moves or travels to an area where rabies is endemic.

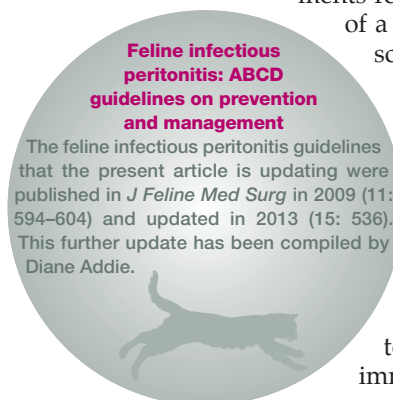
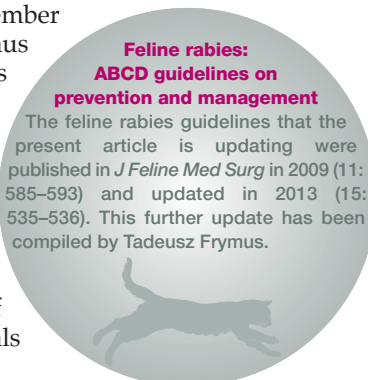
EU Regulation 576/2013 established new rules for the non-commercial movement of pet animals (dogs, cats and ferrets) between EU countries as of 29 December 2014. According to these rules, all such cats should be identified by microchip (or tattoo, if applied before 4 July 2011) and vaccinated against rabies; a 21-day waiting period following primary vaccination is required. This means that for the purpose of travel, cats generally must be at least 15 weeks old, as 12 weeks is the minimum age for rabies vaccination. Some countries accept younger animals without rabies vaccination under certain conditions, but most do not (for details see http://ec.europa.eu/food/animal/liveanimals/pets/nat_rules_dogscatferret_en.htm). According to the recent pet movement regulation, serological testing for rabies neutralising antibodies is no longer required before entry into any EU member state.

Detailed information on the prevention and management of rabies in cats is provided in the ABCD guidelines⁷⁰ and a previous update.¹⁶

Feline infectious peritonitis

Given the number of major recent developments in the field of feline coronavirus (FCoV) and feline infectious peritonitis (FIP), fully updated ABCD guidelines on FIP will be published in the near future. For the purpose of this interim update, some key developments in FIP diagnosis and treatment are outlined below.

Among the most interesting of the developments relating to FIP diagnosis is the advent of a commercially available reverse transcription PCR (RT-PCR) test which distinguishes mutations on the spike of type I FCoV that are associated with the development of systemic spread of the virus.⁷¹ There is a question of this test not being as sensitive as conventional FCoV RT-PCR, not only because it does not detect type II FCoVs, but also because the spike protein is the protein most subject to evolutionary immune pressure, and so the spike gene



is the most variable of the coronavirus genes, and thus primers may not bind, giving a false negative result.

It is the view of the ABCD that RT-PCR is a preferred method of FIP confirmation for effusions, over immunofluorescence of macrophages, which is less widely available and more prone to human error.

In a comparison of commercially available FCoV antibody tests, 16 applications of FCoV antibody tests were considered: sensitivity was deemed extremely important because many uses of FCoV antibody tests involve rule out a diagnosis of FIP or FCoV infection.⁷² An in-house ELISA fared best, both in terms of sensitivity/specificity and quantity of sample required (only 5 µl). The most sensitive rapid immunomigration (RIM) tests were identified. RIM tests fared best in terms of rapidity of result, which is useful when screening a healthy cat, but they may give false negative results if used to support FIP diagnosis on effusion samples, due to binding of virus to antibody, rendering the antibody unavailable for the test antigen.⁷³

For treatment, Polyprenyl Immunostimulant (Sass & Sass) is only for use in non-effusive FIP, having no efficacy in effusive FIP. One study of three non-effusive FIP cases reported survival of 14 months for one cat and over 2 years for two cats [EBM grade IV].⁷⁴ However, a conference abstract report of 58 cats showed only 22% survival at 6 months, with only one cat surviving at a year [EBM grade III].⁷⁵ A placebo-controlled study is required.

Gil et al⁷⁶ showed that in cats with feline

leukaemia virus or feline immunodeficiency virus infection, feline interferon omega therapy resulted in a tendency towards reduced FCoV shedding [EBM grade III].

Chloroquine inhibits FCoV replication in vitro and has anti-inflammatory effects in vivo.⁷⁷ However, reported survival times were only around 30 days at best and the drug increased alanine aminotransferase levels. Thus, the ABCD does not recommend its use until further studies have demonstrated significant benefit.

A placebo-controlled double blind trial on propentofylline showed no efficacy [EBM grade I].⁷⁸

Detailed information on the prevention and management of feline infectious peritonitis is provided in the ABCD guidelines⁷⁹ and a previous update.¹⁶

Influenza A virus infection in cats: ABCD guidelines on prevention and management

The influenza A virus infection in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2009 (11: 615–618) and updated in 2013 (15: 537). This further update has been compiled by Etienne Thiry.



Influenza A virus infection in cats

Recommendations for the prevention of influenza A H5N1 and H1N1 infections in cats were published in the ABCD guidelines⁸⁰ and subsequently updated.¹⁶

Cats were recently found to be susceptible to the H3N2 and H5N2 influenza viruses, the agents of canine influenza in Asia, leading to morbidity and mortality in cats for H3N2, but only to mild clinical signs for H5N2.^{81,82} Experimental infection of cats with the recent H5N8 influenza A virus was successful, but remained subclinical.⁸³

EBM ranking used in this article

Evidence-based medicine (EBM) is a process of clinical decision-making that allows clinicians to find, appraise and integrate the current best evidence with individual clinical expertise, client wishes and patient needs.

This article uses EBM ranking to grade the level of evidence of various statements and recommendations on a scale of I to IV as follows:

- ✦ **EBM grade I** This is the best evidence, comprising data obtained from properly designed, randomised controlled clinical trials in the target species (in this context cats);
- ✦ **EBM grade II** Data obtained from properly designed, randomised controlled studies in the target species with spontaneous disease in an experimental setting;
- ✦ **EBM grade III** Data based on non-randomised clinical trials, multiple case series, other experimental studies, and dramatic results from uncontrolled studies;
- ✦ **EBM grade IV** Expert opinion, case reports, studies in other species, pathophysiological justification. **If no grade is specified, the EBM level is grade IV.**

Further reading

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Feline viral papillomatosis

Papillomaviruses cause cutaneous lesions in man and several animal species, including cats. The ABCD has published guidelines on the prevention and management of feline viral papillomatosis.⁸⁴

In each host, including cats,⁸⁵ different papillomavirus (PV) types exist. To date, four feline PVs from domestic cats have been fully sequenced and classified.⁸⁵

These viruses were designated as *Felis domesticus* PVs (FdPVs), but recently changed to *Felis catus* PVs (FcaPVs).⁸⁶

A clear association between papillomavirus DNA (the *Felis domesticus* papillomavirus 2 – FdPV-2) and squamous cell carcinomas (SCCs)

Feline viral papillomatosis: ABCD guidelines on prevention and management

The feline viral papillomatosis guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 560–562). This update has been compiled by Herman Egberink.



was reported; DNA was detected in all 20 Bowenoid in situ carcinomas (BISCs) examined, and in 17 of 20 cases of invasive SCC.⁸⁷ However, FdPV-2 DNA was also present in 52% of normal skin swabs.⁸⁸ Although FdPV-2 has been detected most frequently in BISCs and SCCs, other PV types have also been identified. Recently, a novel PV type, designated FcaPV-3, was detected in a feline BISC.⁸⁶ In one study, 50% of the sequenced PV DNA was most closely related to human PV DNA.⁸⁹ In another study, PV DNA could not be detected in any of 30 oral SCC samples screened,⁹⁰ which is at variance with earlier observations.

Bartonella species infection in cats

The ABCD guidelines on *Bartonella* species infection in cats⁹¹ list various species and subspecies of *Bartonella* that are confirmed or potential human pathogens: *B bacilliformis*, *B quintana*, *B elizabethae*, *B grahamii*, *B henselae*, *B clarridgeiae*, *B koehlerae*, *B vinsonii* subspecies *berkhoffii*, *B vinsonii* subspecies *arupensis*, *B washoensis* and *B asiatica*. Additionally *B rochalimae* should now be included, for which reservoir hosts may be raccoons, coyotes, red foxes and cats. The vectors are fleas, and humans may be accidental hosts.

The important role of fleas in the transmission of *B henselae* and *B clarridgeiae* among cats has been demonstrated. Using a quantitative molecular approach, *B henselae* DNA was detected in both fleas and their faeces for the entire life span of the arthropod (ie, 12 days) starting from 24 h after the blood meal.⁹²

Recently, the possible role of several bat fly species (*Nycteribiidae*) as *Bartonella* vectors has been studied. It remains a subject of debate, but a reservoir function should be considered in addition to pathogenic, parasitic or mutualistic interactions.⁹³

The role of *Bartonella* as a pathogen after natural transmission is still unclear; however, *B henselae* was found in association with pyogranulomatous myocarditis and diaphragmatic myositis in two cats.⁹⁴

For laboratory diagnosis, a real-time PCR and pyrosequencing-based algorithm was described that allowed rapid differentiation of at least 11 medically relevant *Bartonella* species within 5 h from receipt of the specimens.⁹⁵

Coxiellosis/Q fever in cats

Q fever is a zoonotic disease caused by *Coxiella burnetii*. ABCD guidelines on prevention and management of coxiellosis/Q fever in cats have been published.⁹⁶

Bartonella species infection in cats: ABCD guidelines on prevention and management

The *Bartonella* species infection in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 563–569). This update has been compiled by Fulvio Marsilio.



Francisella tularensis infection in cats: ABCD guidelines on prevention and management

The *Francisella tularensis* infection in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 585–587). This update has been compiled by Maria Grazia Pennisi.



Farm animals and pets are the main reservoir hosts of the bacterium, and exposure of cats is relatively common. In the UK, a seroprevalence as high as 61.5% was recently demonstrated.⁹⁷

A Q fever outbreak among veterinary hospital personnel was linked to a caesarean section on a parturient queen. The breeding queen was *C burnetii* seropositive, and antibodies were demonstrated in 26% of the cats living in the same cattery.⁹⁸

Coxiellosis/Q fever in cats: ABCD guidelines on prevention and management

The coxiellosis/Q fever in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 573–575). This update has been compiled by Herman Egberink.



Francisella tularensis infection in cats

Tularaemia is a potentially fatal zoonosis. Various clinical syndromes occur, but most patients either present with a localised infection of the skin and draining lymph nodes (ulceroglandular form) or with a systemic infection (typhoidal tularaemia). Oropharyngeal and pneumonic forms are rare.

The risk of acquiring the infection from cats is low, but exists for owners of cats with outdoor access, as well as for veterinarians and technicians.⁹⁹ Regular parasitocidal treatment to prevent tick infestations is recommended for outdoor cats. When handling animals with suppurative or draining skin or lymph node lesions in endemic areas, gloves and goggles should be worn. Gloves should be also be worn when examining the oral mucosa. Handling of diagnostic samples by laboratory staff requires adherence to appropriate biosafety procedures.¹⁰⁰

Detailed information on the prevention and management of tularaemia in cats is provided in the ABCD guidelines.¹⁰¹

Mycobacterioses in cats

In recent years, awareness of the importance of mycobacterial infections in humans and animals has been increasing. ABCD guidelines on the prevention and management of mycobacteriosis in cats were published in 2013.¹⁰²

An unusual cluster of *Mycobacterium bovis* infection in cats was recently reported from the UK. Cat-to-cat transmission was suspected, and two humans became infected.¹⁰³ Also nosocomial infection was reported in a cluster of cases that had attended a veterinary practice in Ireland.¹⁰⁴

For diagnostic purposes, the PCR is recommended; it should ideally be performed on fresh tissue samples, but fixed stained smears and formalin-fixed paraffin-embedded tissues can be used with good sensitivity.¹⁰⁵

The zoonotic risk has to be considered when planning therapeutic measures.¹⁰² It is complicated by the fact that confirmation of the mycobacterial species takes time, and antibiotic therapy requires several months. Therefore, euthanasia rather than treatment should be considered as a sensible course of action, in view of the public health implications and the prognostic uncertainties of treatment.

For the tuberculosis complex and non-tuberculous mycobacteria (NTM) groups, double or triple therapy is currently recommended: rifampicin (10–15 mg/kg q24h), plus a quinolone (marbofloxacin [2 mg/kg q24h] or pradofloxacin [3–5 mg/kg q24h]), plus a macrolide (clarithromycin [125 mg/cat q24h or 7–15 mg/kg q24h] or azythromycin [5–15 mg/kg q24h] for 6–9 months. Ideally, the three drugs should be administered during an initial phase of 2 months, followed by two of the drugs for 4–7 months [EBM grade III].^{106,107}

The newer fluoroquinolones (moxifloxacin and pradofloxacin) might be more effective than the older ones.^{108,109} Unpublished clinical experience suggests that pradofloxacin is a good choice; in localised disease, pradofloxacin would be a good initial treatment pending species confirmation [EBM grade IV].¹¹⁰

Treatment of NTM infections is ideally based on culture and susceptibility tests for each case, as different mycobacterial species or strains may have different antibiotic sensitivity. However, this is not always possible, as specific culture systems are unavailable or results take too long.

Disseminated *M avium-intracellulare* complex (MAC) infections usually respond poorly to treatment, and old generation quinolones are not very effective.^{111–113} The recommended first choice treatment is clarithromycin with clofazimine (4–8 mg/kg q24h) or rifampicin or doxycycline (5–10 mg/kg q12h) based on the few cases reported with good outcomes [EBM grade IV].^{114–116} Limited clinical experience with pradofloxacin suggests that it is more effective than the older fluoroquinolones.¹¹⁰

Most cats with feline leprosy can be cured by surgery (small lesions), and treatment with combinations of rifampicin, clofazimine, clarithromycin and pradofloxacin for several months [EBM grade IV].^{117,118} Spontaneous remission has been documented in one cat.¹¹⁹

Keeping the cat indoors and avoiding contact with wild rodents are the only measures for preventing mycobacterial infection.

Potential zoonotic risk

All members of the TB complex are potentially zoonotic, including *M microti*. However, the risk of transmission from cats (and dogs) to humans is low, as cats are spillover hosts.^{107,120} In a recent cluster of feline cases of *M bovis* infection in the south west of England, two people became infected after having been in contact with the cats.¹⁰³ The Public Health Agency in England then changed the risk level of transmission from negligible to low (www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317140243205).

Euthanasia or treatment of cats with confirmed *M bovis* infection should be a consensus decision between the owner and the veterinarian, but due to the risk of cat-to-human transmission (see box above) and antimicrobial resistance, euthanasia has been suggested by some authorities and experts (www.bva.co.uk/News-campaigns-and-policy/Newsroom/News-releases/Updated-statement-on-TB-in-cats/). Similarly, euthanasia might be considered after infection with any of the other potentially zoonotic species (*M tuberculosis*, *M microti* and *M avium*).

Cryptococcosis in cats

Feline cryptococcosis occurs rarely or sporadically, but *Cryptococcus gattii* has a worldwide distribution with a high prevalence along the Pacific coast of North America. It has been reported also from Brazil,¹²¹ and in Europe from Austria, Denmark, France, Germany, Greece, Italy, the Netherlands, Portugal, Spain, Sweden and the United Kingdom.¹²² *C neoformans* var *grubii* also has a worldwide distribution and is commonly isolated from affected individuals of various animal species. *C neoformans* is considered a cosmopolitan opportunistic pathogen in human urban populations, whereas *C gattii* is a true pathogen, more prevalent in rural areas.¹²³

Feline cryptococcosis caused by *C neoformans* or *C gattii* is clinically indistinguishable.

This disease can manifest after a long incubation period¹²⁴ and presents in different clinical forms, including the nasal form, central nervous system (CNS) form (which can derive from the nasal form or occur independently), the cutaneous form and the systemic form.¹²⁵ CNS involvement most likely arises following local dissemination through the cribriform plate.¹²⁶ Recently, otitis interna following systemic spread of the fungus was reported.¹²⁷

Detailed information on the prevention and management of cryptococcosis in cats is provided in the ABCD guidelines.¹²⁵

Cryptococcosis in cats: ABCD guidelines on prevention and management

The cryptococcosis in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 611–618). This update has been compiled by Maria Grazia Pennisi.



Mycobacterioses in cats: ABCD guidelines on prevention and management

The mycobacterioses in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 591–597). This update has been compiled by Albert Lloret.



Sporotrichosis in cats

Sporotrichosis is a deep cutaneous mycosis caused by the dimorphic saprophytic fungus *Sporothrix schenckii*. *S schenckii* is not a unique species but a complex containing at least four distinct species. Most feline cases reported from Brazil are caused by *S brasiliensis*.¹²⁸

The prevalence of the disease varies markedly between regions. In Central and South America, it represents the most common deep mycosis. In Brazil it is endemic, and an important epidemic affecting humans, cats and dogs was reported in Rio de Janeiro.^{129–131} More than 2000 feline cases over 7 years have been seen by just one institution, showing the magnitude of the epidemics and the challenges of disease control.¹³²

Using histopathology and staining procedures, the organisms are readily visualised. Cats with few and well organised granulomas tend to have low numbers of fungal organisms in the lesions. Cats in poor general condition and with large numbers of granulomas have the greatest numbers of fungal organisms.¹³³

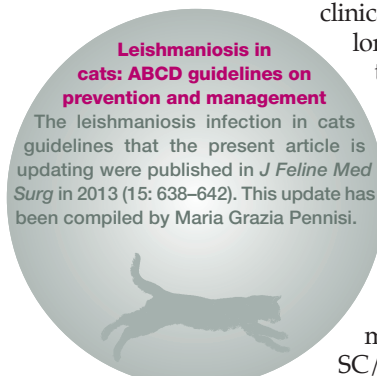
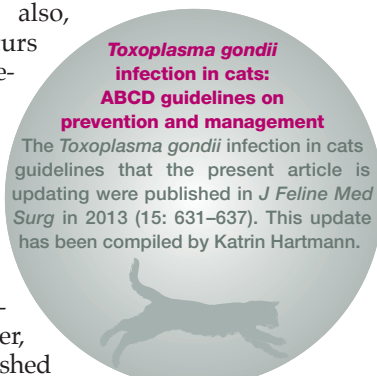
Detailed information on the prevention and management of sporotrichosis in cats is provided in the ABCD guidelines.¹³⁴

Toxoplasma gondii infection in cats

Several antibody tests have been used to detect infection with *Toxoplasma gondii* and to diagnose toxoplasmosis in cats. The indirect immunofluorescence assay can be adapted to detect immunoglobulin M (IgM), IgG and IgA antibodies.

Antibody test results from healthy cats are useful to assess the health risk for humans. An antibody-negative cat could be shedding oocysts (early after infection, before antibodies have developed) or will shed oocysts if exposed; this cat poses the greatest public health risk.

An antibody-positive cat is unlikely to shed oocysts, because antibodies need 2–3 weeks to develop, by which time the infection has been controlled; also, shedding usually occurs only once in the cat's lifetime. Furthermore, a cat with antibodies is unlikely to shed oocysts if re-exposed or immunosuppressed.¹³⁵ In one study, cats inoculated with *T gondii* tissue cysts were orally re-challenged several years later, and a few of them did shed



oocysts after this second challenge (although only low amounts and over a short time).¹³⁶ This, however, has never been shown to occur in naturally infected cats. Thus, the risk of shedding by an antibody-positive cat is very low.

Antibodies are common in both healthy and diseased cats and, therefore, do not prove clinical toxoplasmosis. Not only IgG antibodies, but also antibodies of the IgM class are commonly detected in healthy cats and stay high over long periods; thus their detection is also of no use for diagnosing toxoplasmosis. *T gondii*-specific IgM is detected in the serum of cats with latent or reactivated infection and titres, therefore, do not indicate recent exposure. If increasing IgM titres are detected, however, this can raise the suspicion of clinical toxoplasmosis.

Clinical toxoplasmosis is ideally diagnosed by detection of the organism in muscle biopsies or bronchoalveolar lavage fluid, or by PCR performed on cerebrospinal fluid (CSF) or aqueous humour. During acute illness, tachyzoites can be detected in tissues and body fluids by cytology. They are rarely found in blood, but occasionally in CSF, fine-needle aspirates of organs (eg, lymph nodes), and transtracheal or bronchoalveolar washings, and are common in the peritoneal and thoracic fluid of animals developing thoracic effusions or ascites. Detection of tachyzoites confirms the diagnosis.

A tentative diagnosis can be based on increasing IgM titres, exclusion of other causes of the clinical signs, and a positive clinical response to an anti-*Toxoplasma* drug.^{135,137}

Detailed information on the prevention and management of *T gondii* infection in cats is provided in the ABCD guidelines.¹³⁸

Leishmaniosis in cats

Leishmania infection is less well known in cats than in dogs, but it may be underestimated in endemic areas and is of zoonotic concern. Detailed information on the prevention and management of leishmaniosis in cats was published in the ABCD guidelines.¹³⁹

The information available for treatment is based only on case reports. Despite clinical improvements following long term oral administration of allopurinol (10–20 mg/kg q12h or q24h), the infection is not cleared, and recurrence of clinical signs may occur after cessation of therapy, as in dogs [EBM grade IV].^{140,141} Meglumine antimoniate (5–50 mg/kg or 375 mg/cat q24h SC/IM under different proto-

cols) was used for therapy in four cases and led to good clinical responses, but long term follow-up is lacking [EBM grade IV].¹⁴⁰

Giardiasis in cats

Giardia is a protozoan parasite of the small intestine. Seven genotypes have been identified and designated A to G. Types F and G are the subgroups commonly seen in cats, whereas A and B occur mainly in man and are considered as potentially zoonotic.¹⁴² Giardiasis in cats is not considered a zoonotic risk.^{143,144}

However, recent European studies demonstrated the presence of subgroup A in cats,^{145–147} either alone or as a dual infection (A and F).¹⁴⁶ Genotype B has also been identified in cats, but A is most prevalent, according to a Canadian study.¹⁴⁸ A correlation between body condition score, presence of diarrhoea and infection with *G intestinalis* has been observed;¹⁴⁹ but, in other studies, agent presence has not been notably different in cats with diarrhoea as compared with healthy cats. Coinfections with other enteropathogens have been demonstrated to be frequent in the UK.¹⁵⁰

Detailed information on the prevention and management of giardiasis in cats is provided in the ABCD guidelines.¹⁵¹

Giardiasis in cats: ABCD guidelines on prevention and management

The giardiasis in cats guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 650–652). This update has been compiled by Corine Boucraut-Baralon.



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Conflict of interest

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Prevention of infectious diseases in cat shelters

In shelter situations, infectious diseases are difficult to prevent and they spread quickly.⁵³ In addition, shelters are unstable biological environments; not only are disease outbreaks frequent, but also new pathogens may emerge or virulent variants of endemic pathogens may arise as a result of rapid transmission cycles and forced agent evolution. The virulent systemic feline calicivirus infection is a point in case.¹⁵²

The ABCD guidelines describe the most important factors in minimising the spread of infectious agents in the shelter environment.⁵³ These include: housing in individual sections (quarantine pens for incoming cats, isolation facilities for sick or potentially infectious cats, separate accommodation for clinically healthy, FIV- and FeLV-negative cats, and for pregnant and lactating queens and their kittens); testing for infectious agents; hygiene measures; and stress reduction. Stress is reduced above all by allowing for low animal densities, and by providing adequate bedding and environmental enrichment such as scratching posts, toys and hiding

areas. Newly sheltered cats provided with a hiding box during quarantine had significantly lower stress levels compared with cats without this enrichment.¹⁵³ Animal handling (eg, stroking anxious cats) may have positive effects, as suggested by an increase in secretory IgA and reduced incidence of upper respiratory tract disease.¹⁵⁴

Synthetic pheromones have been used in shelters with the objective of reducing stress. They are expected to alter the emotional state of the cat via the limbic system and the hypothalamus, and have been recommended for the management of anxiety-related behaviours, such as house soiling.¹⁵⁵ Horwitz and Pike¹⁵⁶ have published anecdotal observations that synthetic pheromones are useful when introducing new cats into a household. These data have not been corroborated by impartial, controlled studies. However, based on reports about use of synthetic pheromones in the treatment of undesirable, stress-related behaviour, they may be considered in addition to other stress-reducing measures.

Prevention of infectious diseases in cat shelters: ABCD guidelines

The prevention of infectious diseases in cat shelters guidelines that the present article is updating were published in *J Feline Med Surg* in 2013 (15: 546–554). This update has been compiled by Karin Möstl.



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MATRIX VACCINATION GUIDELINES

2015 ABCD recommendations for indoor/outdoor cats, rescue shelter cats and breeding catteries

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Introduction

It was evident during the preparation of the ABCD vaccination guidelines that no single vaccination protocol would be appropriate for all cats across Europe. Rather, it is important to conduct a vaccination interview in order to devise a strategy appropriate to the lifestyle, geographical location and disease risks relevant to each feline patient. These matrix vaccination guidelines, like the 2013 version, were compiled to assist veterinary surgeons during the vaccination interview, summarising the ABCD's vaccine recommendations. The 'core' vaccines should be administered to all cats, whereas 'circumstantial' vaccines are required under specific circumstances (eg, for cats travelling to areas where rabies is endemic, or cats with outdoor access and therefore at risk of infection with FeLV), and 'non-core' vaccines are recommended only for cats at risk of specific infections.

Abbreviations used in the matrix tables

DOI	Duration of immunity
FCV	Feline calicivirus
FCoV/FIP	Feline coronavirus/feline infectious peritonitis
FeLV	Feline leukaemia virus
FHV	Feline herpesvirus
FPV	Feline panleukopenia virus
MDA	Maternally-derived antibodies
MLV	Modified-live vaccine
PV	Primary vaccination course

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

Overview: In 2013, the ABCD published 'Matrix vaccination guidelines: ABCD recommendations for indoor/outdoor cats, rescue shelter cats and breeding catteries' in a special issue of the *Journal of Feline Medicine and Surgery* (Volume 15, Issue 7, pages 540–544). The ABCD's vaccination recommendations were presented in tabulated form, taking into account that there is no universal vaccination protocol for all cats. To support the veterinarian's decision making, recommendations for four lifestyles were made: for cats with outdoors access, cats kept solely indoors, rescue shelter cats and cats in breeding catteries. This update article follows the same approach, offering current and, where relevant, expanded recommendations.

European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the guidance presented in this article is available at www.abcdcatsvets.org and www.abcd-vets.org





OUTDOOR CATS (cats that have access outdoors and contact with other cats from outdoors)

Vaccination of outdoor cats							Comments
Vaccine/ disease agent	Kitten vaccination course			Adult cat			
	PV1	Primary PV2	PV3	Final PV/ first booster	Vaccinated >3 years ago	Unvaccinated/ no vaccine history	
Core							
FPV	8-9 weeks	12 weeks	16 weeks (in certain situations)	1 year later	One immunisation, boost every 3 years or more	One immunisation; boost 1 year later, then every 3 years or more	Do not use MLV in kittens <4 weeks of age Do not use MLV in pregnant cats
FHV	8-9 weeks	12 weeks	-	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Recovered cats should be vaccinated
FCV	8-9 weeks	12 weeks	16 weeks (if high risk or expected high MDA)	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Recovered cats should be vaccinated with different FCV vaccine strains
FelV	8-9 weeks	12 weeks	-	1 year later	Boost every 2-3 years after 3 years of age	Two immunisations 2-4 weeks apart, boost 1 year later	Cats of uncertain FelV status should be tested prior to vaccination (unless risk of FelV is considered very low) and vaccinated if negative
Rabies virus	12-16 weeks. Single immunisation	-	-	1 year later	Some vaccines' DOI is 3 years, but legislation may require annual boosters	One immunisation	Vaccinate in endemic areas only - refer to national and regional legislation for booster frequency
Circumstantial							
FCoV/FIP	Not before 16 weeks	3 weeks later	-	1 year later	One immunisation, boost annually	Two immunisations, boost annually	Intranasal vaccine available in some European countries. Only vaccinate seronegative cats
<i>Chlamydia felis</i>	8-9 weeks	12 weeks	-	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Where cats are kept together long term, vaccinate regularly
<i>Bordetella bronchiseptica</i>	1 month or older. Single immunisation	-	-	1 year later	One immunisation in high-density populations only, boost annually	One immunisation in high-density populations only, boost annually	Do not use MLV in kittens <4 weeks of age. Consider vaccination when there is contact with dogs. Vaccine available in some European countries. Vaccinate in high-density areas where <i>Bordetella</i> is confirmed
Non-core							

See page 583 for explanation of vaccine categories (core, circumstantial and non-core) and abbreviations. Image courtesy of www.sureflap.co.uk



INDOOR CATS (cats that have no contact with cats from outdoors)

Vaccination of indoor cats							
Vaccine/ disease agent	Kitten vaccination course			Adult cat		Comments	
	PV1	Primary PV2	PV3	Final PV/ first booster	Vaccinated >3 years ago		Unvaccinated / no vaccine history
Core	FPV	8-9 weeks	12 weeks	16 weeks (in certain situations)	1 year later	One immunisation, boost every 3 years or more	Do not use MLV in kittens <4 weeks of age. Pregnant cats should not be vaccinated
	FHV	8-9 weeks	12 weeks	-	1 year later	One immunisation, boost every 3 years*	Recovered cats should be vaccinated
	FCV	8-9 weeks	12 weeks	16 weeks (if high risk or expected high MDA)	1 year later	One immunisation, boost every 3 years*	Recovered cats should be vaccinated with different FCV vaccine strains
CS	Rabies virus	12-16 weeks. Single immunisation	-	-	1 year later	One immunisation. Some vaccines' DOI is 3 years, but legislation may require annual boosters	Only vaccinate if required by local legislation and refer to national and regional legislation for booster frequency
Non-core	FelV	8-9 weeks	12 weeks	-	1 year later	Boost every 2-3 years after 3 years of age	Only vaccinate if there is contact with FeLV-positive cats or those of unknown FeLV status
	FCoV/FIP	Not before 16 weeks	3 weeks later	-	1 year later	One immunisation, boost annually	Intranasal vaccine. Vaccine against FIP is available in some European countries. Only vaccinate seronegative cats
	<i>Chlamydia felis</i>	8-9 weeks	12 weeks	-	1 year later	One immunisation, boost annually	Where cats are kept together long term, vaccinate regularly
	<i>Bordetella bronchiseptica</i>	1 month or older. One immunisation in high-density populations only	-	-	1 year later	One immunisation in high-density populations only, boost annually	Do not use MLV in kittens <4 weeks of age. Consider vaccination where there is contact with dogs. Vaccine available in some European countries. Vaccinate in high-density areas where <i>Bordetella</i> is confirmed

See page 583 for explanation of vaccine categories (core, CS [circumstantial] and non-core) and abbreviations. * Boost annually if using a boarding cattery. Image ©/Stockphoto.com/Kevin Russ



RESCUE SHELTER CATS (cats living in centres for unowned and abandoned cats)

Vaccination of rescue shelter cats

Vaccine/ disease agent	Kitten Primary vaccination course			Adult cat		Comments		
	PV1	PV2	PV3	Final PV/ first booster	Vaccinated <3 years ago		Vaccinated >3 years ago	Unvaccinated/ no vaccination history
Core								
FPV	6 weeks (4 weeks if needed)	3-4 weeks later	3-4 weeks later until 16 weeks	1 year later	Booster vaccinations at 3 year intervals	One immunisation, then at 3 year intervals	One immunisation, boost 1 year later, then at 3 year intervals	Do not use MLV in kittens <4 weeks of age
FHV	6 weeks (4 weeks if needed)	3-4 weeks later	3-4 weeks later until 12 weeks	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Two immunisations 2-4 weeks apart, boost 1 year later	Vaccinate new cats as soon as possible
FCV	6 weeks (4 weeks if needed)	3-4 weeks later	3-4 weeks later until 16 weeks	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Two immunisations 2-4 weeks apart, boost 1 year later	Vaccinate new cats as soon as possible. Due to high antigenic variation of strains, recovered cats should be vaccinated
CS								
Rabies virus	12-16 weeks. Single immunisation	-	-	1 year later	Some vaccines' DOI is 3 years, but legislation may require annual boosters	One immunisation, boost 1 year later	One immunisation, boost 1 year later	In endemic areas: handle all strays with caution, as potentially infected. Vaccinate only in endemic areas - refer to national and regional legislation
Non-core								
FelV	8-9 weeks	12 weeks	-	1 year later	Boost every 2-3 years after 3 years of age	Quarantine and test before two immunisations 2-4 weeks apart, boost 1 year later	Quarantine and test before two immunisations 2-4 weeks apart, boost 1 year later	Not needed if no contact with other cats
FCoV/FIP	First immunisation from 16 weeks	3 weeks later	-	1 year later	-	Two immunisations 2-4 weeks apart, boost 1 year later	Two immunisations 2-4 weeks apart, boost 1 year later	Intranasal vaccine. Vaccination may be considered in cats that are likely to be seronegative
<i>Chlamydia felis</i>	8-9 weeks	12 weeks	-	1 year later	One immunisation, boost annually	Two immunisations 2-4 weeks apart, boost 1 year later	Two immunisations 2-4 weeks apart, boost 1 year later	Where cats are kept close together long term, vaccinate regularly
<i>Bordetella bronchiseptica</i>	One immunisation in cats 1 month or older	-	-	1 year later	One immunisation, boost annually	One immunisation, boost annually	One immunisation, boost annually	Do not use MLV in kittens <4 weeks of age. Vaccine available in some European countries. Vaccinate in high-density areas where <i>Bordetella</i> is confirmed

See page 583 for explanation of vaccine categories (core, CS [circumstantial] and non-core) and abbreviations. Image Image ©iStockphoto.com/Dwight Smith



BREEDING CATTERIES (cats in any multicat environment used for breeding purposes)

Vaccination of cats in a breeding cattery

Vaccine/ disease agent	Kitten vaccination course			Breeding cats	Comments
	PV1	Primary PV2	PV3 Final PV/ first booster		
Core					
FPV	8-9 weeks	12 weeks	16-20 weeks	Boost queens 3 yearly, or annually before breeding if low MDA is a concern	Do not use MLV in kittens <4 weeks of age Pregnant cats should not be vaccinated
FHV	8-9 weeks	12 weeks	-	Boost queens annually and before breeding	Recovered cats should be vaccinated. Consider earlier vaccination in litters from queens that have had infected litters previously. In special circumstances kittens can be vaccinated from 4-6 weeks of age, then every 2 weeks until 12 weeks of age
FCV	8-9 weeks	12 weeks	16 weeks	Boost queens annually and before breeding	Recovered cats should be vaccinated. In special circumstances kittens can be vaccinated from 4-6 weeks of age, then every 2 weeks until 12 weeks of age
CS					
Rabies virus	12-16 weeks. Single immunisation	-	-	Some vaccines' DOI is 3 years, but legislation may require annual boosters of queens	Only vaccinate if required by local legislation
Non-core					
FelV	8-9 weeks	12 weeks	-	Boost queens every 2-3 years after 3 years	Breeding catteries should be FelV negative. Vaccination not needed unless there is access to outdoors and in a high-risk geographical area
FCoV/FIP	Not before 16 weeks	3 weeks later	-	Boost queens annually	Only seronegative cats should be vaccinated. Intranasal vaccine available in some European countries
<i>Chlamydomphila felis</i>	8-9 weeks	12 weeks	-	Boost queens annually	-
<i>Bordetella bronchiseptica</i>	One immunisation in cats 1 month or older	-	-	Boost queens annually	Do not use MLV in kittens <4 weeks of age. Vaccine available in some European countries. Vaccinate in catteries where <i>Bordetella</i> is confirmed

See page 563 for explanation of vaccine categories (core, CS [circumstantial] and non-core) and abbreviations. Image ©iStockphoto.com/oksun70

BLOOD TRANSFUSION IN CATS

ABCD guidelines for minimising risks of infectious iatrogenic complications



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Overview: The availability of blood components has increased the number of indications for transfusing cats, and fresh whole blood is readily accessible to clinicians because it can be taken from in-house donor cats or ‘volunteer’ feline blood donors. A certain amount of risk remains to the recipient cat, as immediate or delayed adverse reactions can occur during or after transfusion, related to immune-mediated mechanisms. This article, however, focuses on adverse events caused by infectious agents, which may originate from either contamination of blood following incorrect collection, storage or transfusion or from transfusion of contaminated blood obtained from an infected donor.

Prevention of blood contamination: In cats, blood cannot be collected through a closed system and, therefore, collection of donor blood requires a multi-step manipulation of syringes and other devices. It is crucial that each step of the procedure is performed under the strictest aseptic conditions and that bacterial contamination of blood bags is prevented, as bacterial endotoxins can cause an immediate febrile reaction or even fatal shock in the recipient cat.

Prevention of disease transmission: With a view to preventing transmission of blood-borne infectious diseases, the American College of Veterinary Internal Medicine has adopted basic criteria for selecting pathogens to be tested in donor pets. The worldwide core screening panel for donor cats includes feline leukaemia virus, feline immunodeficiency virus, *Bartonella* species and feline haemoplasma. The list should be adapted to the local epidemiological situation concerning other feline vector-borne infections. The most practical, rapid and inexpensive measure to reduce transfusion risk is to check the risk profile of donor cats on the basis of a written questionnaire. Blood transfusion can never, however, be considered entirely safe.

Introduction

Over recent decades, small animal transfusion medicine has made significant progress, contributing to the development of emergency medicine and critical care. The availability of blood components has increased the number of indications for transfusing cats and dogs, even if the evidence-based benefit is still lacking in certain cases.¹

Fresh whole blood is readily available to clinicians because it can be taken from in-house donor cats or ‘volunteer’ feline blood donors. Thanks to the commercial availability of in-house typing kits and gel cross-match systems for cats, blood transfusion in veterinary practice has become safer and more accessible.² However, blood transfusion implies a certain amount of risk to the recipient cat and, to some extent, the donor cat as well, which is subjected to an invasive procedure requiring sedation.³ These risks always need to be carefully weighed against the achievable benefits. In terminal patients, blood transfusion should be avoided and other treatment options considered.⁴ Surprisingly, blood transfusion did not reduce the risk of 30-day mortality in humans in a critical care setting in one multi-centre, randomised controlled study.⁵

Immediate or delayed adverse reactions can occur during or after transfusion, related to immune-mediated mechanisms. The severity of these reactions varies from a mild febrile reaction to a severe, life-threatening circulatory overload or haemolytic crisis. The prevention of this risk is not the objective of these guidelines, and guidance is provided elsewhere.^{1,2,4,6–10}

This guideline article focuses on the prevention of transmission of infectious disease related to blood transfusion in cats. Adverse events caused by infectious agents may originate from: contamination of blood following incorrect collection, storage or transfusion; or transfusion of contaminated blood obtained from an infected donor.



European Advisory Board
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Prevention of contamination of donor blood

The blood collection procedure in cats is associated with a greater risk of contamination than in dogs or humans. In cats, blood cannot be collected through a closed system, and therefore a multi-step manipulation of syringes and other devices is required, with the help of several assistants. This increases the risk of contamination. In general, 50 ml of blood is collected from donor cats using three (20 ml) or five (10 ml) different syringes, each containing the appropriate quantity of anticoagulant obtained from a human blood collection bag.⁴ Usually, a T-connector and a three-way tap connect the intravenous (IV) needle to the syringes, which are filled with blood and then gently rotated by an assistant. The blood collected into the syringes is then immediately transferred into a single, plain blood collection bag through the injection port (Figure 1). Finally, blood is transfused through a giving set which is inserted into another port of the bag at the time of the transfusion procedure.

It is crucial that each step of the process is performed under the strictest aseptic conditions, even in an emergency.¹¹ The disposable equipment should be placed on sterile surfaces, and staff should wear sterile gloves and masks. Each syringe should be immediately sealed with its capped needle, both after adding the anticoagulant and after collecting the blood until it is transferred into the bag. Surgical preparation of the ventral neck of the donor is necessary. The longer the delay between blood collection and transfusion, the higher the risk of contamination of the collected blood. Collected blood should be stored at 4°C. However, the blood bag should not be stored once the giving set has been inserted.

Similar principles apply in the case of autologous transfusion, a procedure reported in dogs in emergency situations such as for treatment of haemothorax or haemoperitoneum. Here, blood is collected from the body cavity using cell salvage devices and transfused after appropriate washing.^{10,12}

Bacterial contamination of blood bags can cause an immediate febrile reaction in the recipient if bacterial endotoxins are produced by cold-growing gram-negative bacteria, such as *Pseudomonas* species, or coliforms, such as *Serratia marcescens*. The latter microorganism has been isolated from contaminated feline blood bags and from transfused cats that presented with fever, vomiting, diarrhoea, jaundice and even death.¹³ Fatal endotoxin-related shock is the most dangerous consequence in such cases.

Blood bags should be visually inspected before use and discarded if there is any suspected change in colour or other visible abnormality.¹⁴

Figure 1 Transfer of blood collected with syringes into a single, plain blood collection bag through the injection port. Courtesy of Eva Spada, University of Milan, Milan, Italy



European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the guidance provided in this article is available at www.abcdcatsvets.org and www.abcd-vets.org

Prevention of transmission of blood-borne infectious diseases

Information on feline blood-borne infectious agents is becoming increasingly available, in particular in relation to vector-borne pathogens.^{15,16} In 2005, in a consensus statement on canine and feline blood donor screening for infectious diseases, the American College of Veterinary Internal Medicine (ACVIM) adopted basic criteria for selecting pathogens to be tested in donor pets.¹⁴ Testing is recommended for pathogens in certain circumstances, as highlighted in the box below.

In line with these criteria, the worldwide core screening panel for donor cats (Table 1) includes: feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), *Bartonella* species and feline haemoplasma.^{14,21,22}

When and what to screen for

Testing is recommended for pathogens that meet at least three of the following criteria:

- ✦ Documented clinical disease produced in recipients by blood transmission
- ✦ Possibility of subclinical infections (healthy carrier state)
- ✦ Possibility of cultivation from the blood of an infected animal
- ✦ The disease caused is severe or difficult to clear

Testing is conditionally recommended when:

- ✦ Only experimental transmission by blood is documented
- ✦ The disease caused is not severe in most cases or is easily overcome

Table 1 Core pathogens for worldwide screening of candidate blood donors

Pathogen	Diagnostic tests
Feline leukaemia virus (FeLV)	FeLV provirus PCR*†
Feline immunodeficiency virus (FIV)	Rapid anti-FIV antibody test on blood serum/plasma*
<i>Mycoplasma haemofelis</i> <i>Candidatus Mycoplasma haemominutum</i> <i>Candidatus Mycoplasma turicensis</i>	Blood PCR
<i>Bartonella</i> species	Anti- <i>Bartonella</i> antibodies (IFAT) and/or blood PCR

For more information on these pathogens, see Hosie et al,¹⁷ Lutz et al,¹⁸ Pennisi et al¹⁹ and Willi et al.²⁰ PCR = polymerase chain reaction; IFAT = immunofluorescence antibody test
*Tests for anti-FIV antibodies and FeLV DNA should be confirmed negative at least 3 months after the last exposure
†In life-threatening emergency situations, donors can be screened using rapid FeLV antigen tests, but owners should be informed about the higher risk

Table 2 Pathogens to be considered for screening of candidate blood donors based on local epidemiological information

Pathogen	Diagnostic tests
<i>Cytauxzoon felis</i>	Blood PCR in endemic areas
<i>Babesia</i> species	Blood PCR in endemic areas*
<i>Leishmania infantum</i>	Blood PCR in endemic areas
<i>Ehrlichia</i> species	Blood PCR in endemic areas*
<i>Anaplasma phagocytophilum</i>	Anti- <i>A phagocytophilum</i> antibodies (IFAT) and blood PCR in endemic areas

For more information on these pathogens, see Carli et al,²⁴ Hartmann et al²⁵ and Pennisi et al.²⁶ PCR = polymerase chain reaction; IFAT = immunofluorescence antibody test
*Probably rare and poorly characterised infection of cats in Europe

However, the list of pathogens to be tested in donor cats should be adapted to the local epidemiological situation.²³ Other infectious agents that may be investigated in endemic areas are listed in Table 2.

Two common feline infectious agents – *Toxoplasma gondii* and feline coronavirus (FCoV) – do not meet the ACVIM criteria and are not included in donor screening panels.¹⁴ The presence of antibodies against FCoV in blood products may passively immunise transfused cats. In the case of contact with the virus in the weeks following transfusion, these cats could be exposed to the risk of antibody-dependent enhancement of macrophage infection.^{27,28} Although there have been no reports of feline infectious peritonitis (FIP) following blood transfusion in cats, FCoV-antibody negative blood bank donors are preferred.

Although *Rickettsia felis* and *Rickettsia* of the other spotted fever group can infect cats, the organisms have never been detected by molecular methods in cat blood. At present, there is no indication for testing cats for these pathogens.²⁹

The risk of transmission of pathogens associated with xenotransfusion (transfusion of blood obtained from a different animal species, usually dogs) is theoretically zero for FIV, FeLV and feline haemoplasmas but may be relevant for vector-borne infections, some of which are more common in dogs than in cats.³⁰ Xenotransfusion should be restricted to exceptional circumstances (eg, emergencies in the event of lack of compatible feline blood or oxygen carrier solution), as it is associated with delayed immune-mediated haemolysis and a very short life span of the transfused erythrocytes.³¹

Molecular techniques have significantly increased the sensitivity and specificity of diagnostic testing for the detection of feline blood-borne agents, and their use has increased the safety of blood products. Healthy cats that test negative for FeLV p27 antigenaemia can still harbour provirus integrated in their DNA, which means their blood can transmit FeLV infection to transfused cats.¹⁸ Blood bank donors should, therefore, be tested for FeLV provirus using PCR. In life-threatening emergency situations, transfusions from donors can be screened using rapid FeLV antigen tests, but owners should be informed about the risk.

The screening of blood donors is also influenced by costs. In human medicine, individual blood units are usually tested for several pathogens of major concern (eg, HIV, hepatitis B, hepatitis C, *Treponema pallidum*); while, for cost reasons, no testing is done for other transmissible blood-borne agents from healthy carriers (*Cytomegalovirus*, West Nile virus, prions, *Leishmania*, etc). The preliminary selection of potential human donors is based on history and a risk assessment related to history of travel, sexual behaviour and certain medical procedures.

This is also true in the veterinary field; the most useful, practical, rapid and inexpensive measure to reduce transfusion risk is to check the risk profile of donor cats prior to transfusion, on the basis of a written questionnaire completed by the guardian of the donor cat (see box on page 591). This questionnaire can be presented at the time of obtaining informed consent for blood donation. The ideal low-risk profile of a donor cat is described below.

Ideal profile for a blood donor cat

- ✦ Adult (>3 years old, to reduce the risk of *Bartonella* bacteraemia)
- ✦ Living in the same single-cat household since a kitten (full history directly available from the guardian)
- ✦ Regularly vaccinated and treated against fleas and ticks
- ✦ No history of travel or vector-borne diseases
- ✦ Heartworm prevention in endemic areas*

*Blood from cats infected with heartworm is, however, not infectious following transfusion³²

Risk profile form for candidate blood donors*

If all answers are in the right-hand column, the cat has a low-risk profile for transmission of infectious agents by blood.

Owner:
 Cat's name:
 Breed:
 Gender: M / F Neutered: Yes/No Age:

Circle the correct answer

How long have you owned this cat?	Days	Months	Years
Is (or was) your cat free-roaming or has it (had) any outdoor access?	Yes	Don't know	No
Did you adopt your cat from a shelter?	Yes	Don't know	No
Was your cat a stray?	Yes	Don't know	No
Did you buy your cat from a pet shop or a cat breeder?	Yes	Don't know	No
Is (or was) your cat in contact with other cats?	Yes	Don't know	No
Has your cat ever travelled to other countries?	Yes	Don't know	No
Has your cat had any health problem in the past?	Yes	Don't know	No
Has your cat had any drugs prescribed by a vet?	Yes	Don't know	No
Do you regularly use anti-flea products?	No	Don't know	Yes
Has your cat been vaccinated?	No	Don't know	Yes†
Is your cat eating less than usual?	Yes	Don't know	No
Have you recently seen any unusual behaviour?	Yes	Don't know	No
Has your cat vomited in the last few days?	Yes	Don't know	No
Has your cat had diarrhoea recently?	Yes	Don't know	No
Have you seen any change in urination?	Yes	Don't know	No
Have you seen any change in respiration?	Yes	Don't know	No
Have you noticed sneezing or coughing?	Yes	Don't know	No
Have you seen ocular or nasal discharge?	Yes	Don't know	No

†When was your cat vaccinated last?

Please inform us of **any observed change in the health status of your cat in the next 15 days.**

Date: Signature:

*Available to download as a Word document from the Supplementary material accompanying this article at jfms.com

Risk should be reassessed prior to each transfusion. Risk assessment may eliminate the need to repeat expensive and time-consuming screening for blood-borne pathogens in cats with low-risk profiles. The required frequency of testing varies according

to the pathogen (seasonal exposure or not) and the individual recipient's risk of acquiring the infection. The ABCD does not recommend the use of closed colony donors, which are cats specifically bred for blood banks, as it is preferable from a welfare perspective for cats to live in a more natural environment.

If no feline blood is available from a blood bank, veterinary practitioners should be able to rely on an adequate number of pre-selected potential donors evaluated as being low-risk cats and negative for blood-borne pathogens of interest. Free-roaming cats should never be considered as potential donors. Shelter cats can potentially be considered, according to their history and the quality of management of the shelter. Physical examination performed after history taking should include an accurate observation and combing of the coat to exclude the presence of fleas and ticks. Cats with fleas or ticks should not be considered as donors.¹⁴

Occasional donors recruited in emergency settings always reduce the level of safety of blood transfusion. The need to find a compatible blood donor may rapidly lead to the neglect of important requirements in terms of donor health. Moreover, only in-house tests can be used for assessing donors in emergency cases, which implies they will be screened only for retroviral infections following a physical examination, complete blood count (CBC), biochemical profile and urinalysis. Where this approach is used, records of the donor and recipient cats should be taken; additionally an EDTA blood sample from the donor should be kept (can be the same tube and sample as taken for CBC), stored frozen at -20 °C, for possible further investigations.

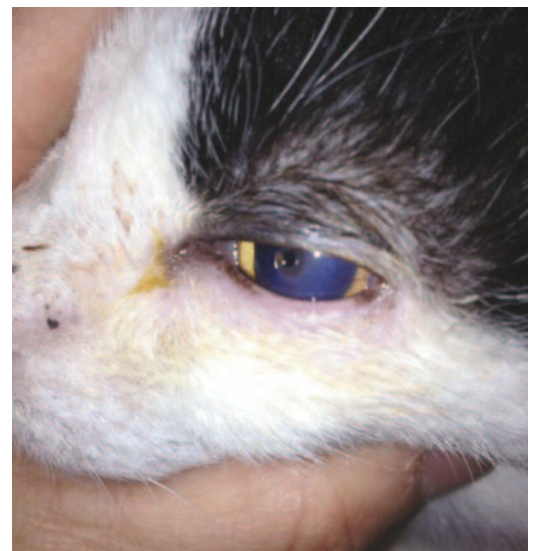


Figure 2 Topical application of autologous blood serum is used empirically as anticollagenolytic treatment in the medical management of corneal lesions, but the procedure requires strict aseptic measure. Courtesy of Maria Grazia Pennisi, University of Messina, Messina, Italy

Other uses of blood products in practice

Topical application of blood serum is used empirically as anticollagenolytic treatment in the medical management of deep corneal ulcers (Figure 2).^{33,34} The autologous preparation is cheap to prepare and easy to administer in practice but strict aseptic conditions are required, as described above for the collection of blood. Sterile disposables (tube, pipette, eye dropper bottle) should be used to prevent bacterial contamination. The preparation should be stored at 4°C and used as soon as possible (preferably within 48 h) because the high administration frequency (up to once an hour) increases the risk of contamination of the contents of the eye drop bottle. In the case of very young kittens, or when it is impractical to bleed the patient, homologous (feline) or even canine serum may be used. The administration of canine serum reduces the risk of feline pathogen transmission to the ocular mucosa and damaged corneal tissue.³⁴ In the case of homologous serum, the donor should be carefully selected, respecting the same criteria as apply for blood transfusion. However, as there have been no controlled studies of the efficacy and safety of this therapy in cats, it should not be encouraged.

Autologous platelet-rich plasma is increasingly used for treating orthopaedic conditions in veterinary practice, including feline practice. The risk of bacterial contamination during preparation of the concentrate must be minimised by strict hygiene.³⁵

The risk of transmitting pathogens using the blood of healthy infected carriers must be minimised, but cannot be eliminated entirely.



Conclusion

Blood transfusion can be a life-saving treatment with a crucial impact on anaesthetic and surgical possibilities or intensive care but it can never be considered totally safe. The development of infectious diseases in recipient cats is an iatrogenic risk that must be minimised by the highest standards of clinical veterinary practice. Despite increasing data on blood-borne infections and the availability of more sensitive diagnostic techniques, the risk of transmitting pathogens using the blood of healthy infected carriers cannot be eliminated entirely. The most cost-effective action is to reduce this risk by the pre-selection of low-risk donors.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

The risks of blood transfusion must always be carefully weighed against the achievable benefits.



KEY POINTS

- ❖ Each step of the blood transfusion procedure should be performed under strict aseptic conditions, even in emergencies.
- ❖ The longer the delay between blood collection and transfusion, the higher the risk of contamination of the collected blood.
- ❖ Blood bags should be visually inspected before use and should be discarded if there is any suspicion of change in colour or other visible abnormality.
- ❖ The core screening panel for donor cats worldwide includes FeLV, FIV, *Bartonella* and feline haemoplasma.
- ❖ Although the onset of FIP following blood transfusion in cats has not been reported, FCoV antibody negative donors are preferred.
- ❖ The most practical, rapid and inexpensive preventive measure is to assess the risk profile of donor cats.
- ❖ Risk assessment may eliminate the need for repeating expensive and time-consuming screening for blood-borne pathogens in cats with a low-risk profile.
- ❖ Free-roaming cats should never be considered as potential donors.
- ❖ Blood transfusion should be avoided in terminal patients.



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DISINFECTANT CHOICES IN FELINE VETERINARY HOSPITALS, SHELTERS AND CAT HOUSEHOLDS

ABCD guidelines on disinfection



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Overview: Regardless of whether a pathogen is viral, bacterial, parasitic, fungal or an emerging unknown, the mainstay of infectious disease control is hygiene, and the cornerstone of good hygiene is effective disinfection.

Challenges and current choices: Certain pathogens present a challenge to kill effectively: parvovirus, protozoal oocysts, mycobacteria, bacterial spores and prions resist most disinfectants but can be eliminated through heat, especially steam, which will kill protozoal oocysts. Heat is the safest and most effective disinfectant, but cannot be universally applied. Temperatures in washing machines and dishwashers should be at least 60°C to eliminate pathogenic spores and resistant viruses. Enveloped viruses are susceptible to most disinfectants; of the non-enveloped viruses, parvovirus is recognised as being the most difficult to eradicate. Sodium hypochlorite is recommended for many applications: cleaning of floors, laundry, food preparation surfaces and utensils. Skin scrubs and rubs containing alcohols are more effective than those containing chlorhexidine, and less subject to contamination.

Disinfectants to avoid: Deficiency of the enzyme UDP-glucuronosyl transferase renders the cat susceptible to the toxic effects of phenol-based disinfectants (including the essential oils of tea tree and clove), so these should be avoided in feline environments. Quaternary ammonium compounds (eg, benzalkonium chloride) are also probably best avoided.

The future: Veterinary disinfection approaches in the future may include use of ultraviolet radiation and, increasingly, silver.

Introduction

Infectious disease is a major challenge for the domestic cat (*Felis catus*). In nature, a solitary creature, the cat has been forced, by domestication, to live sometimes in unnaturally dense populations (eg, shelters or breeding households), which results in exposure to unnaturally high doses of pathogens at a time when stress may already be compromising the cat's immune system and ability to deal with it. Hygienic routines and disinfection are the method of choice for eliminating meticillin-resistant *Staphylococcus aureus* (MRSA) or virulent systemic feline calicivirus (VS-FCV) from premises, and are especially important in situations where there is an emerging, or unknown, contagion, and neither vaccination nor specific testing are available.

There are three priorities when choosing disinfectants for use around the cat: the first, obviously, is efficacy. The second is safety for the cat: the idiosyncrasies of the feline metabolism render the cat especially sensitive to many things that are perfectly safe for other species, such as phenol-based disinfectants. The third, which is outwith the scope of this article but also very important, is safety for humans; especially in veterinary hospitals and shelters, where exposure is likely to be a daily occurrence and long term. Cleaning chemicals have been associated with airway irritation, asthma, contact dermatitis and even, with prolonged exposure, neoplasia. The strongest airway irritants in cleaning products are bleach (sodium hypochlorite), which releases chlorine gas, hydrochloric acid and alkaline agents (ammonia and sodium hydroxide), which are commonly mixed together.¹

The mainstay of infectious disease control is hygiene, and the cornerstone of good hygiene is effective disinfection.



European Advisory Board
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European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the guidance provided in this article is available at www.abcdcatsvets.org and www.abcd-vets.org

Cleaning agents are divided into sensitisers (amine compounds, quaternary ammonium compounds [QACs], scents containing terpenes, isothiazolinones, formaldehyde) and irritants (chlorine, ammonia, hydrochloric acid, monochloramine, sodium hydroxide, QACs).¹

Different pathogens require different approaches for effective disinfection; thus recommendation of a single disinfectant for all purposes is not possible. In addition, there is no single solution for all applications: for example, steam cleaning, which is necessary to eliminate protozoal oocysts from a premises,² is not feasibly applied to the hands of a veterinary surgeon or the skin of a cat. Although hand hygiene (Figure 1) has been recognised as the most important tool in nosocomial infection control since Semmelweis observed its immense effect on the incidence of childbed fever in 1847 (cited in Kampf and Kramer³), obtaining compliance remains a challenge over 150 years on.^{4,5} Apparently people are more willing to use a hand rub than to wash their hands in water.³

For each class of pathogen, certain members have been identified as the most difficult to kill; for example, of the viruses, parvovirus is the most resistant – thus, if a disinfectant kills parvovirus, it is likely to kill most other viruses as well. There are many publications reporting on the virucidal activity of disinfectants against feline calicivirus (FCV), as this pathogen is often used as a surrogate for human norovirus,⁶ which is difficult to grow in cell culture. Details of any special disinfection requirements for a particular feline pathogen are given in the respective ABCD guidelines.

By contrast, some organisms will die outside the host without any intervention (eg, feline leukaemia virus, feline herpesvirus). Survival times outside the host are presented elsewhere.^{7,8}

These disinfection guidelines are intended for the general veterinary practitioner. Special areas, such as the disinfection of blood for transfusion, bone marrow/organs for transplant, and specialised equipment, such as endoscopes, will not be covered. For a review of endoscope disinfection, see Greene et al.⁹



Figure 1 Hand sanitisers are located by all of the doors of the University of Berne Veterinary Hospital. Courtesy of Dr Diane Addie

Definition and principles of disinfection

Disinfection is a potent means of reducing the number of pathogens on a surface: it minimises the risk of infection for animals and humans that come into contact with that surface. Disinfection does not result in sterility, which can be achieved by other methods, and only for very confined surfaces (eg, on instruments) or liquids (eg, infusion solutions).

Disinfection is always non-specific: it does not inactivate specific pathogens. A good disinfectant will kill most of the bacteria on a surface, including the pathogenic ones. Therefore, it is important that a disinfectant is capable of substantially reducing the bacterial burden on a surface; this is defined in most test protocols as a reduction in the number of infectivity by at least 4 log₁₀.

Disinfection can be achieved by various methods: bacteria, viruses and other pathogens can be damaged and inactivated by physical treatment (which is basically heat and radiation) and also by chemical means. The latter is the most common approach to disinfection and can be applied to virtually all surfaces.

Physical disinfection

Heat and steam

Heat is by far the most broad-spectrum method of disinfection. Moist heat is more effective than dry heat, especially under pressure. When used correctly, steam under pressure (ie, autoclaves) is also the most efficient means of achieving sterility.⁹ Steam cleaners are widely available and can be used on soft furnishings (eg, carpet), as well as floors and work surfaces.

In veterinary hospitals, shelters and the home, heat can be used in dishwashers, washing machines and incinerators to inactivate infective agents. Introduction of a dishwasher was one of the measures that ended an outbreak of MRSA in a human neonatal hospital.¹⁰ Household dishwashers modified to achieve a temperature of 71°C were even proposed as a substitute for autoclaving in smaller surgeries.¹¹ However, care must be taken that the dishwasher itself does not become a source of cross-contamination.¹² Sterilisation efficacy is dependent on the duration of exposure of the pathogen to heat, and on whether or not a chemical disinfectant is also used.

Human safety needs to be considered. Zoonotic infections may be indirectly transmitted to laundry workers; albeit from a human source (ie, not zoonotic in this particular example), there is a report of *Salmonella*

being transmitted to laundry workers.¹³ One heavily contaminated item can contaminate an entire laundry load, as viruses can be transferred from contaminated to uncontaminated laundry during washing.^{14,15} It has been demonstrated that *Cryptosporidium* species oocysts can attach to fabrics during machine washing.¹⁶ In a human hospital, a nosocomial outbreak of *Microsporium canis* infection was linked to laundry contamination.¹⁷

The temperature needed for decontamination depends on the duration of the wash cycle and the detergent type.¹⁵ For mycotic contaminants, Ossowski and Duchmann¹⁸ found that reliable decontamination was achieved by laundering at 60°C, regardless of the textiles and detergents used. Moriello recommends two washings and stresses the importance of not overloading washing machines to be rid of *M. canis* spores.¹⁹ Nims and Plavsic report that 60°C (or higher) is the optimal temperature for inactivating FCV.²⁰ Temperatures of 56°C and above will kill 99% of *Giardia* cysts.²¹ Addition of sodium hypochlorite with detergent significantly reduced the numbers of viruses in laundry¹⁴ and the addition of activated oxygen bleach increased efficacy against a number of bacteria.¹⁵ However, parvovirus can resist temperatures of 80°C for at least an hour.²²

Microbial size is an important determinant in the fabric attachment–detachment process during the machine washing cycle, with larger microorganisms showing greater transfer to, and retention on, fabric swatches than smaller ones. Transfer efficiencies are higher for cotton towelling than for other fabric types, both before and after the washing machine spin cycle, indicating that it is not only the properties of the microorganism that influence transfer efficiency but also the properties of the fabric.¹⁶

Ultraviolet-C radiation

Ultraviolet light radiation in the C range (UV-C; typically 254 nm) and B range (UV-B; 280–320 nm) has been investigated for disinfecting water, food preparation surfaces²⁰ and hospital rooms. UV-C-emitting devices were shown

to significantly reduce the bioburden of important pathogens (*Clostridium difficile* and vancomycin-resistant enterococci, though not *Acinetobacter*) in real-world settings such as hospital rooms.²³

Parvoviruses and circoviruses appear to be more susceptible to UV-C inactivation than are the caliciviruses.²⁰

Chemical disinfection

Both pure active substances and commercial disinfectants can be used for efficient disinfection, provided they are applied at an effective microbicidal concentration. Commercially available products usually contain a combination of various active substances. Side effects are minimised but, above all, they are efficacy tested and the microbicidal concentration is determined by an independent body.

In Europe, chemical disinfectants are considered as biocides and need to be licensed. The licensing procedure is complex and expensive (see box below), and will inevitably lead to a substantially reduced supply of available products in the future. It will, therefore, become even more important to choose the right disinfectant for a given purpose.

Chemical disinfectants for use in veterinary practices

In veterinary practice, cleaning and disinfection of the surfaces (floors, walls, tables, etc) in various areas of the clinic has to be performed on a regular basis, up to several times a day (Figure 2). In both the veterinary clinic and shelter setting, special attention has to be given to the use of products with proven efficacy against a broad spectrum of microorganisms and viruses, that are safe for use with animals (and used in compliance with local regulations).

Figure 2 (a) The waiting room at the University of Barcelona Veterinary Hospital: note the easily cleaned chair, with as few legs as possible to facilitate floor disinfection. Hygiene is given priority over appearances in this spotless hospital. (b) Rounded corners where floor meets wall in this veterinary hospital consulting room in Stromsholm, Sweden, likewise facilitate floor cleaning. Images courtesy of Dr Diane Addie



Efficacy testing and licensing of biocides

In Europe, chemical disinfectants are considered as biocides and are licensed under the EU Biocides Regulation (Regulation 528/2012).

All disinfectants affect the environment to a varying degree and will react with inert materials, such as the surface to be disinfected. The licensing procedure, therefore, includes tests for the ecotoxicological effect of the biocide, as well as the potential to harm animals and humans, and to be compatible with various materials.

The licensing procedure has been in place since September 2013, and the first licensed commercial chemical disinfectants will begin to be sold this year. Up until now, interim regulations have allowed use of the established disinfectants and voluntary efficacy testing; the latter performed according to established guidelines, such as those from the German Veterinary Medical Society (DVG) and the Association for Applied Hygiene (VAH), or other national or international test protocols.

Alcohol

Rubbing alcohol (USP)/surgical spirit (BP) is used primarily for topical application, especially following a chlorhexidine- or iodine-based scrub prior to surgery, or is applied immediately after a dog or cat bite (it stings, but is remarkably effective in preventing bacterial infection sequelae). It is prepared from a special denatured alcohol solution and contains approximately 70% v/v of pure, concentrated ethanol (ethyl alcohol) or isopropyl alcohol (isopropanol). Individual manufacturers can use their own 'formulation standards' in which the ethanol content usually ranges from 70–99% v/v. It is colourless. Instruments (eg, thermometers) may be disinfected by immersion in alcohol-based solutions: contamination of such solutions has rarely been reported.²⁴

Alcohols have a non-specific mode of action, consisting mainly of disrupting the cell membrane or virus envelope, as well as denaturation and coagulation of proteins. Cells are lysed, and the cellular metabolism disrupted.³ In terms of bactericidal activity, the following ranking has been generally established: n-propanol > isopropanol > ethanol. Bactericidal activity is higher at 30–40°C than at 20–30°C. In terms of virucidal activity, ethanol is superior to the propanols.³ In one study, alcohols, and particularly ethanol, exhibited poor activity against all non-enveloped viruses.²⁵ In another, parvovirus resisted exposure to alcohol for 5 mins.²⁶ Taken orally, concentrated alcohols are lethal.

Park et al²⁷ evaluated seven hand sanitisers containing various active ingredients, such as ethanol, triclosan and chlorhexidine, and compared their virucidal efficacy against FCV and a GII.4 norovirus faecal extract. Based on the results of a quantitative suspension test, only one ethanol-based product (72% ethanol, pH 2.9) and one triclosan-based product (0.1% triclosan, pH 3.0) reduced the infectivity of FCV (by ≥ 3.4 log units). FCV is susceptible to low pH.

Chlorine releasers

Sodium hypochlorite

Sodium hypochlorite (bleach) has been used as a disinfectant for more than 100 years. It has many of the properties of an ideal disinfectant (see box),²⁸ and is relatively safe around cats, which is why sodium hypochlorite-based disinfectants are widely used, both in the veteri-

The priorities when choosing disinfectants for use around the cat are efficacy, safety for the cat and safety for humans.



nary surgery and in the home. Rapid inactivation on contact with matter means that items must be first cleaned before they can be effectively disinfected using sodium hypochlorite.

The efficacy of sodium hypochlorite in cleaning and disinfection processes depends on the concentration of available chlorine and the pH of the solution. Hypochlorous acid (HOCl) is a weak acid and dissociates to the hypochlorite ion (OCl⁻) and proton (H⁺), depending on the solution pH. It is generally believed that HOCl is the active compound in the germicidal action, whereas the concentration of OCl⁻ is a key factor determining the cleaning efficiency. This implies that the optimal pH for the germicidal activity of sodium hypochlorite differs from that for its cleaning activity.²⁹ Activity is reduced in the presence of heavy metal ions, biofilms, organic material, low temperature, low pH or UV radiation.²⁸

Hypochlorites are lethal to most microbes, although viruses and vegetative bacteria are more susceptible than endospore-forming bacteria, fungi and protozoa. Clinical uses in healthcare facilities include hyperchlorination of potable water to prevent *Legionella* species colonisation, chlorination of water distribution systems used in haemodialysis centres, cleaning of environmental surfaces, disinfection of laundry, local use to decontaminate blood spills, disinfection of equipment, decontamination of medical waste prior to disposal and dental therapy. Despite the increasing availability of other disinfectants, disinfectants based on hypochlorites continue to find wide use in hospitals.²⁸

Household bleach (0.0314%, 0.0933% and 0.670% sodium hypochlorite, pH 8.36–10.14) produced a >5 log reduction in *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella typhimurium* pathogens after 1 min at 25°C.³⁰

Oxidising agents

Hydrogen peroxide

Hydrogen peroxide is often flushed directly into contaminated or infected wounds where its effervescent action and increased oxygenation retard anaerobic bacteria. It should not be used on closed wounds because of the risk of embolism.⁹ It is also used as a disinfectant for nebuliser and anaesthetic equipment.⁹ Hydrogen peroxide is not very stable and dissociates into H₂O and O₂.

After 1 min at 25°C, 3% hydrogen peroxide (pH 2.75) achieved a >5 log reduction in both *S typhimurium* and *E coli* O157:H7. Compared

Properties of an ideal disinfectant

- ❖ Broad antimicrobial activity
- ❖ Rapid bactericidal action
- ❖ Reasonable persistence in treated potable water
- ❖ Ease of use
- ❖ Solubility in water
- ❖ Relative stability
- ❖ Relative non-toxicity at used concentrations
- ❖ No poisonous residues
- ❖ No colour
- ❖ No staining
- ❖ Low cost
- ❖ Ready availability

From Rutala and Weber²⁸

with 1 min at 25°C, greater reductions in *L monocytogenes* ($P < 0.05$) were obtained after 10 mins of hydrogen peroxide treatment at an initial temperature of 55°C.³⁰

Potassium peroxymonosulfate

Potassium peroxymonosulfate is an oxidising disinfectant that is usually combined with a surfactant and inorganic buffer in commercially available preparations.⁹ It is highly bactericidal and virucidal, even against parvovirus (when exposed for 10 mins).⁹ However, there is concern that it can corrode surfaces.

Potassium peroxymonosulfate has been shown to significantly reduce FCV titres.^{20,31}

Peracetic acid

Peracetic acid (peroxyacetic acid or PAA) is an organic compound with the formula $\text{CH}_3\text{CO}_3\text{H}$; it is generated in situ by some laundry detergents. It is a weaker acid than acetic acid, and is always sold in solution with acetic acid and hydrogen peroxide to maintain the stability of the peracid. It is corrosive due to the acetic acid; however, additives in some commercial products reduce this side effect.

Faecal indicator bacteria (*Enterococcus faecium*), virus indicator (male-specific [F⁺] coliphages [coliphages]), and protozoa disinfection surrogate (*Bacillus subtilis* spores [spores]) were tested by Park et al.³² Scanning electron microscopy revealed that peracetic acid targets the external layers of spores. Concentrations of 5 ppm (contact time: 5 mins), 50 ppm (10 mins) and 3000 ppm (5 mins) were needed to achieve a 3 log reduction of *E. faecium*, coliphages and spores, respectively.

Peracetic acid concentrations as low as 0.0025% were effective in decreasing *Salmonella* species artificially applied to chicken carcasses, while concentrations of 0.02% were effective in decreasing *Campylobacter* species, extending the shelf-life of the carcasses to 15 days.³³

Pruss et al.³⁴ studied the antimicrobial efficacy of a peracetic acid–ethanol sterilisation (PES) procedure in allogenic avital bone transplants against three enveloped viruses (human immunodeficiency virus type 2, Aujeszky's disease virus, bovine virus diarrhoea virus) and three non-enveloped viruses (hepatitis A virus, poliovirus, porcine parvovirus). PES led to a reduction in virus titres of more than 4 log₁₀. Only hepatitis A virus showed a reduction below 4 log₁₀ (2.87) with residual infectivity. For *Staphylococcus aureus*, *E. faecium*, *Pseudomonas aeruginosa*, *Bacillus subtilis* (including spores), *Clostridium sporogenes*, *Mycobacterium terrae*, *Candida albicans* and *Aspergillus niger*, a titre reduction below the detection level (5 log₁₀) was achieved after an incubation time of 2 h.

Disinfection is a potent means of reducing the number of pathogens on a surface. It does not result in sterility and is always non-specific.



Aldehydes

Chlorhexidine

Chlorhexidine gluconate is widely used as a patient/surgeon skin scrub, and for hand hygiene (both wet washing and rubs). Its antimicrobial activity occurs more slowly than that of alcohols. Both chlorhexidine and povidone–iodine cause an immediate reduction in bacteria; however, the reduction when using chlorhexidine is more dramatic. Povidone–iodine shows a lack of cumulative and residual activity in comparison with chlorhexidine.³⁵

Resistance to chlorhexidine has been described.^{36,37} Also, multiple nosocomial outbreaks have been linked to contaminated chlorhexidine.²⁴ Most reports have been traced to the use of contaminated water to prepare diluted preparations and/or the practice of reusing bottles to dispense chlorhexidine without adequate disinfection. Although most outbreaks have occurred with solutions containing less than 2% chlorhexidine, an outbreak has been reported with solutions of 2–4% chlorhexidine.²⁴

Chlorhexidine was shown to be ineffective against FCV.²⁷

Jarral et al conclude their review of 593 papers thus: '[T]here is no evidence suggesting the use of chlorhexidine during hand scrub reduces surgical site infections, which perhaps explains why guidelines from the World Health Organization, the Centers for Disease Control and Prevention and the Association for Perioperative Practice do not recommend one specific antimicrobial over another for hand scrub.'³⁵

Iodine/iodophors

Iodine has broad-spectrum activity against gram-positive and gram-negative bacteria, fungi, protozoa and, to some extent, viruses.^{9,24} Destruction of bacterial spores requires moist contact for more than 15 mins.⁹ Iodine is widely used as a preoperative scrub on patients' skin. It has a synergistic effect when combined with alcohol and, since it is only slightly soluble in water, it tends to be dissolved in alcohol.

Iodophors are less irritating to skin than iodine compounds,²⁴ and are non-staining.

Iodine surgical scrub was effective in killing MRSA³⁸ and parvovirus.²²

Quaternary ammonium compounds/benzalkonium chloride

The QACs are chemicals that alter the surface tension of an organism and are classed as cationic detergents. They are used for disinfection but are inactivated by organic material, soap and hard water. They are fungicidal, bactericidal and virucidal against some

enveloped viruses at medium concentrations, but there is no evidence that they are effective against parvovirus.⁹ Benzalkonium chloride was unable to eradicate a mature *Salmonella* biofilm (though reduced an immature one).³⁹ Scorza and Lappin⁴⁰ claimed that the compound Roccal (Winthrop Laboratories, New York) was effective at inactivating *Giardia* cysts.

Bacterial adaptation to QACs is documented. Worryingly, exposure to gradually increasing concentrations of this type of disinfectant results in reduced susceptibility not only to the QACs themselves but also to antibiotics, as well as cross-resistance to phenicol compounds (florfenicol and chloramphenicol) in 90% of *E coli* strains.⁴¹ Extensive use of QACs at subinhibitory concentrations may lead to the emergence of antibiotic-resistant bacteria and may represent a public health risk.⁴¹

Household products

Sodium bicarbonate

The advantages of sodium bicarbonate over the available chemical disinfectants for food contact surfaces are its safety, ready availability and low cost. Sodium bicarbonate at concentrations of 5% and above was found to be the most effective, with 4 log₁₀ (99.99%) reduction in FCV titres on food contact surfaces with a contact time of 1 min. Virucidal efficacy was enhanced when sodium bicarbonate was used in combination with aldehydes or hydrogen peroxide.⁴² However, sodium bicarbonate was shown to be ineffective against *L monocytogenes*, *E coli* O157:H7, and *S typhimurium*, even after 10 mins at 55 °C.³⁰ Therefore, since bacterial reduction is important in the disinfection of food contact surfaces, it is preferable to use a cat-safe disinfectant (eg, sodium hypochlorite) and thoroughly wash it off (preferably with very hot [$>60^{\circ}\text{C}$] water).

Acetic acid (household vinegar)

Cheap and readily available, household vinegar (2.5% and 5% acetic acid) can be used for cleaning as well as for cooking. After 1 min at room temperature (25°C) undiluted vinegar (pH 2.58) reduced *S typhimurium* by over 5 logs; and at a starting temperature of 55°C, exposed for 10 mins, it significantly reduced *L monocytogenes*.³⁰ However, acetic acid fumes make it fairly unpleasant to work with and it is unlikely that it would be chosen in practice over a commercially available disinfectant.

Citric acid (lemon juice)

A 5% solution of citric acid reduced *L monocytogenes* after 10 mins at an initial temperature of 55°C.³⁰ However, little is known about the general disinfectant properties of citric acid.

Recent advances in nanotechnology have paved the way for using pure silver against a wide array of pathogens – particularly multiresistant bacteria, which are hard to treat with available antibiotics.



Essential oils

Essential oils have been shown to have some effect against *M canis* in vitro and in vivo.⁴³ A mixture composed of 5% *Origanum vulgare*, 5% *Rosmarinus officinalis* and 2% *Thymus serpyllum*, in sweet almond oil, was administered to seven infected, symptomatic cats: four of the seven cats recovered.⁴³ Vázquez-Sánchez et al⁴⁴ evaluated the potential of 19 essential oils in removing the foodborne pathogen *S aureus* from food-processing facilities: thyme oil was the most effective. Thosar et al⁴⁵ evaluated five essential oils against four common human oral pathogens (*S aureus*, *Enterococcus faecalis*, *E coli* and *C albicans*); eugenol oil (oil of cloves), peppermint oil and tea tree oil exhibited significant inhibitory effects.⁴⁵

However, the antimicrobial activity of essential oils is due to a number of small terpenoids and phenol compounds;⁴⁵ since these are toxic to cats, essential oils should only ever be used under supervision of a qualified veterinary surgeon. Essential oil toxicity has been reported (see Table 1, page 601).^{46–48}

Silver compounds

Silver has been used for centuries for making cutlery and dishes, based on an innate understanding of its antimicrobial action. The antibacterial, antifungal and antiviral activities of silver have generated a lot of interest in recent years. A wide variety of applications of silver has recently emerged for consumer products, ranging from disinfecting medical devices, textiles, cosmetics and home appliances to water treatment. The antimicrobial action of silver or silver compounds is proportional to the bioactive silver ion (Ag^+) released and its availability to interact with bacterial or fungal cell membranes. Silver metal and inorganic silver compounds ionise in the presence of water, body fluids or tissue exudates. The silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes. Bacterial (and probably fungal) sensitivity to silver is genetically determined and relates to the level of intracellular silver uptake and its ability to interact with and irreversibly denature key enzyme systems.⁵⁴

Recent advances in nanotechnology have enabled the production of pure silver as nanoparticles, which are more efficient than silver ions. This has paved the way for new strategies for using pure silver against a wide array of pathogens – particularly multiresistant pathogens, which are hard to treat with available antibiotics.⁵⁵ It is believed that the silver nanoparticles are able to interact with disulphide bonds of the glycoprotein/protein

Toxicity of disinfectants to cats

Due to the cat's fastidious eating habits, there are fewer feline toxicity incidents than there are canine.^{48,58–61} Nevertheless, cats spend an estimated 5–25% of their waking time in grooming; hence disinfectants used in the cat's environment (home, shelter, veterinary surgery, etc) must be safe in case inadvertent ingestion via grooming occurs. Additional sources of toxicity include transdermal absorption;^{50,62} or inhalation of irritant or toxic fumes. The cat may present with caustic burns to the paws or other areas that are in direct contact with disinfectant, and/or ulceration of the tip of the tongue and oesophagus through attempting to groom the toxin off.^{9,63,64}

Possible poisoning by household products was the second most common reason (after ingestion of drugs) for telephone calls to the Kansas State University between 2009 and 2012: 15.5% of 1616 calls were related to potential poisoning of dogs and cats by household products; and, of those, 17 calls related to cats and household cleaners.⁵⁸ However, it is worth emphasising that in most reports on domestic animal poisoning, disinfectants do *not* play a major role – the major culprits being human medications, ethylene glycol, lead, lily plants and topical pesticides.^{59,61,64–71}

Deficiency of the enzyme UDP-glucuronosyl transferase renders cats extremely sensitive to the adverse effects of

phenol-based products (see below). Actual case reports of disinfectant toxicity in the literature are few and far between, with most published papers on toxicity in the cat having been deliberately perpetrated in the name of science. Disinfectant toxicity in cats is summarised in Table 1.

Susceptibility to phenols

The domestic cat (*Felis catus*) shows remarkable sensitivity to the adverse effects of phenolic compounds, including acetaminophen and aspirin, as well as structurally related toxicants found in the diet and environment.⁷² This idiosyncrasy results from pseudogenisation of the gene encoding UDP-glucuronosyltransferase (UGT) 1A6, the major species-conserved phenol detoxification enzyme.⁷² Glucuronidation is quantitatively the most important of the six routes by which xenobiotics (toxins) are conjugated, and therefore eliminated, from the body.⁵¹ Cats have a carnivorous diet and, as a result of lack of exposure to plant-based toxins (phytoalexins), have presumably lost the need to metabolise these toxins via glucuronidation, which is common in most herbivores and omnivores.⁷²

contents of microorganisms such as viruses, bacteria and fungi.⁵⁵ Silver nanoparticles are attractive because they are non-toxic at low concentrations and have broad-spectrum antibacterial action against at least 12 species of bacteria including multiresistant MRSA, multidrug-resistant *P aeruginosa*, ampicillin-resistant *E coli* O157:H7 and erythromycin-resistant *Streptococcus pyogenes*.⁵⁵

There is a growing trend for developing food-packaging materials with antimicrobial properties. Martínez-Abad et al⁵⁵ incorporated silver ions into polylactic acid (PLA) films. The films demonstrated strong antimicrobial efficacy against *Salmonella enterica* and FCV in vitro, with increasing effects at higher silver concentrations. In vivo, antimicrobial activity was very much dependent on the food type and temperature: in lettuce samples incubated at 4°C for 6 days, 4 log colony forming units of *Salmonella* were inactivated for films with 1.0 wt % and no infectious FCV was reported under the same conditions. On paprika samples, no antiviral effect was seen on FCV infectivity and films showed less antibacterial activity on *Salmonella*.

Advances in biotechnology have enabled incorporation of ionisable silver into fabrics for clinical use to reduce the risk of nosocomial infections and for personal hygiene.⁵⁴ Although veterinary use of silver has not yet taken off, in 2012, Woods et al⁵⁷ reported the use of a combination of nanocrystalline silver dressing and subatmospheric pressure

Antiparasitic disinfection in cat husbandry relies on thorough cleaning and, whenever possible, heat treatment to minimise the number of infectious parasites.



therapy to treat a resistant wound infection, following tumour removal and radiation therapy, in a difficult-to-manage surgical site in a cat.

Chemical disinfectants against parasites

In Europe there is no uniform protocol for efficacy testing of chemical disinfectants against parasitic infections. The only guideline available is from the German Veterinary Medical Society (DVG), with the test organisms being oocysts of the coccidia species *Eimeria tenella* and eggs of the nematode *Ascaris suum*. The specific context for this testing is the disinfection of large animal housing. The disinfectants that pass this test are exclusively products based on cresols and phenols – substances that are considered highly toxic for cats. Products based on other active substances, such as aldehydes and peracetic acid, have not been tested against these agents or have been shown not to be efficacious (U Truyen, personal communication).

Antiparasitic disinfection in cat husbandry has, therefore, to rely on thorough cleaning and, whenever possible, heat treatment to minimise the number of infectious parasites.

Summary

Table 2 presents a summary of the disinfectants discussed in these guidelines. The unique metabolism of cats requires that extra caution is taken when using disinfectants around them.

Table 1 Reported toxicity in cats associated with disinfectant use

Substance	Clinical signs	Treatment	Reference
Benzalkonium chloride	Chemical burns when put undiluted onto skin, conjunctiva or mucosae. Cats also developed oral and oesophageal ulceration after licking treated skin		Greene et al ⁹
Hexachlorophene*	Hindlimb paralysis in 3–5 days. Cardiovascular collapse, corneal ulcers, trembling, lethargy and weakness. Status spongiosis, astrocytosis, and microgliosis of the cerebral and cerebellar white matter and corticospinal tracts	Slow IV administration of 30% urea (2 g/kg in 10% invert sugar)	Hanig et al ⁴⁹ Thompson et al ⁵⁰
Phenol	Dark green urine Carcinogen		Garg ⁵¹ Shukla ⁵²
Pine oil containing disinfectant (eg, Pine-Sol; Clorox)	Unresponsive pupils and extreme ataxia were observed prior to death. Pathological changes consisted of severe acute centrilobular hepatic necrosis and renal cortical necrosis		Rousseaux et al ⁵³
Essential oils in flea treatment (peppermint oil, cinnamon oil, lemongrass oil, clove oil, thyme oil)	In a study of 39 cats and 9 dogs with a history of exposure to natural flea preventives, the onset of adverse effects (agitation, anorexia, erythema, fasciculation, hiding, hyperactivity, hypersalivation, hypothermia, lethargy, panting, retching, seizures, tachycardia, tremors, vocalisation, vomiting, weakness) occurred within 24 h in 39 of 44 animals. The duration of signs in 24 animals ranged from 30 mins to 149 h. The products were used as per label in 77% of animals (n = 37). Death (1 cat; n = 1/28; 4%) or euthanasia (1 cat and 1 dog; n = 2/28; 7%) was reported in three animals	Of 28 animals with known outcome, 50% (n = 14) recovered with bathing alone while others received intravenous fluids, muscle relaxants, and anticonvulsive medications	Genovese et al ⁴⁶
Tea tree oil	Clinical signs (increased salivation/drooling, signs of CNS depression or lethargy, paresis, ataxia, tremors, hypothermia, coma, dehydration, elevated AST and ALT) developed within 2–12 h and lasted up to 72 h. A significant association with severity of illness was found for age and weight, with a higher prevalence of major illness in younger and smaller cats	Wash off oil, activated charcoal per os, dexamethasone	Bischoff and Guale ⁴⁷ Khan et al ⁴⁸

*Now banned worldwide because of its high rate of dermal absorption and subsequent toxic effects
CNS = central nervous system, AST = aspartate aminotransferase, ALT = alanine transaminase

Table 2 Recommended disinfection for use around the cat (continued on page 602)

Disinfectant	Concentration/dilution	Uses	Comments
Heat and steam	Recommended temperature–pressure–exposure time to produce sterilisation with an autoclave is 121°C at 15 psi for 15 mins or 126°C at 20 psi for 10 mins. Prions require a heat of 130°C for 30–60 mins to inactivate. ⁹ For washing machines/dishwashers, a 30 min cycle at 60°C is required	Instruments, floors, work surfaces, dishes, bedding	The most effective, safe and broad spectrum of disinfection methods. Moist heat (steam) is the most effective for eliminating protozoal oocysts such as <i>Toxoplasma</i> and <i>Isospora</i> . In outbreaks of enteric infections, cardboard litter trays, which can be incinerated, can be used
Sodium hypochlorite (bleach)	5–6% bleach diluted at 1:32 or less, depending on use*	Water decontamination, cleaning surfaces, food utensils, litter trays, floors, laundry, instruments and foot baths*	The best all-round chemical disinfectant. Inactivated by organic debris. One of the few chemicals that will inactivate parvovirus and kill clostridial spores. Loses activity if stored for a long time. ⁹ Caution: can release toxic chlorine gas
Alcohol		Hand rubs are more likely to be used than hand washes ³ and reduce bacterial and viral titres more effectively	Contamination of alcohol-based solutions has rarely been reported. ²⁴ Ineffective against parvovirus ²⁶
Ethanol	70–90% concentration for 1 min – the higher the concentration, the more effective. At least 90% concentration required for MRSA control ³⁸	Used along with isopropanol in rubbing alcohol/surgical spirit and in hand sanitisers	More effective against FCV than isopropanol, ⁷³ but poor activity against all non-enveloped viruses. ²⁵ No sporicidal activity

Table 2 Recommended disinfection for use around the cat (continued from page 601)

Disinfectant	Concentration/dilution	Uses	Comments
Isopropanol	40–60% concentration for 1 min	Used along with ethanol in rubbing alcohol/ surgical spirit and in hand sanitisers	Less effective than ethanol against FCV ⁷³
Hydrogen peroxide		Initial flush for wounds for its effervescent action and oxygenation, which retards anaerobes	Do not use in closed wounds (risk of air embolism)
Sodium bicarbonate	5% for 1 min is effective against FCV ⁴²	Can be used on hands, and food surfaces and containers	Cheap and safe, but not effective against some bacteria, ³⁰ so not recommended
Acetic acid (household vinegar)	Undiluted vinegar (pH 2.58) (2.5% and 5% acetic acid) for 1 min at room temperature will reduce <i>Salmonella typhimurium</i> , and at a starting temperature of 55°C for 10 mins will reduce <i>Listeria monocytogenes</i> ³⁰	Food surfaces and containers	No information about activity against viruses/parasites. Unlikely to be used in practice due to odour
Citric acid	5% citric acid solution for 10 mins	Food surfaces and containers	Reduces <i>L. monocytogenes</i> after 10 mins at an initial temperature of 55°C. ³⁰ Efficacy against other pathogens unknown
Chlorhexidine	0.5% in water, saline, lactated Ringer's solution or alcohol ⁹	Preoperative skin scrub and hand wash. Gives up to 2 days' antiseptic protection of skin after a single application ⁹	Does not inactivate FCV ²⁷ or dermatophytes (though works with miconazole). Should never be used in the ear (ototoxic) ⁷⁴ or eye ⁹ Skin irritant at ≥4% concentration ³
Iodine/iodophors	1–10% solution applied topically	Preoperative patient/surgeon skin scrub. 1:50 dilution of povidone-iodine for ocular preoperative surface disinfection. Hand rub	Can be skin irritant. Iodine surgical scrub has proven effective in killing MRSA. ³⁸ Synergistic effect when used with alcohol
Potassium peroxymonosulfate		Cleaning surfaces and instruments Foot baths	Bactericidal and virucidal, even against parvovirus (10 mins exposure). Good activity in presence of organic material. Can even be used on carpets. However, can corrode surfaces. Proven efficacy against FCV
Quaternary ammonium compounds (eg, benzalkonium chloride)	0.001% to 1%	Used as soap and antiseptic. Have unusual ability to kill <i>Giardia</i> cysts at 4°C and room temperature	Algicidal, fungicidal, bactericidal and virucidal against some enveloped viruses. Do not reliably inactivate FCV, herpesvirus and parvovirus. Harbour opportunistic bacteria (eg, <i>Serratia</i> species). ^{9,24} Inactivated by organic materials, soap and hard water. Concern about widespread use leading to antibiotic resistance, ⁴¹ so not recommended , except possibly where there is <i>Giardia</i> infection
Phenol-based; eg, hexachlorophene, essential oil of tea tree or clove (eugenol)			Not recommended around cats: toxic and caustic
Ultraviolet-C radiation	Fluence ≥30 mJ/cm ²	For reducing bacterial contamination in whole rooms	FCV is more resistant than parvovirus to UV-C. ²⁰ Effective against enterococci and <i>C. difficile</i> but not <i>Acinetobacter</i> . ²³ Decreased efficacy in presence of organic material ²⁰
Silver compounds		Impregnated wound dressings	Safe antimicrobial but at present in cats has only been used in wound dressings

This table lists disinfectants used in veterinary practices and around the home, showing the most notoriously difficult to eradicate pathogens as sentinels for efficacy

*For a detailed examination of the uses of bleach, see table 93-1 in Greene et al.⁹ FCV = feline calicivirus

KEY POINTS

- ❖ Disinfectants should be chosen on the basis of efficacy, safety for cats and safety for human users.
- ❖ Cats are susceptible to phenol-based disinfectants, including certain essential oils.
- ❖ Heat, especially moist heat, is the safest and most effective disinfectant. Temperatures in washing machines and dishwashers should be at least 60°C to eliminate pathogenic spores and resistant viruses.
- ❖ Alcohols, and particularly ethanol, have poor activity against all non-enveloped viruses.
- ❖ Bleach is effective and relatively safe, but its activity is reduced by the presence of heavy metal ions, biofilms, organic material, low temperature, low pH or UV radiation.
- ❖ Enveloped viruses are susceptible to most disinfectants; of the non-enveloped viruses, parvovirus is universally recognised as being the most difficult to eradicate.
- ❖ Skin scrubs and rubs containing alcohols are more effective than those containing chlorhexidine, and less subject to contamination.
- ❖ Quaternary ammonium compounds (eg, benzalkonium chloride) are probably best avoided.
- ❖ The future of veterinary disinfection may include ultraviolet radiation and more use of silver.



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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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FELINE INJECTION-SITE SARCOMA

ABCD guidelines on prevention and management



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Overview: In cats, the most serious of adverse effects following vaccination is the occurrence of invasive sarcomas (mostly fibrosarcomas): so-called ‘feline injection-site sarcomas’ (FISSs). These develop within the skin at sites of previous vaccination or injection. They have histological characteristics that are distinct from those of fibrosarcomas in other areas and they behave more aggressively. The rate of metastasis ranges from 10–28%.

Pathogenesis: The pathogenesis of these sarcomas is not yet definitively explained. However, chronic inflammatory reactions are considered the trigger for subsequent malignant transformation. Injections of long-acting drugs (glucocorticoids, penicillin, lufenuron and others) have been associated with sarcoma formation. Adjuvanted vaccines induce intense local inflammation and seem therefore to be particularly linked to the development of FISS. The risk is lower for modified-live and recombinant vaccines, but none are risk-free.

Treatment and prevention: Aggressive, radical excision is required to avoid tumour recurrence. The prognosis improves if additional radiotherapy and/or immunotherapy (recombinant feline IL-2 is commercially available in Europe) are used. For prevention, administration of irritating substances should be avoided. Vaccination should be performed as often as necessary and as infrequently as possible. Non-adjuvanted, modified-live or recombinant vaccines should be selected in preference to adjuvanted and inactivated vaccines. Injections should be given at sites at which surgery would likely lead to a complete cure; the interscapular region should generally be avoided. Post-vaccination monitoring should be performed.

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Introduction

Recently, vaccination of cats has received scientific and public attention linked to the supposition that a range of rare adverse effects can arise following vaccination. In cats, the most serious of these adverse consequences is the occurrence of invasive sarcomas (mostly fibrosarcomas), so called ‘feline injection-site sarcomas’ (FISSs), that can develop within the skin at sites of previous vaccination. Despite extensive research on the pathogenesis of these sarcomas, there is no definitive causal relationship that explains their occurrence and the direct link to vaccination. The most accepted hypothesis suggests that a chronic inflammatory reaction at the site of injection provides a trigger for subsequent malignant transformation.

Epidemiology and characterisation

In 1991, an increased incidence of tumours in cats that developed at injection sites was first reported in the United States.¹ This observation was connected to an increased use of rabies and feline leukaemia virus (FeLV) vaccinations.^{2,3} As a consequence, these tumours were first called feline ‘vaccine-associated sarcomas’. However, the subsequent finding that other, non-vaccinal injectables can also cause this type of tumour has led to reclassification of these neoplasms as ‘feline injection-site sarcomas’ (FISSs). These tumours seem to be unique to cats,⁴ although comparable tumours have been reported in ferrets⁵ and very occasionally in dogs.⁶

FISSs occur at sites typically used for vaccination and injections, such as the interscapular region (Figure 1), the lateral thoracic or abdominal wall, the lumbar region, and the area of the semimembranosus and semitendinosus muscles. FISSs are most commonly located in the subcutis, but also can occur intramuscularly.^{7,8}

FISSs can occur as early as 4 months and up to 3 years after an injection. They are characterised by invasive local growth in the subcutis, often with spread along fascial planes.⁹ Most FISSs are fibrosarcomas,¹⁰ but other malignancies, such as osteosarcomas,¹¹ chondrosarcomas,⁷



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rhabdomyosarcomas,⁷ malignant fibrous histiocytomas,^{7,11} and myofibroblastic sarcomas⁸ have also been described.

FISSs have histological characteristics that are distinct from those of fibrosarcomas in other areas. Typically there is perivascular infiltration of lymphocytes and macrophages at the tumour periphery, a central area of necrosis, inflammation and local infiltration of tumour cells (Figure 2).^{10,12} FISSs behave more aggressively than sarcomas at other sites.¹³ The rate of metastasis ranges from 10–28%.^{14,15} The lung is the most common site of metastasis, followed by regional lymph nodes and abdominal organs, such as the kidney, spleen, intestine and liver.^{16,17}

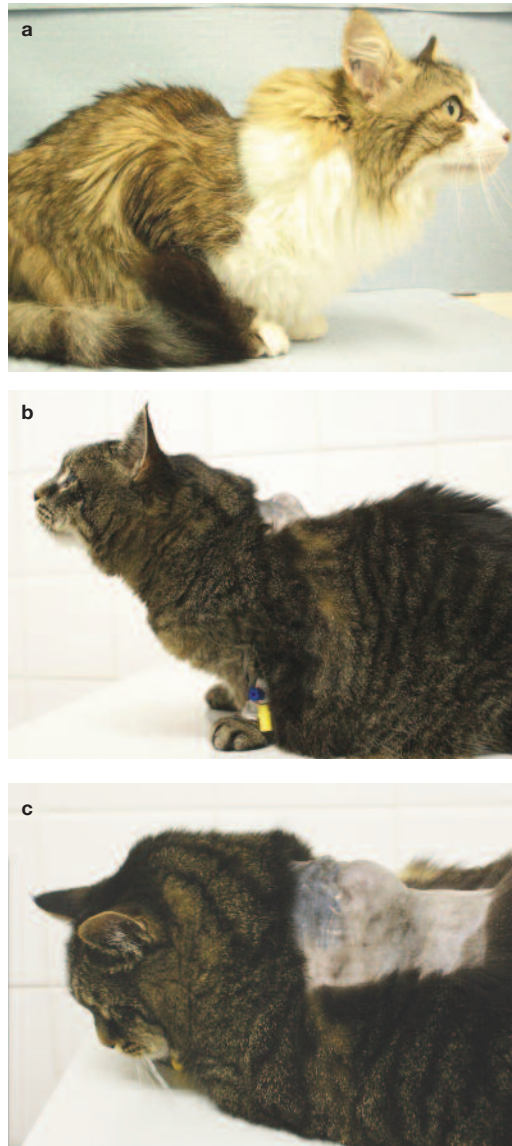
In the past 20 years, an epidemiological association has been demonstrated between vaccination and the later development of FISS.^{3,13,18–21} The incidence of FISS has been estimated at 1–4 in every 10,000 vaccinated cats in the USA,^{22,23} and the ratio of injection-site to non-injection-site sarcomas increased from 0.5 in 1989 to 4.3 in 1994.¹⁰ In one study in the USA, reported rates of reaction were 0.3 FISSs per 10,000 vaccinations and 11.8 postvaccinal inflammatory reactions per 10,000 vaccinations in cats.²² If inflammatory reactions are a necessary prelude to FISS, then these rates suggest that 1 in 35–40 inflammatory reactions develop into FISS. In the UK, the incidence of FISSs seems to be relatively low (incidence risk of FISS per year was estimated to be 1/16,000–50,000 cats registered by practices, 1/10,000–20,000 cat consultations, and 1/5000–12,500 vaccination visits).²⁴ One reason for the low rate might be that rabies vaccination is not a routine procedure for cats in the UK. One study in Canada investigated the annual prevalence of feline postvaccinal sarcomas among 11,609 feline skin mass submissions from 1992 to 2010 and revealed no decrease in disease prevalence or increase in age of affected cats in response to change in vaccination formulation or recommended changes in feline vaccination protocols.²⁵

Pathogenesis

Despite extensive research, there is no definitive proof of the pathogenesis of FISS. The most widely accepted hypothesis suggests that a chronic inflammatory

FISSs are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

Figure 1 (a–c) Cats with feline injection-site sarcoma. Courtesy of Johannes Hirschberger, Ludwig Maximilians University, Munich, Germany

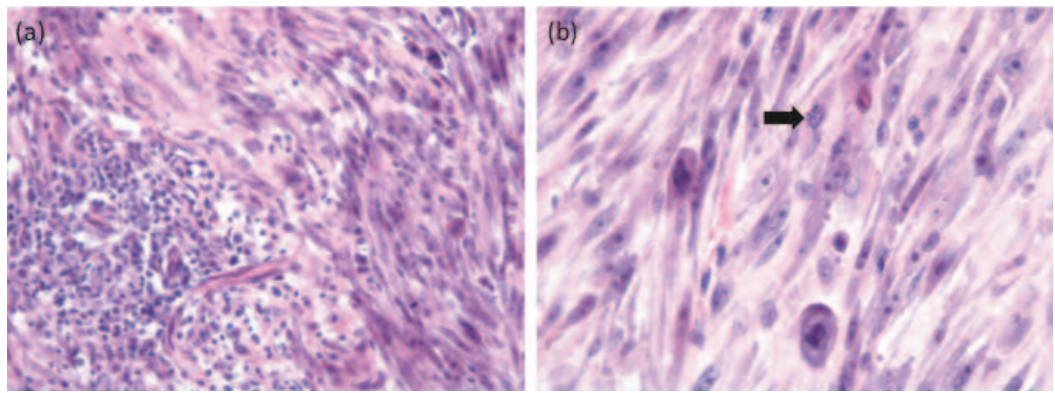


reaction at the site of an injection acts as a trigger for subsequent malignant transformation. Adjuvanted vaccines seem to be particularly linked to the development of FISS due to the more intense local inflammation associated with such products. This idea is supported by frequent identification of adjuvants in histological or ultrastructural investigations of these sarcomas.^{12,18}

Many data suggest an association between vaccination and FISS in cats. Aluminium, a vaccine adjuvant, has been found in biopsy samples of FISS.²⁶ In most inactivated vaccines, an adjuvant is added to enhance the inflammation at the site of injection, which is intended and necessary when applying a killed agent in order to trigger the necessary immune response. However, this inflammation might potentially lead to malignant transformation. Traces of adjuvants can be seen in the inflammatory reaction, specifically accumulated within macrophages or multinucleate giant cells, and later in histological sections of FISS in the transformed fibroblast.¹⁸ Intracellular crystalline particulate material was found in an ultrastructural study in five of 20 FISSs investigated, and in one of the five cases was identified as aluminium-based.¹² Although no specific vaccine or adjuvant has been incriminated,²⁷ local irritation from adjuvant is thought to stimulate mainly fibroblasts to the point that malignant transformation occurs.

At first, only rabies and feline leukaemia virus (FeLV) vaccines were identified as risk factors,^{3,13,23} but subsequently other vaccines, including vaccines against feline panleukopenia virus (FPV), feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) were also found to be involved in the development of FISS in some cases.^{13,23,28–30} In addition to vaccines, injections of long-acting drugs, such as glucocorticoids, penicillin, lufenuron,^{27,31,32} cisplatin³³ and meloxicam,³⁴ have been associated with sarcoma formation. One study found that the frequency of administration of long-acting corticosteroid injections (dexamethasone, methylprednisolone and triamcinolone) was significantly higher in cats with FISS in the interscapular region than in control cats.³⁵ Fibrosarcomas

Figure 2 Histological sections of a 2 cm diameter mass removed from the lateral thorax of a 13-year-old domestic shorthair cat. A similar interscapular mass had been removed from this cat 2 months previously. (a) A focus of lymphoplasmacytic inflammation is contained within the surrounding sarcoma. (b) Higher magnification of the neoplastic tissue reveals a pleomorphic population of neoplastic spindle cells with occasional giant nuclei and irregular mitotic activity (arrow). Haematoxylin and eosin stain. Courtesy of Michael Day, School of Veterinary Sciences, University of Bristol, UK



were also reported: at the site of a deep, non-absorbable suture in one cat;³⁶ around a surgical swab in the abdomen of one cat;³⁷ adjacent to the site of microchip implantation in two cats;^{38,39} and associated with a subcutaneous fluid port device.^{38,39} This suggests that all inflammatory reactions, theoretically, have the potential to lead to the development of FISS by triggering uncontrolled proliferation of fibroblasts and myofibroblasts, which, in some cases, results in malignant transformation.

Although many causes of inflammation are associated with FISS development, the risk seems to be higher for vaccines compared with other injections; among vaccines, the risk seems to be higher when adjuvanted vaccines are used. Srivastav et al³⁵ compared associations between vaccine types and other injectable drugs with the development of FISS in a case-control study of 181 cats with soft tissue sarcomas (cases), 96 cats with tumours at non-vaccine regions (control group 1), and 159 cats with basal cell tumours (control group 2). There was a clear association between the administration of various types of vaccines and other injectable products (eg, long-acting corticosteroids) and FISS development. Of 192 cats with sarcoma, 101 had vaccinations at the site of tumour development during the preceding 3 years, and 23 had received other injections.³⁵ This study also showed that adjuvanted inactivated vaccines were significantly more commonly associated with FISS development than other vaccines; of 35 vaccinated cats with sarcoma on the hindlimb, 25 had received adjuvanted vaccines, seven cats had received modified-live virus (MLV) vaccines (FPV, FHV-1 and FCV), and only one cat had received a recombinant vaccine. These findings also indicated that no vaccines were risk-free.³⁵

The mechanism by which the inflammatory reaction causes tumour formation is not fully understood. Growth factors promote proliferation, can induce malignant transformation,

and also can be involved in the regulation of angiogenesis. Overexpression of growth factors and oncogene activation have been demonstrated in cats with FISS and are suspected to play a role in tumour development.⁴⁰⁻⁴²

As vaccination against FeLV is associated with a higher risk of FISS, some studies looked at a possible role of FeLV and its mutant feline sarcoma virus (FeSV) in the development of FISS, but could not detect either FeLV or FeSV in the tumours.⁴³ Furthermore, no other viruses, including feline immunodeficiency virus, feline foamy virus, polyomaviruses or papillomaviruses were detected in tumour tissues.⁴⁴⁻⁴⁷ No evidence has been found to implicate replication or expression of endogenous retroviruses in FISS formation.^{45,46}

The observation that not all cats develop FISS after vaccination suggests that there might be a genetic predisposition. It has been suggested that there is a higher incidence of FISS in siblings of affected cats, and that some cats tend to develop more than one FISS. Alterations with unknown relevance such as hyperploidy,⁴⁸ translocations⁴⁹ and triploidy⁵⁰ of oncogene and tumour suppressor loci have been found on extra chromosomes and monosomic chromosomes in affected cats. Mutations have been identified in the tumour suppressor gene p53, which is implicated in cancer initiation and progression in sarcoma tissue of cats with FISS.⁵¹⁻⁵⁵ A case-control study (50 domestic shorthair cats with a confirmed diagnosis of FISS and 100 disease-free matched controls) investigating a possible association between polymorphisms in the genomic sequence of the feline p53 gene and a predisposition to FISS, found a strong association between FISS and the presence of specific nucleotides at two of the polymorphic sites.⁵⁶ However, another study, conducted in Munich, Germany, could not reproduce these findings and observed no association with the polymorphisms described.⁵⁷

European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the guidance provided in this article is available at www.abcdcatsvets.org and www.abcd-vets.org

Management

Appropriate treatment should first include staging and careful planning of the surgery, because aggressive, radical excision is crucial to avoid tumour recurrence. The prognosis improves if, in addition to radical surgery, adjunctive treatments such as radiotherapy or immunotherapy are used. Preoperatively, (contrast-enhanced) computed tomography (CT) or magnetic resonance imaging (MRI) should be obtained for staging, and to determine the extent of the tumour and the size of the radiation field required to maximise the chance of a successful outcome.⁵⁸ It was shown that the actual size of tumours determined by CT could be twice that estimated at physical examination.^{59,60} Surgeons should attempt to achieve complete, en bloc, surgical tumour resection with at least 3 cm (ideally, 5 cm) margins⁶¹ [EBM grade III] and the removal of one fascial plane underlying the tumour, because incomplete resection can result in recurrence as early as 2 weeks after surgery [EBM grade III].^{28,62} Treatment using surgical excision alone has a recurrence rate of up to 70%, with tumour regrowth usually occurring in the first 6 months after surgery [EBM grade III].¹³ Tumour-free margins are very important for a longer disease-free interval, which was 700 days when complete tumour excision was accomplished, but only 112 days for incomplete resection [EBM grade III].⁶³ However, even with clean surgical margins, the recurrence rate can be as high as 50% [EBM grade III].⁶⁴

Preoperative or postoperative radiation therapy significantly decreases recurrence rates and prolongs remission times,^{16,63,65} while the benefit of chemotherapy is not proven as large prospective randomised controlled trials are lacking. One non-randomised study found no significant difference between control cats (surgery alone) and cats treated with surgery and doxorubicin [EBM grade III],⁶⁶ while a recent study demonstrated chemotherapy benefits compared with historical controls using a combination of neoadjuvant and adjuvant chemotherapy (three epirubicin doses before and after surgery) [EBM grade III].⁶⁷ Chemotherapy mainly remains an option for palliative treatment in cats with non-resectable FISS, when radiation therapy is not available.

Additional immunotherapy appears to be promising.⁶⁸⁻⁷⁰ Results of prospective randomised controlled studies of cytokine gene transfer techniques for adjuvant-immunological treatment of FISS showed reduced recurrence rates. In cats receiving gene therapy by the peritumoural administration of histoincompatible Vero cells expressing human interleukin-2 (hIL-2) in addition to surgery and

EBM grades

The ranking system for grading the level of evidence of various statements within the management and prevention sections of this article is described on page 574 of this Special Issue.

radiation therapy, only 5/16 (31%) had FISS recurrence, while 11/16 control cats (69%) that had surgery and radiation therapy, but no immunotherapy, had FISS recurrence within 16 months [EBM grade I].⁷¹ Use of neoadjuvant gene therapy using a non-viral vector that expresses feline granulocyte-macrophage colony-stimulating factor (GM-CSF) or a combination of the feline genes GM-CSF, interleukin (IL)-2 and interferon- γ (IFN- γ) was well tolerated by cats [EBM grade I]^{68,69} and showed promising results. Recombinant feline IL-2 is now commercially available in Europe for the treatment of FISS in combination with surgical excision and radiation therapy. In a randomised controlled clinical trial, administration of a recombinant canarypox virus expressing feline IL-2 was well tolerated and resulted in a significantly longer median time to relapse and a significant reduction in the risk of relapse at 1 year and 2 years [EBM grade I].⁷⁰

Prevention

Prevention consists of three general considerations (see below).

Key considerations in the prevention of FISS

- ✦ Injections in cats should always be given at sites at which surgery (such as amputation of a limb or excision of lateral abdominal skin) would likely lead to a complete cure with the least complicated surgical procedure
- ✦ General recommendations to reduce the inflammatory reaction at injection sites should be followed, such as avoiding the administration of irritating substances
- ✦ It is advised to vaccinate only as often as necessary and as infrequently as possible (eg, according to the principles of current vaccination guidelines, avoiding FeLV vaccination in FeLV antigen-positive, FeLV PCR-positive or FeLV antibody-positive cats)

Choice of injection site

In general, injecting distally in a leg aids, where necessary, in the subsequent treatment of sarcoma by amputation of the leg (because these tumours are very difficult to excise completely and often recur after resection).²⁰ Administration of vaccines (or other injections) between the scapulae is generally contraindicated because tumour resection is almost impossible in this location.

To assess the acceptance of the recommendations of the Vaccine-Associated Feline Sarcoma Task Force (VAFSTF), published in 1996, a study involving 392 cats with FISSs compared the anatomical locations of tumours between cases with FISS diagnosed before and after publication of these recommendations.⁷² The proportions of FISS significantly decreased in the interscapular (53% to 40%) and right and left thoracic (10% to 4% and 9% to 1%, respectively) regions, whereas

the proportions of FISS significantly increased in the right thoracic limb (1% to 10%) and the combined regions of the right pelvic limb with the right lateral aspect of the abdomen (13% to 25%) and the left pelvic limb with the left lateral aspect of the abdomen (11% to 14%). Thus, while veterinarians are complying with vaccination recommendations to some extent, a high proportion of tumours still developed in the interscapular region. There was also an increase in lateral abdominal FISSs, which could be attributable to aberrant placement of injections intended for the pelvic limbs. It remains the case that only administration of vaccines as distally as possible on a limb allows for complete surgical margins if limb amputation is required [EBM grade III].⁷³ Current data in Europe shows a similar situation. In a study examining the location of FISSs in cats presented to the oncology service at the University teaching hospital in Munich, most still occurred between the scapulae (40%), followed by the right (19%) and left thoracic walls (13%).⁷⁴

Unfortunately, there is still insufficient clinical information to enable evidence-based vaccine site recommendations. The majority of safety and efficacy data comes from licensing studies in which vaccines are administered subcutaneously in the interscapular region (which should not be used for any injection in the clinical setting). Current research indicates that radical surgical resection of injection-site sarcomas including margins of at least 3 cm, but preferably 5 cm [EBM grade III],⁶¹ is associated with the highest response rate and long-term survival [EBM grade III].¹⁵ With this in mind, the Feline Vaccination Advisory Panel of the American Association of Feline Practitioners (AAFP) conducted an informal survey of veterinarians whose practices focused on radiation (12), surgical (36), and medical (44) oncology for opinions on what the preferred vaccination sites should be.⁶² These experts agreed that distal to the stifle, followed by distal to the elbow, were their preferred sites. Nearly as popular was the tail. Respondents frequently commented that vaccines should be administered as low on the leg as possible. They added that vaccination of cats resting in a crouched position often resulted in inadvertent injection of the skin fold of the flank, leading to tumours that were difficult to resect.⁶² This is reflected in a recent paper that found an increase in lateral abdominal injection-site sarcomas since the publication of the VAFSTF's vaccination recommendations in 1996.⁶¹

Based on these expert opinions, the AAFP now recommends in its new guidelines,⁶² consistent with the earlier (2006) guidelines,⁷⁵ that vaccines against FPV, FHV-1 and FCV should be administered below the right elbow; FeLV vaccines should be administered below the

Although many causes of inflammation are associated with FISS development, the risk seems to be higher for vaccines (particularly adjuvanted vaccines) compared with other injections.



left stifle; and rabies vaccines should be administered below the right stifle.⁶² So far, vaccination in the tail has not been considered a practical option. However, a recent pilot study demonstrated that vaccination in the tail was well tolerated and that tail-vaccinated cats developed an antibody response comparable to that observed following injection of the vaccine distally in the leg [EBM grade II].⁷⁶ Further studies are warranted to confirm whether this would be an alternative option leading to equal protection rates.

Alternative recommendations are made by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association, which recognises the practical difficulties often faced by veterinarians attempting vaccination into limbs or the tail. The advice of the VGG is that an optimum site for vaccine delivery (and surgical resection of a FISS that might arise) is the skin over the lateral abdomen. This is a procedure that appears well tolerated in the majority of cats.

As a general recommendation, recording the sites of injections in the patient's medical records is important. In addition, post-vaccination monitoring plays an vital role (see box).

Recommendations for reducing inflammatory reactions

In terms of preventing inflammatory reactions at injection sites, there are a few recommendations to follow. Cats should receive as few subcutaneous injections as possible. Intramuscular injections in cats should be avoided because intramuscular tumours develop with a similar frequency, but are more difficult to detect early. Whenever feasible, cats should receive drugs orally or intravenously. The subcutaneous injection of long-acting irritating substances (such as long-acting glucocorticoids) should be avoided.

One study examined potential risk factors when administering vaccines²⁷ and few factors

Post-vaccination monitoring

Veterinarians should instruct their clients to monitor vaccination (and other injection) sites for swelling or lumps in order to detect potential sarcomas early and at a time while they still can be removed successfully.

Practitioners and owners should follow the '3-2-1' rule.

Incisional wedge biopsies or total removal and histological examination of any mass is warranted if the mass

is still present **3 months** after vaccination, if the mass becomes larger than **2 cm** in diameter, or is increasing in size **1 month** after vaccination.

Practitioners and owners should follow the '3-2-1' rule.

In general, a diagnostic work-up is warranted when any cutaneous mass is noted in a cat. FISSs are usually firm, indolent, seemingly well-circumscribed, subcutaneous masses that are often not freely moveable.

were associated with the development of FISS. It was observed that the size of the needle and the syringe, the velocity of injection, and whether manual pressure was applied after injection or not, played no role. In contrast, the temperature of the vaccine made a significant difference, with cold vaccines being associated with a higher risk of FISS development than vaccines at room temperature.²⁷ Thus, vaccines should be taken out of the refrigerator about 15 minutes before injection, but not much longer, to avoid reduction in vaccinal efficacy.

If available, intranasal or oral vaccines would be preferable over injectable vaccines in cats. However, in most countries only injectable vaccines are available. Therefore, vaccines are preferred that cause the least subcutaneous inflammatory reaction. Vaccines without adjuvants should be used rather than adjuvant-containing vaccines, which means that MLV or recombinant vaccines without adjuvant (eg, canarypox-vectored vaccine) are preferred over inactivated vaccines with adjuvants.

It has been shown that recombinant canarypox-vectored vaccines cause less inflammation at the injection site. This was demonstrated in rats,⁷⁷ and in a study in cats, in which the typical granulomatous inflammation did not develop at the injection site when using these particular vaccines.⁷⁸ An extensive study investigating the subcutaneous tissue response following administration of a single dose of multi-component vaccines confirmed these findings.⁷⁹ Three groups of 15 cats were injected with one of three vaccines or saline as a negative control; cats in group A received a non-adjuvanted recombinant canarypox-vectored FeLV vac-

Recording the sites of injections in the patient's medical records is important.



cine; cats in group B received an FeLV vaccine with a lipid-based adjuvant; and cats in group C were vaccinated with an FeLV vaccine adjuvanted with an alum-Quil A mixture. On days 7, 21 and 62 post-vaccination, significantly less inflammation was associated with administration of the non-adjuvanted recombinant canarypox-vectored vaccine. The inflammation was most severe in the cats receiving the aluminium-based adjuvant. Cats receiving adjuvanted vaccines had evidence of residual adjuvant material accumulated within macrophages even at 62 days post-vaccination.⁷⁹ In a case-control study investigating associations between vaccine types and development of FISS, adjuvanted inactivated vaccines were significantly more commonly associated with sarcoma development than other vaccines; of 35 vaccinated cats with sarcoma on the hind limb, 25 cats had received adjuvanted vaccines, seven cats had received MLV vaccines (FPV, FHV-1 and FCV), while only one cat had received a recombinant canarypox-vectored vaccine [EBM grade III].³⁵

Vaccination schedules

Finally, to prevent development of FISS, cats should be vaccinated no more than necessary. Therefore, long vaccination intervals should be applied in adult animals, vaccines (such as rabies vaccines and FPV vaccines) that are licensed for 3 year or even 4 year boosters should be preferred, no FeLV or rabies vaccinations should be administered to indoor-only cats, and immune cats should not be vaccinated (eg, if antibodies are detected). This confirms the necessity of individual vaccination schedules.

KEY POINTS

- ❖ Vaccination of cats provides essential protection and should not be stopped because of the risk of feline injection-site sarcoma (FISS).
- ❖ Vaccines are not the only injectable medical products associated with FISS.
- ❖ An individual vaccination schedule is important. Cats should be vaccinated no more than necessary, in accordance with current guidelines.
- ❖ Appropriate sites for injection should be selected. The interscapular region should generally be avoided. Vaccines should be injected at a site from which a mass can easily be surgically removed, such as distally on a leg or in the skin of the lateral abdomen.
- ❖ Vaccines should be brought to room temperature prior to administration, but should not be kept unrefrigerated for hours.
- ❖ Whenever possible, subcutaneous, rather than intramuscular, injection should be performed.
- ❖ The preference is for: non-adjuvanted vaccines over those containing adjuvant; modified-live vaccines or recombinant vaccines over inactivated vaccines; and vaccines with a long duration of immunity.
- ❖ Post-vaccination monitoring should be performed. Any lump at the site of injection that is still present 3 months after vaccination, that is larger than 2 cm in diameter, or that it is increasing in size 1 month after vaccination should be surgically removed.

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BORNA DISEASE VIRUS INFECTION IN CATS

ABCD guidelines on prevention and management



Hans Lutz, Diane Addie, Corine Boucraut-Baralon, Herman Egberink, Tadeusz Frymus, Tim Gruffydd-Jones, Katrin Hartmann, Margaret J Hosie, Albert Lloret, Fulvio Marsilio, Maria Grazia Pennisi, Alan D Radford, Etienne Thiry, Uwe Truyen, Marian C Horzinek and Karin Möstl

Overview: Borna disease virus (BDV) has a broad host range, affecting primarily horses and sheep, but also cattle, ostriches, cats and dogs.

In cats, BDV may cause a non-suppurative meningoencephalomyelitis ('staggering disease').

Infection: The mode of transmission is not completely elucidated. Direct and indirect virus transmission is postulated, but BDV is not readily transmitted between cats. Vectors such as ticks may play a role and shrews have been identified as a potential reservoir host. Access to forested areas has been reported to be an important risk factor for staggering disease.

Disease signs: It is postulated that BDV may infect nerve endings in the oropharynx and spread via olfactory nerve cells to the central nervous system. A strong T-cell response may contribute to the development of clinical disease. Affected cats develop gait disturbances, ataxia, pain in the lower back and behavioural changes.

Diagnosis: For diagnostic purposes, detection of viral RNA by reverse transcription PCR in samples collected from cats with clinical signs of Borna disease can be considered diagnostic. Serology is of little value; cats without signs of Borna disease may be seropositive and yet not every cat with BDV infection has detectable levels of antibodies.

Human infection: A hypothesis that BDV infection may be involved in the development of selected neurological disorders in man could not be confirmed. A research group within the German Robert Koch Institute studied the potential health threat of BDV to humans and concluded that BDV was not involved in the aetiology of human psychiatric diseases.

Background

Borna disease virus (BDV) historically has affected horses and sheep (for a review see Ludwig and Bode¹). The disease was first described in 1855 in horses which became severely sick, near the German town of Borna (cited in Lundgren et al²). More recently, BDV has been described as the causative agent of a viral meningoencephalitis in cattle, ostriches, cats and dogs.¹ In the mid-1970s, staggering disease – a non-suppurative meningoencephalomyelitis – was described in Swedish cats (cited in Lundgren et al² and Cubitt and de la Torre³). Later, it was found that antibodies recognising BDV were common to these cases.⁴ Finally, in 1995, BDV was confirmed as the aetiological agent of staggering disease.²

Aetiological agent

BDV is an enveloped virus with a helical capsid and a single-stranded RNA genome. The genome comprises 8900 bases and, based on sequence analysis, it was assigned to the order of *Mononegavirales* as the only member of the *Bornaviridae* family.^{3,5} BDV particles are spherical and have an average diameter of approximately 100 nm. The genome encodes six known proteins including an envelope protein of 56 kd. Interestingly, BDV can infect a number of brain-derived cell types, but it does not usually induce any cytopathic effect.

Epidemiology

The mode of transmission of BDV has not been completely elucidated. It is postulated that transmission occurs through direct contact with an infected animal or indirectly by contact with secretions of an infected animal. In addition, the local occurrence of disease in forested areas in Sweden suggests that vectors such as ticks may play a role in transmission. In 2006, a shrew (*Crocidura leucodon*) was identified as the reservoir host in an area of Switzerland where BDV is prevalent in horses and sheep.⁶ Shrews could also serve as reservoirs for BDV infection in cats. BDV infection appears not to be readily transmitted between cats.

Feline BDV infection has been reported in many countries, including Germany, Switzerland, Belgium, the United Kingdom, Japan, the



Philippines, Indonesia, Australia and Finland (cited in Ludwig and Bode¹ and Someya et al⁷). The fact that BDV was also shown to be present in horses in North America and several other species in Western China suggests that cats in the USA and China might also be affected by BDV. Clinical staggering disease has been mainly observed in Sweden, Austria, Germany, Switzerland and Liechtenstein.

The seroprevalence in cats with neurological disease in different countries has been reported to vary widely, between 0 and 67%. In healthy cats, the occurrence of BDV antibodies is much lower, varying between 2% and over 40%.⁸ Access to forested areas was reported to be an important risk factor for staggering disease, since 68% of all clinical cases occurred in cats with access to forests.⁴ Staggering disease shows a clear peak in frequency in the spring.⁹ So far, an association between BDV infection and gender has not been described. The findings on the age distribution of BDV infection are controversial. A recent study in Japan found no age predisposition in BDV infection although cats younger than 1 year were already found to be affected.⁷

Pathogenesis and clinical signs

It is postulated that BDV may infect nerve endings in the oropharynx, nose and/or intestinal tract. The virus is thought to migrate along the nerves to the central nervous system (CNS),¹⁰ where it leads to lymphocytic inflammation and neuronal degeneration. A strong T-cell response to the virus is believed to be responsible for the development of clinical signs but other factors may also be important for disease development.¹⁰ Affected cats develop gait disturbances, ataxia, pain in the lower back and behavioural changes. In some cases, cats lose the capacity to retract their claws. Clinical signs will usually progress and cats will eventually die after developing severe paralysis of the hind legs. However, some cats will recover partially or even completely. Subclinical infections can also occur.

Immune response

CD8+ lymphocytes stimulated by BDV have been found in peripheral blood, spleen and brain.¹¹ These findings suggest that a successful immune reaction usually allows infected cats to control the infection. A weak innate immune response to BDV infection was recently described in rat brain cell cultures.¹² It is, therefore, expected that a weak innate immune response may likewise contribute to disease development in cats.

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Clinical staggering disease has been mainly observed in Sweden, Austria, Germany, Switzerland and Liechtenstein.

Diagnosis

Diagnosis on the basis of clinical signs alone is not possible as there are several other viral infections (feline immunodeficiency virus, feline leukaemia virus and feline coronavirus) that can lead to similar clinical signs. Detection of antibodies to BDV by ELISA or indirect immunofluorescence in cats exhibiting clinical signs typical of BDV infection permits a tentative diagnosis.¹³

However, the diagnostic sensitivity of the detection of antibodies, at 81%, means that not every cat with BDV infection will have detectable levels of antibodies.¹³ The reason for this is unclear. It is speculated that different strains of BDV exist which are sufficiently different from the antigen used in the assay and therefore remain undetected. Alternatively, some cats may not be capable of mounting an immune response that is serologically detectable.

The diagnostic specificity of antibody detection is also very low, as many seropositive cats may be completely healthy.¹³ In the absence of clinical signs of Borna disease, diagnostic serology is of little value.

Detection of viral RNA by reverse transcription PCR in pooled samples of blood, serum, urine, conjunctival, nasal, oral and anal swabs collected from cats with clinical signs of Borna disease can be considered diagnostic.¹³

Currently, the most reliable means of diagnosis of Borna disease is considered to be pathology and histopathology.

Pathology

In cats with end-stage staggering disease, mild neutropenia is observed in about a third of the affected population. No other changes in clinical or biochemical parameters are observed. The most important histopathological findings include perivascular cuffing in the hippocampus, basal ganglia, cerebellum, cerebrum and grey matter of the brainstem.⁹ In addition, plasma cells have been frequently seen in the close vicinity of neurons,¹⁴ indicative of an inflammatory reaction and thereby explaining the clinical findings in cats with staggering disease.

Prevention

Currently, no vaccine is available for the prevention of staggering disease. As the exact modes of transmission are still not completely clear, it is difficult to make specific recommendations for preventive measures. Cats without access to a rural environment are probably at lower risk of BDV infection compared with those with unlimited access to such areas. In

Zoonotic aspects

As BDV persistently infects the CNS of many animal species, it was postulated that this virus might also infect humans. Indeed, it was shown that humans can be seropositive for BDV and that the frequency of BDV antibodies was increased in human patients with chronic neurological disorders. Specifically, among 70 psychiatric patients, 20% were found to be seropositive, compared with a few percent of the normal population. This led to the hypothesis that BDV infection may be involved in the development of selected neurological disorders,^{15,16} and triggered the creation of a research group within the German Robert Koch Institute in the 1990s to study the potential health threat of BDV to humans.

In 2007, this research group published a statement that (1) the methods providing seropositive results in human blood were not adequate to substantiate the presence of antibodies to BDV; and (2) the RNA sequences found in human blood and tissue were the consequence of BDV contamination in the laboratory of the respective research laboratory. Therefore, it was concluded that BDV was not involved in the aetiology of human psychiatric diseases and after dozens of careful studies the research group ended its activity.

For details see http://www.rki.de/DE/Content/Forsch/Forschungsschwerpunkte/NeueRisiken/NeuartigeErreger/Einstellung_Projekt_Bornavirus.html.

areas where staggering disease is known to occur, it might therefore be recommended that cats should be kept indoors. However, limiting outdoor access should be carefully weighed against the risk of BDV infection. For many cats, outdoor access is an important component of their wellbeing.

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Conflict of interest

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KEY POINTS

- ❖ Borna disease virus (BDV) is the aetiological agent of staggering disease, seen in several animal species, including horses, sheep and cats.
- ❖ Transmission probably occurs through direct contact or indirectly via the secretions of an infected animal.
- ❖ Ticks may play a role in transmission.
- ❖ Infection starts in the olfactory nerve cells and then spreads to the central nervous system.
- ❖ Signs include an abnormal gait, ataxia progressing to paralysis, lower back pain and behavioural changes.
- ❖ Serological tests are of little diagnostic value.
- ❖ Detection of viral RNA by reverse transcription PCR in pooled samples of body secretions is diagnostic.
- ❖ Pathology and histopathology are considered the most reliable diagnostic methods.
- ❖ BDV is not involved in the aetiology of psychiatric disease in humans.





WEST NILE VIRUS INFECTION IN CATS

ABCD guidelines on prevention and management

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Introduction

West Nile virus (WNV) is a zoonotic mosquito-borne virus belonging to the family *Flaviviridae*, genus *Flavivirus*, in the Japanese encephalitis antigenic group. It is an enveloped virus containing a single molecule of linear, positive-sense, single-stranded RNA. Several phylogenetic lineages can be distinguished but most isolates can be assigned to lineages 1 and 2. WNV has a broad host range, comprising mainly birds and mosquitos, but also mammals (including humans), reptiles, amphibians and ticks. It can cause disease in humans, horses and several species of birds. The severity of disease depends on the (neuro)virulence of the infecting virus strain. Disease is uncommon in other wild and domesticated animals, and has been incidentally reported in alpacas, sheep, reindeer, dogs and also cats.¹

Epidemiology

WNV was first identified in 1937 from the blood of a febrile patient in the West Nile district of Uganda. Since then, the virus has spread from Africa via migratory birds to other parts of the world including Central and Southern Europe, Asia and Australasia.^{1,2} In 1999, the virus was introduced into North America, in the city of New York, causing encephalomyelitis in horses, birds and humans. Since then, the virus has spread across the USA and parts of Latin America and Canada.³

WNV is maintained in a bird–mosquito–bird transmission cycle. The most important vectors are bird-feeding mosquitos of the *Culex* genus. More than 300 species of birds have been reported to be infected with WNV, but maintenance and amplification mainly involve passerine birds.⁴ These birds develop viraemia levels that are sufficient to infect mosquitos feeding upon them. Mortality differs between bird species. High mortality is seen especially in corvids and robins. In other species of wild birds, and also chickens and pigeons, infection remains sub-clinical. In these latter species a low-magnitude viraemia develops which is unlikely to be sufficient to again infect mosquitos. Humans and other mammals also develop low levels of viraemia and are therefore considered dead-end hosts and not important as virus reservoirs.¹

Overview: West Nile virus (WNV) is a zoonotic mosquito-borne virus with a broad host range that infects mainly birds and mosquitos, but also mammals (including humans), reptiles, amphibians and ticks. It is maintained in a bird–mosquito–bird transmission cycle. The most important vectors are bird-feeding mosquitos of the *Culex* genus; maintenance and amplification mainly involve passerine birds. WNV can cause disease in humans, horses and several species of birds following infection of the central nervous system.

Infection in cats: Cats can also be infected through mosquito bites, and by eating infected small mammals and probably also birds. Although seroprevalence in cats can be high in endemic areas, clinical disease and mortality are rarely reported. If a cat is suspected of clinical signs due to an acute WNV infection, symptomatic treatment is indicated.

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Pathogenesis and clinical signs

Infection occurs mainly through inoculation of virus by a mosquito bite. Initial target cells are keratinocytes and skin-resident dendritic cells (DCs).⁵ The latter migrate to draining lymph nodes where initial replication occurs. From there, the virus spreads to visceral organs, including the spleen. The target cells in the spleen and other visceral organs are thought to be DCs, macrophages and neutrophils. Viral replication leads to viraemia. The virus enters the central nervous system (CNS), resulting in inflammation of the medulla, brainstem and spinal cord.⁶

Similar to other mammals and birds, cats can be infected through mosquito bites but also orally by eating infected small mammals and probably also birds as evidenced by serological studies and experimental infections.⁷ In a serological survey conducted in St Tammany Parish, Louisiana, USA, 9% of cats were shown to be seropositive, with stray cats having almost three times the WNV seroprevalence as family cats, although the difference was not significant.⁸ Seropositive cats were also identified in other areas of the USA and in China, with seroprevalence rates ranging from 10–15%.^{9,10}

Although seroprevalence of WNV infection in endemic areas can be high, clinical disease and mortality are rarely reported. It seems that most infections are subclinical in nature.

In 33 cats from Germany with non-suppurative meningoencephalitis of unknown origin, positive immunostaining was detected for WNV antigen in four cats. All four cats had shown neurological signs. However, WNV infection could not be confirmed by PCR analysis and the positive WNV reactions might have been due to infections with cross-reactive agents or caused by molecular mimicry of host-derived antigens.^{11,12}

During the 1999 outbreak of West Nile virus infections in New York, mortality was observed in humans, horses and one cat 2000.¹³ In cats that were experimentally infected through mosquito bites, mild, non-specific signs including lethargy and a modest decrease in appetite were observed during the first week after challenge. No neurological signs occurred.⁷ Infection could also be established after oral exposure through ingestion of infected mice. The magnitude and duration of viraemia was similar to that in cats infected by mosquito bites. However, clinical signs were not observed. The level of viraemia in cats was higher than that in dogs included in

EBM grades

The ranking system for grading the level of evidence of a statement within the treatment and prevention section of this article is described on page 574 of this Special Issue.

the same study. The duration of viraemia ranged from 3.5–4.5 days. The level of viraemia observed in cats might be high enough to infect mosquitos at low efficiency. However, cats are not considered to be epidemiologically important amplifying hosts.⁷

Diagnosis

Since feline WNV infection is mostly subclinical, the need for specific diagnostic tests for cats is limited. In humans and horses, a diagnosis of WNV infection can be established through detection of the virus or virus-specific antibodies. Acute infection can be confirmed by detecting virus-specific immunoglobulin M antibodies, although antibodies may be absent in the early phase of infection. A significant rise in WNV antibodies in paired acute and convalescent sera can be determined as evidence of acute infection. A test for neutralising antibodies can also be performed, but this requires special facilities and will not be offered by most laboratories. Other serological assays are performed, such as an epitope-blocking ELISA.¹⁴ If they are to be used in the diagnosis of infection in cats these assays need to be standardised for use with feline sera.

Virus can be detected by reverse transcription PCR in blood samples or infected tissues at necropsy. However, viraemia is short-lived and PCR might be negative in a patient at the time of clinical presentation. Virus can also be detected in tissues by *in situ* hybridisation and immunohistochemistry.

Treatment and prevention

There is no specific treatment for WNV infection. In a cat with clinical signs suspected to be due to WNV infection, symptomatic treatment is indicated.

Several vaccines are available for protection against WNV infection in horses. The efficacy of a recombinant canarypox-vectored WNV vaccine was also studied in dogs and cats.¹⁵ As expected, clinical signs did not develop in any of the cats (control and vaccinated animals) after challenge. However, the vaccinated cats were shown to develop virus-neutralising antibodies and were protected against viraemia after challenge. This demonstrates the potential of the vaccine for protection against infection in cats [EBM grade II]. A commercial vaccine for cats is not available. Since current WNV strains cause no or only minimal and occasional clinical signs, a feline vaccine is currently not required.

Mosquito control can reduce the risk of infection. Similar control measures as implemented for prevention of mosquito bites in humans and horses might be taken.



Although seroprevalence of WNV infection in endemic areas can be high, clinical disease and mortality in cats are rarely reported.

KEY POINTS

- ❖ West Nile virus (WNV) has a broad host range, infecting mainly birds and mosquitos, but also mammals (including humans), reptiles, amphibians and ticks.
- ❖ WNV is maintained in a bird–mosquito–bird transmission cycle.
- ❖ Infection in cats occurs through mosquito bites or ingestion of infected small mammals and probably also birds.
- ❖ Clinical disease and mortality is rarely reported in cats.
- ❖ Experimentally infected cats show mild, non-specific signs including lethargy and loss of appetite.
- ❖ If a cat is suspected of clinical signs due to acute WNV infection, symptomatic treatment is indicated.
- ❖ Since current WNV strains cause no or only minimal and occasional clinical signs, a feline vaccine is currently not required.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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STREPTOCOCCAL INFECTIONS IN CATS

ABCD guidelines on prevention and management



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Overview: *Streptococcus canis* is most prevalent in cats, but recently *S equi* subsp *zooepidemicus* has been recognised as an emerging feline pathogen.

S canis infection: *S canis* is considered part of the commensal mucosal microflora of the oral cavity, upper respiratory tract, genital organs and perianal region in cats. The prevalence of infection is higher in cats housed in groups; and, for example, there may be a high rate of vaginal carriage in young queens in breeding catteries. A wide spectrum of clinical disease is seen, encompassing neonatal septicaemia, upper respiratory tract disease, abscesses, pneumonia, osteomyelitis, polyarthritis, urogenital infections, septicaemia, sinusitis and meningitis.

S equi subsp zooepidemicus infection: *S equi* subsp *zooepidemicus* is found in a wide range of species including cats. It was traditionally assumed that this bacterium played no role in disease of cats, but it is now considered a cause of respiratory disease with bronchopneumonia and pneumonia, as well as meningoencephalitis, often with a fatal course. Close confinement of cats, such as in shelters, appears to be a major risk factor. As horses are common carriers of this bacterium, contact with horses is a potential source of infection. Additionally, the possibility of indirect transmission needs to be considered.

Diagnosis: Streptococci can be detected by conventional culture techniques from swabs, bronchoalveolar lavage fluid or organ samples. Also real-time PCR can be used, and is more sensitive than culture.

Treatment: In suspected cases, treatment with broad-spectrum antibiotics should be initiated as soon as possible and, if appropriate, adapted to the results of culture and sensitivity tests.

Introduction

Although different streptococci have been isolated occasionally from cats, including *Streptococcus agalactiae*, *S pneumoniae* and *S suis*, the most prevalent species is *S canis*.¹ *S equi* subspecies *zooepidemicus* has been recognised as an emerging pathogen in dogs, and also recently in cats.^{2,3}

Streptococcus canis

S canis is a beta-haemolytic Lancefield group G gram-positive bacterium that is considered part of the commensal mucosal microflora of the oral cavity, upper respiratory tract, genital organs and perianal region in cats. *S canis* infection seems to be sporadic in single-cat households, especially in older cats.¹ Young queens (up to 2 years of age) may carry *S canis* in the vagina, and the prevalence of infection is generally higher in cats housed in groups. Up to 70–100% of young queens in breeding

catteries may carry this bacterium in the vagina, resulting in infection of the kittens, but also in the transfer of passive immunity against *S canis* via colostrum. Various factors, including the level of maternally derived antibodies, immune response, age, infection pressure, stress and probably also the strain virulence, determine whether the bacteria cause disease or not.

Contamination of the umbilical vein may lead to a generalised infection resulting in neonatal septicaemia.¹ In 3- to 7-month-old kittens, a subclinical infection of the pharynx and tonsils may



Figure 1 In adult cats, *S canis* infection is usually opportunistic as a result of wounds, surgery, immunosuppression or viral infection. In this shelter cat, viral infection was severely complicated by secondary bacterial infection due to poor hygienic conditions. Courtesy of Tadeusz Frymus, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland

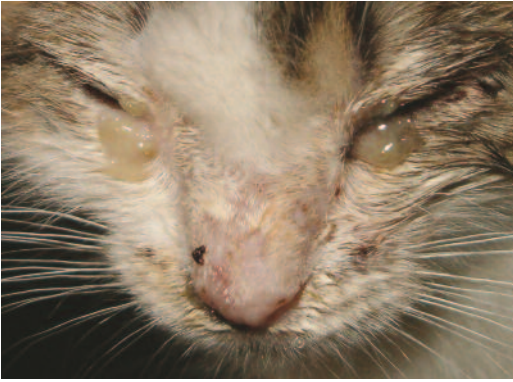


Figure 2 In some cats suffering from upper respiratory tract disease, *S canis* can be isolated from the nasal cavity. Courtesy of Tadeusz Frymus, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland

induce cervical lymphadenitis. In older cats, the infection is usually opportunistic, as a result of wounds, surgery, immunosuppression or viral infection (Figure 1). In up to 10% of cats suffering from chronic upper respiratory tract disease, *S canis* can be isolated from the nasal cavity (Figure 2).⁴ Conditions associated with this pathogen include abscesses, pneumonia, discospondylitis, osteomyelitis, polyarthritis, urogenital infections, necrotising fasciitis (toxic shock syndrome), sinusitis and meningitis. Outbreaks of fatal disease in cats have been reported in shelters and breeding colonies,⁴ as all of these conditions may result in septicaemia and embolic lesions, especially of the lung and heart.¹

Microscopic examination of exudates or tissue reveals gram-positive cocci (usually in chains), and culture can confirm the diagnosis. *S canis* is generally sensitive to penicillins, and early antibiotic administration is the basis of therapy.

More information can be found in a review by Greene and Prescott.¹

Streptococcus equi subsp zooeidemicus

Agent and host susceptibility

S equi subsp *equi* (commonly referred to as *S equi*) and *S equi* subsp *zooeidemicus* (*S zooeidemicus*) are beta-haemolytic gram-positive Lancefield group C bacteria, and the most important equine streptococci worldwide.⁵ *S equi* is an obligate agent causing strangles, the most frequently diagnosed infectious disease of horses, and one which is both devastating and highly contagious. *S equi* is host-restricted, infecting equids almost exclusively.

S zooeidemicus is regarded as a mucosal commensal, most notably in equids, with a potential to cause serious opportunistic disease secondary to viral infections, heat exposure, trans-

***S zooeidemicus* was thought to play no role in diseases of cats until an outbreak was described in a shelter in Israel in 2010. Cases have since been described in the USA and Canada.**



Figure 3 Purulent nasal discharge and cough may be early signs of *S equi* subsp *zooeidemicus*-related disease in cats. Courtesy of Tadeusz Frymus, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland



portation or other stressful situations.⁶ Believed to be part of the normal microflora of the upper respiratory airways and lower reproductive tract, this bacterium is frequently isolated from suppurative discharge in horses including cases of mixed bacterial/viral infection of the upper airways.^{5,6} However, in contrast to *S equi*, *S zooeidemicus* strains are highly diverse and are not restricted to causing disease in horses. These strains have been found in a wide range of other species including pigs, cattle, sheep, goats, poultry, dogs, cats, guinea pigs, seals, dolphins, monkeys, llama and farmed red deer.^{2,7,8-17} Occasionally, glomerulonephritis, rheumatic fever, meningitis or purulent arthritis caused by *S zooeidemicus* have been reported in humans.¹⁸⁻²⁰ Many of these zoonotic infections have resulted from contact with horses or from the consumption of unpasteurised milk of cows or goats.

There is increasing evidence that the veterinary importance of *S zooeidemicus* may be underestimated, and concern has been expressed that this bacterium may be 'potentially more than just an opportunist'.²¹ Several outbreaks in species other than horses have been described. In Asia, pandemics have occurred in pigs.^{14,22} Also in companion animals, the incidence of infections by this agent has apparently increased. Since 2003, several outbreaks of an acute *S zooeidemicus*-related severe haemorrhagic canine pneumonia have been described in many countries.²³⁻²⁷ This disease is highly contagious and often fatal. The most prominent signs reported were a sudden-onset fever, dyspnoea, and haemorrhagic nasal discharge. Haemorrhagic pneumonia and pleural effusion were recognised post mortem. Most outbreaks occurred in shelters, where *S zooeidemicus* infection caused many deaths. Kennels and research facilities were also involved;²⁸ in addition, individually housed dogs were occasionally affected.^{20,27}

Feline *S zooeidemicus*-related disease

It was thought that *S zooeidemicus* played no role in diseases of cats until an outbreak was described in 2010 in a shelter in Israel.² Early clinical signs included an effusive purulent nasal discharge and cough (Figure 3), progressing to sinusitis, dyspnoea, pneumonia and death. The vaccination status of the shelter cats was unknown. Between June 2006 and January 2008, 78 dead cats from the shelter, which housed approximately 700 animals, had been submitted for post-mortem examination. In 39 of these, the major necropsy findings were severe, acute and



Figure 4 In cats succumbing to fatal *S equi* subsp *zooepidemicus* infection, the major necropsy finding is severe, acute and diffuse bronchopneumonia. Courtesy of Karolina Kozłowska, Warsaw, Poland

diffuse bronchopneumonia (Figure 4) or bronchoalveolar pneumonia, either suppurative or necrosuppurative. Interstitial multifocal pyogranulomatous pneumonia was present in a few cats, pleuritis in four cases, and pyothorax in one animal. Pyogranulomatous meningoencephalitis was recorded in four cats. Necrosuppurative peritonitis was present in one case. The most common histopathological lesions were a diffuse mixed infiltrate of neutrophils, histiocytes and lymphocytes, thickening of the interalveolar septa and multifocal bacterial colonies with coccoid forms.²

S zooepidemicus was the main pathogen isolated, both from the dead cats with signs of respiratory disease as well as from nasal and pharyngeal swabs or bronchoalveolar lavage fluid samples obtained from sick animals.² In the dead cats, *S zooepidemicus* was isolated from the lungs in all cases, and additionally from the sinuses in a few. The bacterium was also cultured from the pleura in two of four cases of pleuritis, from the brain in three of four cases of meningoencephalitis and from the peritoneum in one case of peritonitis. Usually *S zooepidemicus* was isolated alone, or was dominant in mixed cultures. However, the bacterium was not isolated from any of the 29 dead cats without clinical and pathological signs of respiratory disease, and from only two of 10 animals in which respiratory disease was suspected prior to death, but no gross pathological signs were found on necropsy.²

S zooepidemicus could also be isolated from cats showing vague signs of respiratory disease, which possibly shed the organism long before being detected.² This might suggest subclinical carriage. In the few cases with lesions suggesting feline infectious peritonitis, the presence of feline coronavirus (FCoV) was ruled out by immunohistochemistry. Tests for feline herpesvirus (FHV) and feline calicivirus (FCV) were not performed but, based on clinical signs, the authors suspected that the cat population in this

shelter was infected with both viruses. They assessed the hygiene and ventilation in this cattery as being adequate and the facilities as not overcrowded. This could mean that *S zooepidemicus* may become persistent in a cattery in spite of sufficient hygiene practices and treatment. The authors speculated that the transfer to this shelter of a group of cats from another cattery (closed due to poor conditions) prior to the disease outbreak might have induced stress that facilitated this epidemic. However, the source of infection remained unknown. The cats had no contact with horses.²

In 2010, a fatal *S zooepidemicus* infection in two mature domestic cats housed in separate shelters was also described in Canada.²⁹ Both animals had been resident for several months in the shelter prior to a sudden onset of a peracute disease with non-specific clinical signs, and blindness in one cat, followed by death within 24 h. Post-mortem examination revealed rhinitis and meningitis, and *S zooepidemicus* was cultured from the nasal cavity and brain. Both cats had tested negative for feline leukaemia virus (FeLV) antigen and were seronegative for feline immunodeficiency virus (FIV) antibodies. PCR of lung, nasal mucosa and brain, performed post mortem, revealed that both cats were also negative for FCV and FCoV, and one was positive for FHV. Interestingly, other cats in these shelters remained normal. Neither of the cats that succumbed, nor their shelter attendants, had had contact with horses.

The pathogenic role of *S zooepidemicus* in cat colonies was revealed following a recent investigation of cat hoarding.³ In this study, about 2000 cats were removed from four sanctuaries following reports consistent with animal hoarding. During intake examination, 27% of the animals (366/1368) showed respiratory disease. A subset of 81 cats with respiratory signs was tested for infectious agents by PCR, and 55% were positive for *S zooepidemicus*.

A case of acute *S zooepidemicus* meningoencephalitis was also described in an exclusively indoor cat in the USA in 2011.³⁰ It was likely secondary to otitis media/interna, as identified by computed tomography. The patient presented with neurological signs of a central vestibular lesion and left Horner's syndrome. Cerebrospinal fluid analysis revealed marked neutrophilic pleocytosis; *S zooepidemicus* was isolated in pure culture, while PCR results for *Toxoplasma gondii*, FCoV and FeLV were negative, as was antigen enzyme immunoassay for *Cryptococcus* species. A bulla osteotomy and debridement was performed and, in accordance with resistance profile results, the cat was treated with trimethoprim-sulfamethoxazole for 8 weeks. The patient recovered fully.

European Advisory Board on Cat Diseases
The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the feline streptococcal infections in cats guidelines is available at www.abcdcatsvets.org and www.abcd-vets.org

In addition to the infections of domestic cats reviewed above, a fatal suppurative meningoventriculitis with intralesional *S zooepidemicus* has been described in an elderly, captive snow leopard in Japan.³¹ This animal had had no contact with horses, but defrosted horse meat was fed routinely and was presumed to be the source of infection.

Epidemiology in small animals

It is generally considered that, in contrast to *S canis*, *S zooepidemicus* is not part of the normal microflora of dogs and cats.^{32–35} Nevertheless, both canine and feline subclinical infections have been observed.^{2,20,23,36} *S zooepidemicus*-related diseases secondary to viral infections have been described in dogs, especially in cases of distemper and canine influenza virus (CIV) infection.³⁷ The bacterium may also act as a primary cause of canine pneumonia, sometimes with a peracute course, although experimental infections have not been performed.³⁸

Contact with horses, which are common carriers of this bacterium, is a potential source of infection.³⁶ Dogs experimentally infected with CIV and then kept together with healthy horses acquired *S zooepidemicus* pulmonary infection.³⁹ The possibility of indirect transmission should also be taken into consideration, as equine streptococci may survive outdoors for up to several days, and indoors for probably longer.⁴⁰ It has been speculated that contact with staff members could explain outbreaks in canine research facilities and urban kennels, where direct contact with horses is excluded.¹⁰ Certainly *S zooepidemicus* is able to spread between dogs through direct contact, and outbreaks in shelters usually affect large numbers of animals within a short time.

Similar probably applies in cats. It has been postulated that close confinement of animals, such as in shelters, research laboratories and other facilities, appears to be the major risk factor for the development of *S zooepidemicus*-associated disease in dogs and cats.^{23,29} Co-infection with other respiratory pathogens, as well as the age and health of the animal on entry to the facility, has been shown to be unrelated to later colonisation of the respiratory tract by *S zooepidemicus* in dogs.^{23,28} The role of infected dogs as a source for feline infections is not known; however, in one shelter, canine haemorrhagic pneumonia caused by this bacterium did not spread to cats located in an adjacent building of the same facility.²⁶

In contrast to *S canis*, *S zooepidemicus* is not part of the normal microflora of cats.



Close confinement of animals, such as in shelters, appears to be the major risk factor for development of *S zooepidemicus*-associated disease in cats.

Pathogenesis in small animals

The pathogenesis of *S zooepidemicus* infection in small animals is poorly understood. The existence in dogs of both subclinical and clinical infections of different severity suggests that some isolates might be more pathogenic than others.

In many dogs, the rapid onset of disease and progression of clinical signs are similar to human toxic shock syndrome caused by *Streptococcus pyogenes*.⁴¹ Toxic shock is characterized by a hyperreactive inflammatory response, resulting in increased vascular permeability, vasodilation, increased coagulation and migration of inflammatory cells to the site of infection.⁴² Pyrogenic exotoxins produced by some streptococci, including *S pyogenes*, act as superantigens by binding simultaneously to major histocompatibility complex class II receptors on macrophages and T-cell receptors, bypassing conventional antigen presentation, and leading to the activation of a large proportion of T lymphocytes.⁴³ The resulting hyperproduction of proinflammatory cytokines has been linked to increased virulence and has also been suggested to contribute to the pathogenesis of some streptococcal diseases. Marked elevation of the mRNA of some proinflammatory cytokines was also observed in dogs with *S zooepidemicus*-induced pneumonia, and three superantigen genes were prevalent among canine isolates of the bacterium.⁴¹

So far, no clinical signs similar to the toxic shock syndrome have been described in cats. Various typing methods have been used to determine the virulence factors and genetic relationships among different *S zooepidemicus* isolates; M-like protein, IgG-binding proteins and fibronectin-binding protein appear to be the main virulence factors for this bacterium.^{44–46} To date, the factors underlying the differences in pathogenicity of some isolates/genotypes in cats and dogs remain unknown.

Diagnosis

A tentative diagnosis of a streptococcal infection can be made based on the history, clinical signs, lesions and the presence of gram-positive coccus chains in the lesions. *S zooepidemicus* can be isolated from nasal and pharyngeal swabs, as well as from bronchoalveolar lavage fluid samples, from cats with respiratory disease, and from lung samples or other lesions in fatal cases.² Selective media for gram-positive organisms, such as Columbia agar with 5% sheep or horse blood containing colistin and nalidixic acid, should be used. If Lancefield group C streptococci grow, the presence of *S zooepidemicus* can be confirmed by biochemical methods (eg, API

KEY POINTS

- ❖ *Streptococcus equi* subsp *zooepidemicus* is an emerging pathogen in cats.
- ❖ Infection is highly contagious and often fatal.
- ❖ In cats, the pathogen mainly affects the respiratory tract, and clinical signs include purulent nasal discharge, coughing, sinusitis, dyspnoea, pneumonia and death.
- ❖ Meningoencephalitis has also been described.
- ❖ Horses are common carriers of this bacterium, and contact with these animals is a potential source of infection.
- ❖ Close confinement of cats, such as in shelters, research laboratories and other facilities, appears to be the major risk factor for infection.
- ❖ In the case of respiratory disease, *S zooepidemicus* can be isolated from nasal and pharyngeal swabs as well as from bronchoalveolar lavage fluid; in fatal cases, these bacteria can be isolated from lung samples or other lesions.
- ❖ In suspected cases, treatment with broad-spectrum antibiotics should be initiated as soon as possible and then adapted according to the results of culture and sensitivity tests, where required.



EBM grades

The ranking system for grading the level of evidence of various statements within the treatment section of this article is described on page 574 of this Special Issue.

20 Strep; bioMérieux). In contrast to *S equi*, *S zooepidemicus* has the ability to ferment ribose, sorbitol and lactose, properties that are commonly used to discriminate the subspecies.⁴⁷ Real-time PCR was found to be more sensitive than conventional culture for diagnosis and differentiation of *S equi* and *S zooepidemicus*.⁴⁸

Treatment

There is only one report of effective treatment in cats, involving a case of acute *S zooepidemicus* meningoencephalitis.³⁰ Trimethoprim-sulfamethoxazole administered over several weeks was the main antibiotic [EBM grade IV]. In suspected cases, treatment with broad-spectrum antibiotics should be initiated as soon as possible, and then adapted, if required, in the light of the results of culture and sensitivity tests. *S zooepidemicus* isolates from dogs were found to be susceptible to penicillin, ampicillin, amoxicillin and enrofloxacin [EBM grade IV].²⁶ Some isolates were found to be resistant to tetracycline and doxycycline [EBM grade IV].^{25,28}

Prevention

There is little data about the management of *S zooepidemicus* infections in feline shelters. However, sick cats should be isolated and staff should wear protective clothing when caring for them. Hands, premises and all contaminated equipment should be thoroughly cleaned and disinfected. Quaternary ammonium disinfectants, phenol-based solutions or oxidising agents are generally recommended.

Though significant attempts have been made, there are no *S zooepidemicus* vaccines available for any species.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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LUNGWORM DISEASE IN CATS

ABCD guidelines on prevention and management



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Overview: Cardiopulmonary nematodes are emerging parasites of cats in Europe. A number of helminth parasites may be involved. The most prevalent lungworm in domestic cats is *Aelurostrongylus abstrusus*. *Oslerus rostratus* and *Troglostrongylus* species are found mainly in wild cats. The trichurid *Capillaria aerophila* has a low host specificity and is not uncommon in cats. Additionally the lung flukes *Paragonimus* species are reported in many species outside of Europe, including cats.

Clinical signs: Lungworm infections may be asymptomatic, or cause mild to severe respiratory signs, dependent on the worm species and burden; mixed infections are observed. Kittens can be vertically infected and may develop a more severe disease. Affected cats show a productive cough, mucopurulent nasal discharge, tachypnoea, dyspnoea and, in severe cases, respiratory failure and death.

Management: Early diagnosis and treatment greatly improves the prognosis. First-stage larvae can be easily detected in fresh faecal samples; the Baermann migration method is the enrichment technique of choice, but takes 24 h. Lungworm larvae can be found in tracheal swabs and bronchoalveolar lavage fluid, but with less sensitivity than in faeces. Molecular methods have been developed that exhibit high specificity and sensitivity, and allow diagnosis in the prepatent phase. Treatment options include fenbendazole paste, milbemycin oxime/praziquantel and various spot-on formulations. Severe cases should receive prompt medical care in an intensive care unit.

Prevention: Avoiding predation is at present the only preventive measure for pulmonary worms with indirect life cycles.

Zoonotic risk: *C aerophila* has zoonotic potential, causing severe pulmonary disease in humans. Some *Paragonimus* species are also of zoonotic concern.

Introduction

Cardiopulmonary nematodes are emerging parasites of cats and dogs in Europe and have received growing attention from researchers in recent years.^{1–4} Significant progress has been made, mainly in the diagnosis and treatment of infection.

Disease agents

Infection of the lower respiratory tract can be caused by a number of parasitic nematodes. Certain metastrongyloid worms are commonly defined as lungworms because the adult stage is located in the lungs of their hosts, but actually some trichurids and flukes also live in the respiratory system.^{1–3,5} *Aelurostrongylus abstrusus* (*Strongylida*, *Angiostrongylidae*) is the most well known feline lungworm and is regarded as the most prevalent in domestic cats. It is small (5–10 mm) and very narrow (less than 100 µm) and capable of colonising the respiratory bronchioles and alveolar ducts of domestic cats and other felids worldwide.^{2,6} Other respiratory mollusc-borne metastrongylids are commonly reported at necropsy in wild felids but are considered very rare in domestic cats. *Oslerus rostratus* (*Strongylida*, *Filaroididae*) exceeds 30–40 mm in length and infects the bronchial submucosa mainly in wild cats such as bobcats or in feral cats.^{5,7–10} *Troglostrongylus* species (*Strongylida*, *Crenosomatidae*) is reported in a wide variety of wild cats and occasionally in domestic cats;^{4,9,11–14} these worms vary in length, according to the species, from about 10–25 mm and are up to 0.5 mm in width. They are located in the trachea and bronchi or even the bronchioles for the smallest species (*T brevior*).^{12,15}

The trichurid *Capillaria aerophila* (syn *Eucoleus aerophilus*) has a low host specificity and is not uncommon in cats and dogs as well as wild carnivores.¹⁶ It is also a zoonotic parasite, causing a potentially severe pulmonary disease in humans.¹⁷ *C aerophila* is found in the submucosa of the trachea, bronchi and bronchioles.^{2,16}

Mixed infections by respiratory nematodes are sometimes reported^{7,9,14,18–20} and both *Troglostrongylus* species and *O rostratus* may be more prevalent than presumed in domestic cats since there is a risk



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that these infections are being misdiagnosed as *A abstrusus* because of morphometric similarities of their first-stage larvae (L1) in faeces.^{3,4}

Paragonimus species are lung flukes reported in many animals, including cats and humans, and some species are of zoonotic concern. Many species are found in cats, including *P kellicotti*, and between one and 10 adults measuring 8–18 mm x 4–8 mm live in subpleural cysts or bullae.¹

Life cycle and transmission

A abstrusus, *O rostratus* and *Troglostrongylus* species all have an indirect life cycle involving terrestrial molluscs. Eggs of *A abstrusus* laid by female worms hatch in the respiratory tract and L1 larvae are coughed up, swallowed and eliminated in the environment with the faeces. They can actively enter slugs or snails where they moult into the infectious L3 stage.^{21–24} The biological cycle in the intermediate host is influenced by environmental temperature: a higher rate of larval development is observed at warmer temperatures.²³ The L3 larvae are also found in a wide range of paratenic hosts (rat, mouse, lizard, frog, bird) commonly predated by cats.^{1,5,22} The ingestion of L3 larvae by the cat is the best recognised means of transmission of lungworms, but vertical transmission via the placenta or milk cannot be excluded, as adult egg-laying worms have been found in kittens as young as 8 weeks of age.¹⁴ Experimentally, it has been demonstrated that egg production starts 4–6 weeks after infection and may last for months, although it can be irregular.^{6,25–28}

Vertical transmission of *T brevior* was recently observed in a queen and patent infection was detected in 1-month-old kittens.^{13,14,29} *T brevior* and *A abstrusus* larvae may develop simultaneously in the same mollusc host (*Helix aspersa*) and overwinter for at least 120 days.²⁴ Very recently environmental contamination has been suggested as an alternative means of transmission for both *A abstrusus* and *T brevior* L1 on the basis of an experimental study;³⁰ live larvae were found in the pedal mucus excreted by *H aspersa* and in the water where the snails died.

C aerophila has a direct life cycle and eggs laid by female worms in the respiratory tract are swallowed and reach the environment in the faeces. After 30–45 days, embryonated eggs become infective when ingested by cats. Earthworms are facultative paratenic hosts.¹⁶ When cats ingest infective eggs or earthworms carrying larvae, the larvae migrate to the lung and develop into the adult stage in 3–6 weeks.³¹

The life cycle of *Paragonimus* species is asso-

European Advisory Board on Cat Diseases
The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

The latest version of the lungworm disease in cats guidelines is available at www.abcdcatsvets.org and www.abcd-vets.org



Difficulties with morphometric differentiation of L1 larvae have meant that many cases of (co)infection by other metastrongylids may have been erroneously attributed to the better-known *A abstrusus* lungworm.

ciated with freshwater environments and is complex as it involves two intermediate hosts. Motile miracidia are released from eggs when swallowed and then passed in faeces from infected cats and penetrate aquatic snails; cercarial stages developed in snails will move from them, actively entering the second intermediate host (crab or crayfish). Cats are infected after eating the second intermediate host where metacercariae finally develop. Young flukes develop from metacercariae in the cat intestine, and cross the intestinal wall and the diaphragm to the pleural cavity where they penetrate the lung parenchyma and become reproducing adults in about 6 weeks.¹

Epidemiology

Feline lungworm infection is receiving increasing attention.^{2,6} *A abstrusus* is a well recognised agent of lower respiratory tract disease in cats.^{1,2} Epidemiological studies and case reports have confirmed the presence of the parasite in the Americas, Europe, Asia and Australia.^{1,14,32–40} Prevalence rates vary and endemicity is linked to climatic and ecological factors that may influence: (a) the vitality and developmental capacity of L1; (b) the presence of suitable intermediate hosts in the environment; and (c) the number of days needed for development of the infective stage (L3). The diagnostic method used in epidemiological studies and the characteristics of the population investigated heavily influence the results.^{2,37,41,42} Feral and free-roaming cats are at higher risk because of their predator activity, as are cats with respiratory signs and young cats.^{43,44} In Tirana (Albania), post-mortem examination of the lungs of 18 feral cats revealed that nine (50%) were positive for *A abstrusus*.⁴⁵ Use of a low-sensitivity diagnostic method, such as the standard faecal flotation technique, showed a prevalence rate of 1–25% in a general cat population (see Table 1).^{14,46–49}

O rostratus is considered an uncommon parasite in domestic cats, but the prevalence in feral cats was found to be 24% in Majorca (Spain). It was also reported in a cat in northern Spain.^{7,8} Very recently, the incidental occurrence of a few adult *O rostratus* worms was reported in Sicily (Italy) at the necropsy of an adult cat that had died following a road traffic accident.¹⁰

C aerophila has a sporadic occurrence in cats, dogs and humans in Europe. In central Italy, a prevalence of 3–14% was found in the feline population.^{2,16,36}

Single cases of *Troglostrongylus* species infection were recently reported in cats from Ibiza (Spain), central and southern Italy and Crete (Greece).^{9,12,13,15,19,20,29,50} The first epidemiologi-

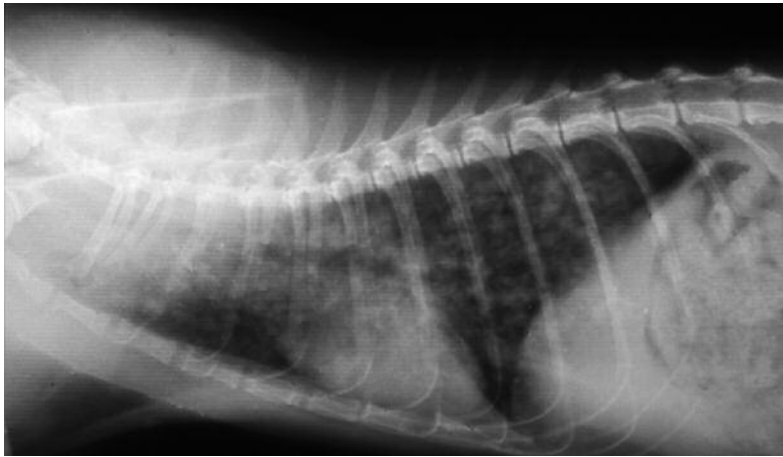


Figure 1 Right lateral thoracic radiograph of a kitten with severe aelurostrongylosis, showing a diffuse focal alveolar pattern. Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

cal data on *T brevior* in domestic cats were recently provided in Sardinia (Italy) where 6.5% of a sample of 107 cats tested positive compared with 25.2% that tested positive for *A abstrusus*,¹⁴ confirming that *Troglostrongylus* is not a negligible lungworm of domestic cats.

The recent development of molecular assays specific for mollusc-borne feline lungworms sharing the same ecological niches, as well as for *C aerophila*, is likely to be of great value for epidemiological investigations, overcoming the difficulties of copromicroscopy for differentiating the metastrongylid L1 larvae.^{3,9,50–52}

Paragonimus species infections are reported in cats from the Americas, Africa and Asia.^{1,53,54} Paragonimiasis is most prevalent in cats and dogs in some parts of Asia.⁵⁵

Prevalence rates and/or documented cases of *A abstrusus*, *C aerophila*, *O rostratus* and *Troglostrongylus* species in some European countries are shown in Table 1.

Pathogenesis

The severity of lesions depends on the worm species and burden. Kittens also seem to develop a more severe disease.^{18,26,27,67} This may be explained by the smaller lung volume and small diameter of the trachea and bronchi, which are more easily blocked by worms, in particular for the larger *Troglostrongylus* species. The immature immune system also seems to facilitate infection: experimental reinfection of kittens with *A abstrusus* L3 larvae about one year after the initial symptomatic infection failed to induce respiratory signs or lung lesions.²⁵ In cats with natural aelurostrongylosis, the more severe

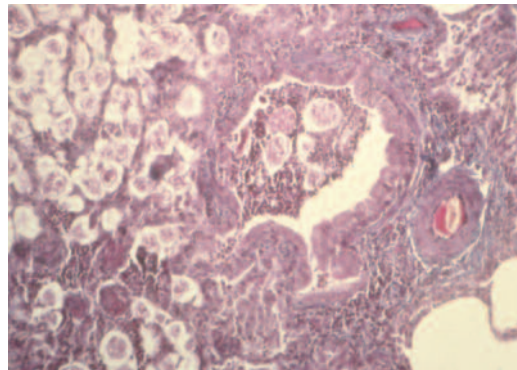


Figure 2 Alveolitis with larval accumulation, bronchiolitis and bronchiectasis in the lung of a cat with aelurostrongylosis (haematoxylin and eosin stain). Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

radiological abnormalities and the higher larval burdens were found in younger animals (Figure 1).⁶⁸

An infective dose of <100 L3 *A abstrusus* larvae does not induce clinical signs but infective doses of 800–3200 larvae severely affect the lung and may even be lethal.^{69,70} However, at normal infective doses, the individual immune response significantly disrupts the parasite life cycle.²⁸ Cats repeatedly infected with a low number of larvae do not develop clinical disease when challenged with a high dose.⁷¹

The role of immunity is confirmed also by the protective effect of passive immunisation in experimentally infected kittens. In some cases it can halt the parasite life cycle and the patent phase of infection does not occur.^{28,72}

It has been recognised for a long time that eosinophilia is evident 2–6 weeks after the ingestion of L3 larvae of *A abstrusus* and that immune-mediated reactions of types I, III and IV are associated with alveolar, interstitial, peribronchial and vascular lesions and may lead to the death of parasites several months later.^{1,73} A more recent experimental study provides more detailed information

on the clinical signs, haematology, biochemistry, coagulation analysis, computed tomography, coprology and post-mortem findings in young adult cats.^{28,70} Infected cats had moderate, non-specific clinical signs (fever, lethargy, weight loss, lymph node enlargement) and respiratory signs (dyspnoea, respiratory sounds, cough). Leucocytosis, massive and persistent eosinophilia and, in some cases, severe lymphocytosis were the most frequently observed abnormalities but no changes were detected on serum biochemistry. Various coagulation abnormalities were found, with a frequent

serum biochemistry. Various coagulation abnormalities were found, with a frequent

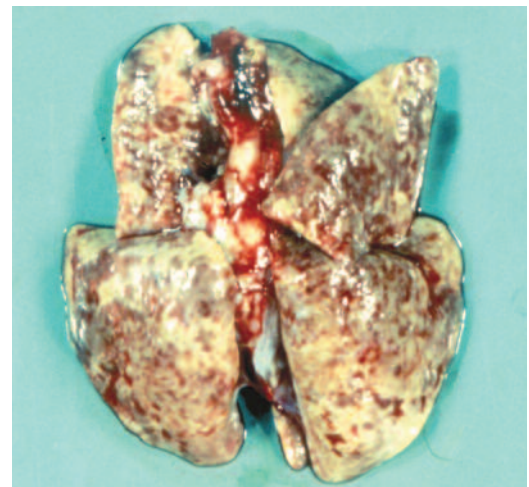


Figure 3 Multifocal subpleural nodules and haemorrhages in a severe case of aelurostrongylosis. Courtesy of Maria Grazia Pennisi, Department of Veterinary Sciences, University of Messina, Italy

Table 1 Prevalence rate (%) and documented cases of *A abstrusus*, *C aerophila*, *O rostratus* and *Troglostrongylus* species infection in various European countries. Case reports of *A abstrusus* respiratory disease exist also from the UK, Ireland, France, Switzerland, Belgium, Denmark, Poland and Greece^{2,6}

Country	References	<i>A abstrusus</i>	<i>O rostratus</i>	<i>Troglostrongylus</i> species	<i>C aerophila</i>
Italy	Brianti et al (2008) ⁴⁷ Traversa et al (2008) ⁴² Iorio and Traversa (2008) ⁴³ Mugnaini et al (2012) ⁵⁶ Riggio et al (2013) ⁵⁷ Spada et al (2013) ⁵⁸ Brianti et al (2014) ¹⁰ Tamponi et al (2014) ¹⁴ Giannelli et al (2014) ¹⁵ Varcasia et al (2015) ²⁰	1.2–25.2% (CR)	(CR)	6.5% (CR)	1.2–14.3% (CR)
Spain	Miró et al (2004) ⁴⁶ Jefferies et al (2010) ⁹	1% (CR)	24% (CR)	(CR)	1.3%
Greece	Diakou et al (2014) ²⁹		–	(CR)	–
Portugal	Payo-Puente et al (2008) ⁵⁹	17.4%	–	–	0.3–0.6%
Netherlands	Robben et al (2004) ⁶⁰	2.6% (CR)	–	–	–
Germany	Taubert et al (2009) ⁶¹ Becker et al (2012) ⁶² Barutzki and Schaper (2013) ⁴⁴	0.7–6.5% (CR)	–	–	0.2%
Croatia	Grabarević et al (1999) ⁶³	22%	–	–	–
Albania	Knaus et al (2011) ⁴⁵	50%	–	–	–
Romania	Mircean et al (2010) ⁴⁸	5.6%	–	–	3.1%
Hungary	András and Péter (2002) ⁶⁴ Capari et al (2013) ⁶⁵	14.5% (CR)	–	–	3.8%
Bulgaria	Stoichev et al (1982) ⁶⁶	33.3%	–	–	–

CR = case report

occurrence of low fibrinogen values suggesting an increased consumption of coagulation factors. Imaging changes in the thorax were related to the dose and consisted of pulmonary nodules, bronchial pattern and lymphadenomegaly and were found even in a cat that did not develop a patent infection.⁷⁰

A abstrusus eggs accumulate in alveoli and bronchioles, inducing an inflammatory reaction in the lung (Figure 2). Multiple subpleural nodules (Figure 3) are caused by the granulomatous reaction surrounding clusters of eggs and adult worms, and emphysema is due to parasitic accumulation in the alveolar spaces. Bronchitis is severe and diffuse, usually manifested by bronchial and peribronchial lymphoid hyperplasia, hypertrophy of the smooth muscle layer and mucosal hyperplasia with increased mucous cell secretion in the bronchi. Vascular and perivascular changes are also seen, with hypertrophy and hyperplasia of pulmonary arteriolar smooth muscle, subendothelial fibrosis associated with eosinophilic infiltrates, endothelial and perivascular hyperplasia. Pulmonary hypertension may be the consequence of lung dis-

Kittens appear to develop more severe lungworm disease.



ease and arteriolar and bronchial changes may persist after the parasite dies, mimicking the changes found in feline asthma.^{27,73–75} Bacterial complication is frequent and can be associated with pleural effusion.²⁶ *Salmonella typhimurium*, *Pseudomonas* species and *Escherichia coli* have been isolated in some cases and infection with enteric bacteria probably results from larvae migrating from the intestine.^{54,76}

In a kitten with severe pulmonary aelurostrongylosis, enteritis and mild diarrhoea were associated with the presence of a high number of L1 larvae invading the small intestinal mucosa.⁴⁰

Lethal *T brevior* infection was associated in three kittens with catarrhal bronchitis occluding the lumen together with the adult worms, and multifocal pulmonary haemorrhages, consolidation and emphysematous foci.^{12,15}

O rostratus does not seem to be associated with severe pathological changes in domestic cats, as few adult worms are found embedded in bronchial or peribronchial tissues inside pseudocysts.^{7,10} *C aerophila* usually induces chronic bronchitis.^{16,77}

The penetration of *Paragonimus* species in the lung is associated with haemorrhagic foci, usually in the diaphragmatic lobe. Fluke cysts enter the bronchi and may evolve into bullae, with a consequent risk of pneumothorax.

Clinical signs

Although the majority of the publications in the literature concern *A abstrusus*, it has been suggested that many cases of infection or co-infection by other metastrongylids may have been erroneously attributed to the better-known *A abstrusus* because of difficulties with the morphometric differentiation of L1 larvae.^{3,4,9} Genetic characterisation of larvae now offers new insights and is likely to allow more accurate diagnosis.

Lungworm infections may be asymptomatic, or cause mild to severe respiratory signs due to bronchopneumonia, sometimes complicated by pleural effusion or pneumothorax.^{26,67,78,79} A productive cough is, therefore, the main clinical sign, together with mucopurulent nasal discharge, tachypnoea, dyspnoea with laboured, abdominal breathing and end-inspiratory crackles upon auscultation. In more severe cases, respiratory failure causes cyanotic mucosae and respiratory acidosis.^{9,18,27,42,80}

Diagnostic imaging (eg, thoracic radiography or computed tomography) reveals bronchial thickening and poorly defined, small nodules during the patent phase. These findings may persist after clearing the infection and should be differentiated from other chronic bronchial disease such as asthma.^{59,81} Imaging changes may be evident even before the patent phase of disease.^{28,70}

Mixed lungworm infections are increasingly reported but they do not necessarily have a more severe clinical picture or poorer outcome.



Right-sided cardiomegaly associated with eccentric hypertrophy and secondary to pulmonary hypertension has been described in two kittens affected by a severe bronchopneumonia caused by *A abstrusus*.²⁷ Both kittens presented with heart murmurs with maximum intensity on the right hemithorax due to tricuspid and pulmonary regurgitation. One of the kittens died but, in the surviving kitten, the heart murmur disappeared several months after parasitological and clinical cure. Echo-Doppler examination confirmed the resolution of pulmonary hypertension.²⁷ It is, therefore, advisable to investigate for the presence of lungworm infection in cases of right heart disease associated with signs of pulmonary hypertension in outdoor cats. In a study of 54 cats that died during anaesthesia in spay-neutering programs in the USA, 9% of post-mortem investigations revealed the presence of *A abstrusus*.⁸² Stray outdoor cats, such as those included in trap–neuter–release programs, are at higher risk of lungworm infection.

Eosinophilia is a frequent abnormality but is not found consistently in cell blood counts or in bronchoalveolar lavage (BAL) cytology.^{18,67,76,83}

Troglostrongylus species was considered the cause of death of parasitised kittens presenting with a cough and severe respiratory failure at diagnosis, but cases of asymptomatic infection have also been reported.^{12,13,15,19,84}

Capillaria infection may induce coughing (mostly dry cough), sneezing and wheezing in cats but asymptomatic carriers have also been reported.^{16,85}

Mixed infections are increasingly reported but they do not necessarily have a more severe clinical picture or poorer outcome.^{19,84,85}

Baermann method

The Baermann method separates live larvae from a faecal sample as they are attracted by humidity (hydrotropism). It can be performed using an in-house system (Figure 4).

- ❖ Fill a large (60 ml) syringe with tap water
- ❖ Connect the cone of the syringe to a rubber tube, which is clamped at the end
- ❖ Orientate the syringe vertically
- ❖ Fill a cheesecloth pouch with approximately 5–10 g of faeces
- ❖ Clamp the pouch and dip it in the water-filled syringe
- ❖ After 24 h any live larvae will have passed into the water and sedimented at the bottom of the system
- ❖ Collect a few millilitres of the water in a tube and centrifuge (400 g x 2 mins). Discharge the supernatant and put one drop of the sediment fluid on a microscope slide. Cover with a coverslip and examine under a microscope at x 100 magnification

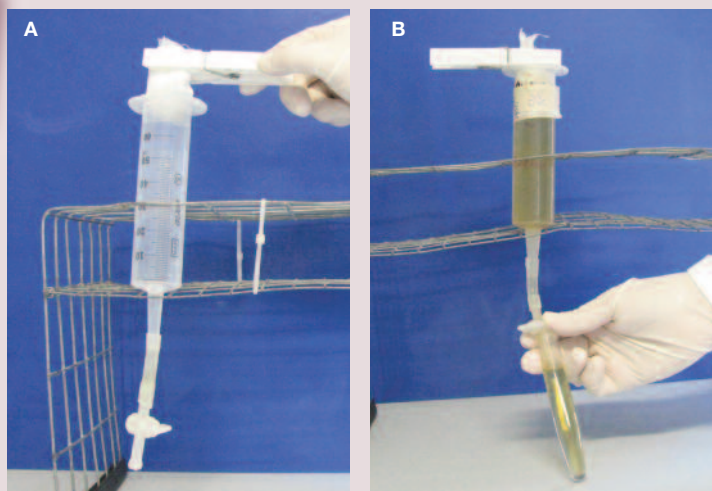


Figure 4 (A,B) Baermann apparatus. Courtesy of Emanuele Brianti, Department of Veterinary Sciences, University of Messina, Italy

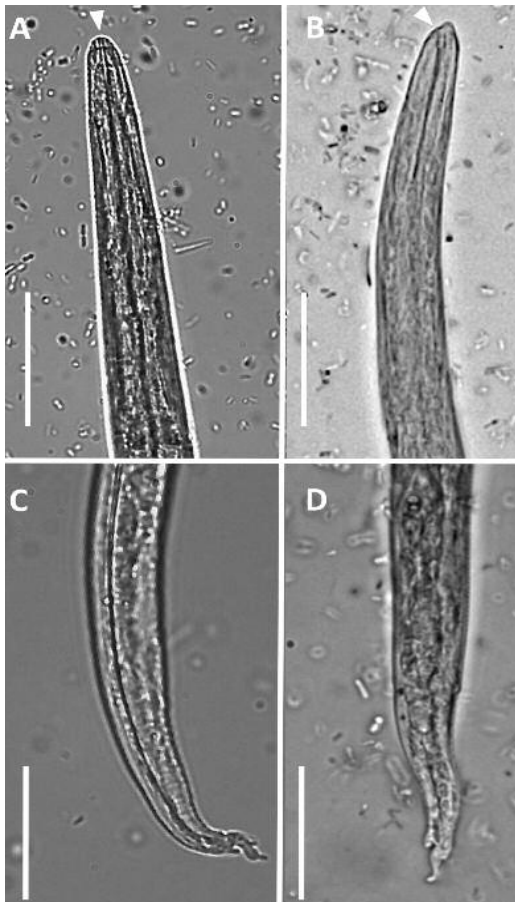


Figure 5 First-stage (L1) larvae of *Aelurostrongylus abstrusus* (A and C) and *Troglostrostrongylus brevior* (B and D) viewed by light microscopy (scale bars = 25 μ m). (A) Anterior extremity of *A. abstrusus*, lateral view. Note the terminal oral opening (arrowhead). (B) Anterior extremity of *T. brevior*, lateral view. Note the pointed head and the subterminal oral opening (arrowhead). Morphology of the tail of *A. abstrusus* (C) and *T. brevior* (D) showing a dorsal spine at the end of the tail.

Courtesy of Emanuele Brianti, Department of Veterinary Sciences, University of Messina, Italy

Diagnosis

L1 larvae are very active in the faeces and are readily detected in fresh faecal samples. Care should be taken to prevent soil contamination of samples, as the presence of free-living nematodes may lead to misdiagnosis. L1 larvae can be observed in direct faecal smears or by the flotation technique. Note that, in the latter method, high specific gravity concentrated salt or sugar solutions may induce osmotic damage to the larvae, making identification difficult.¹ The Baermann migration method is considered the enrichment technique of choice for metastrongyloid lungworms, and is based on the positive hydrotropism observed in live nematode larvae (see box and Figure 4, page 630).^{41,42,86} It can provide quantitative information on the number of larvae found in each gram of faeces, which correlates well with the severity of disease.^{47,68} Unfortunately, 24 h are necessary to obtain the result

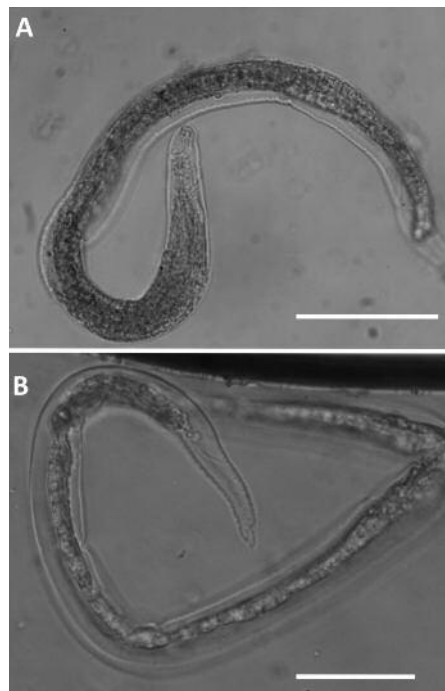


Figure 6 First-stage (L1) larvae of *Oslerus rostratus* viewed by light microscopy (scale bars = 100 μ m). Note the morphology of the cephalic (A) and caudal (B) regions. Courtesy of Emanuele Brianti, Department of Veterinary Sciences, University of Messina, Italy

and the test should be repeated three times in the event of negative results, for optimum sensitivity.

A newer parasitological device for multi-valent quantitative estimation of eggs, larvae and oocysts, named FLOTAC, was evaluated for suitability in the diagnosis of *A. abstrusus* infection. The authors reported that it was more sensitive than the Baermann method.⁸⁷ However, the major limitation of copromicroscopy in general is the impossibility of making a diagnosis in the prepatent period, which lasts about 1–2 months, or when egg shedding has stopped but parasites persist and clinical signs are manifest. A well-trained observer is required to distinguish between the different strongyloid L1 forms on the basis of their morphometric and morphological characteristics (Figures 5 and 6).^{3,12}

Lungworm larvae can be found in tracheal swabs or wash and BAL cytology but with less sensitivity than in faeces, so there is no benefit in using these more invasive procedures that risk severe respiratory disease.⁴¹ Antibodies to *A. abstrusus* can be detected as early as 3 weeks postinfection using an immunofluorescence antibody test, but past and currently active infections cannot be differentiated by serology.⁸⁸

Significant progress has been made diagnostically with the advent of molecular methods. A nested-PCR assay specific for *A. abstrusus* has been validated on different biological samples (faeces, flotation supernatant, Baermann sediment and pharyngeal swabs) collected from cats with natural infections. A specificity of 100% and a sensitivity of up to 96.6% were recorded and the best results were obtained using pharyngeal swabs.⁵¹ This method allows early diagnosis in the prepatent phase, with a potential positive impact on prognosis. Molecular techniques are expected to significantly improve the understanding of lungworm infections. A new multiplex PCR has also been developed for the simultaneous detection of *A. abstrusus* and *T. brevior*.⁵⁰

Capillariosis is diagnosed by standard faecal flotation but molecular techniques are also available for screening and for human cases.^{2,52}

Paragonimiasis is diagnosed using a formalin-ether sedimentation technique.⁵³ Molecular methods are available for epidemiological purposes in cats and are used for human cases.^{89,90}

EBM grades

The ranking system for grading the level of evidence of various statements within the treatment and prevention sections of this article is described on page 574 of this Special Issue.

Table 2 Drugs used for the treatment of pulmonary nematode infections in cats

Drug	Formulation	Dosage	Efficacy
Fenbendazole	Oral paste	50 mg/kg q24h for 3 days	<i>A abstrusus</i> (CS)
Imidacloprid 10% + moxidectin 1%	Spot on	Licensed dosage 1 administration for <i>C aerophila</i> and <i>A abstrusus</i> (CS) 1–3 administrations for <i>A abstrusus</i> (CR)	<i>A abstrusus</i> (CS, CR) <i>C aerophila</i> (CS)
Emodepside 2.1% + praziquantel 8.6%	Spot on	Licensed dosage Repeated after 15 days (CR)	<i>A abstrusus</i> (CS, CR) <i>T brevior</i> (CR) <i>C aerophila</i> (CR)
Fipronil 8.3% + (S)-methoprene 10% + eprinomectin 0.4% + praziquantel 8.3%	Spot on	Licensed dosage	<i>A abstrusus</i> (EI, CR) <i>T brevior</i> (CR)
Milbemycin oxime (4 mg) + praziquantel (10 mg)	Tablet	1 tablet/2 kg every 15 days for three doses	<i>A abstrusus</i> (CR)
Selamectin	Spot on	Licensed dosage for two to three doses	<i>A abstrusus</i> (CR)

CS = controlled study, CR = case report, EI = experimental infection

Treatment

Information on the efficacy of various drugs in the treatment of feline lungworm infection is available from controlled studies and clinical case reports (Table 2). Oral administration of fenbendazole has been suggested, with different doses and durations of therapy (from 20 mg/kg for 5 days to 50 mg/kg for 15 days), but an oral paste is licensed in the UK for treating aelurostrongylosis in cats at 50 mg/kg q24h for 3 days [EBM grade III].²

Off-label use of ivermectin has been reported, with inconclusive results, and should not be considered because of the risk of toxicity, principally in kittens [EBM grade III].⁶⁷

Two spot-on formulations administered at the recommended dosage were compared with a 3 day course of fenbendazole therapy and were found to be effective and safe in the treatment of 12 naturally infected cats each: one formulation contained imidacloprid 10% and moxidectin 1% (Advocate; Bayer), the other emodepside 2.1% and praziquantel 8.6% (Profender; Bayer) [EBM grade I].^{91,92} The moxidectin formulation proved the most efficacious of the three protocols, with 100% efficacy after 30 days [EBM grade I].⁹² In an additional controlled study, the imidacloprid 10% and moxidectin 1% spot-on formulation was significantly effective also against *C aerophila* infection [EBM grade I].⁸⁵ A new spot-on combination of fipronil 8.3%, (S)-methoprene 10%, eprinomectin 0.4% and praziquantel 8.3% (Broadline; Merial) was evaluated under experimental conditions and found to be highly effective for both the prevention and treatment of *A abstrusus* infection

Bacterial secondary infections may contribute to the severity of lungworm disease and require broad-spectrum antibiotic and corticosteroid therapy.



[EBM grade II].⁹³ Very recently the same product was found effective for treating cats with natural disease caused by *A abstrusus* (18 cats), as well as *T brevior* (three cats) or both lungworms (two cats) [EBM grade III].⁹⁴

In a case series study, cats with natural infection treated with the combination of imidacloprid 10% and moxidectin 1% were rechecked at day 14, and those still found positive (4/7) were retreated and checked 1 week later. At that stage, one cat remained positive and was treated for a third time. At the end of the study (day 50), two negative faecal tests had been obtained for all treated cats, confirming the efficacy of the treatment with this combination [EBM grade III].⁴⁷

A combination of milbemycin oxime (4 mg) and praziquantel (10 mg) (Milbemax; Novartis) was administered as a single oral dose (half a tablet per kg) three times, 15 days apart, to a kitten with *A abstrusus* bronchopneumonia and pulmonary hypertension, achieving parasitological and clinical cure [EBM grade IV].²⁷ Efficacy of standard topical administration of selamectin spot-on formulation (6 mg/kg) (Stronghold; Zoetis) was reported in a case report and in two case series.^{67,83,95} In one case series, selamectin was effective in one of four cats at day 30 and in two of the three cats retreated and followed up at day 60 [EBM grade III].⁶⁷ In the second case series, treatment was effective in nine of 10 cats [EBM grade III].⁹⁵ Capillariasis was successfully treated in a cat with two injections of abamectin (14 days apart) at a dose of 0.3 mg/kg [EBM grade IV].⁹⁶

Information on the treatment of *Troglostrongylus*, as well as on mixed infections, is

derived from case reports only. Cases of severe respiratory disease associated with *Troglostrongylus* infection were not cured by imidacloprid 10% and moxidectin 1% or febendazole treatment [EBM grade IV].¹² A combination of milbemycin oxime (4 mg) and praziquantel (10 mg) was administered as a single oral dose (half a tablet per kg) in two kittens with mixed infections caused by *A abstrusus* and *T brevior*. The asymptomatic kitten was cured but the sibling with severe respiratory disease died 2 days later [EBM grade IV].¹⁹ Mixed *T brevior*/*A abstrusus* and *T brevior*/*C aerophila* infections were cured in two kittens using the emodepside 2.1% and praziquantel 8.6% spot-on combination; in one case, two administrations were required to clear *Troglostrongylus* larval shedding [EBM grade IV].⁸⁴

Bacterial secondary infections may contribute to the severity of lungworm disease and broad-spectrum antibiotics should always be given together with corticosteroids at anti-inflammatory doses in cases with signs of bronchopneumonia. Pleural effusion and pneumothorax require immediate resolution by thoracocentesis, and medical care in an intensive care unit (oxygen administration) is required for all cats with respiratory failure.

Molecular techniques are expected to significantly improve the understanding of feline lungworm infections.



Prognosis

In cases of *A abstrusus* infection, a delay in diagnosis and treatment may lead to fatal cardiopulmonary lesions, while early diagnosis and treatment greatly improves the prognosis. The level of larval burden determined by the Baermann test is usually related to the severity of the disease but the prognosis should be based mainly on physical examination (severity of dyspnoea and occurrence of cyanosis) and radiographic findings (severity of diffuse bronchial, alveolar and interstitial disease).

Prevention

Stray and free-roaming cats have a higher risk of becoming infected with lungworms in endemic areas.⁴³ Avoiding predation is at present the only preventive measure for metastrongyloid or trematode pulmonary

KEY POINTS

- ❖ *Aelurostrongylus abstrusus* (*Strongylida*, *Angiostrongylidae*) is the most well known feline lungworm and is regarded as the most prevalent worldwide in domestic cats.
- ❖ Other lungworms in the cat include *Oslerus rostratus*, *Troglostrongylus* species, *Capillaria aerophila* and *Paragonimus* species.
- ❖ *A abstrusus*, *O rostratus* and *Troglostrongylus* species may cause mixed infections as they share the same intermediate and paratenic hosts.
- ❖ Lungworm infections may be asymptomatic, or cause mild to severe respiratory signs due to bronchopneumonia, sometimes complicated by pleural effusion or pneumothorax (nasal discharge, tachypnoea, dyspnoea, coughing). The disease can be fatal.
- ❖ Kittens may be vertically infected and develop a more severe disease at an early stage, due to the smaller diameter of the respiratory tract and their immature immune system.
- ❖ It is advisable to investigate for the presence of lungworm infection in outdoor cats with right-sided heart disease associated with signs of pulmonary hypertension.
- ❖ Stray outdoor cats are at higher risk of lungworm infection.
- ❖ The Baermann migration method is considered the enrichment technique of choice, but takes 24 h to produce results and false negatives may occur.
- ❖ The major limitation of copromicroscopy is that it is not diagnostic in the prepatent period, which lasts about 1–2 months.
- ❖ A nested-PCR assay specific for *A abstrusus* has been validated.
- ❖ Treatment options include fenbendazole paste, milbemycin oxime/praziquantel and various spot-on formulations (imidacloprid 10 %/moxidectin 1%; emodepside 2.1%/praziquantel 8.6%; fipronil 8.3%/(S)-methoprene 10%/eprinomectin 0.4%/praziquantel 8.3%; or selectamin).
- ❖ In severe cases, broad-spectrum antibiotics should be given, together with corticosteroids.
- ❖ *C aerophila* has zoonotic potential and sporadic cases of human capillariasis, manifesting with a productive cough, haemoptysis and lung lesions, have been described.



worms with indirect life cycles. The prophylactic activity of some molecules used to treat nematode respiratory infections – as for *A. vasorum* infection in dogs – is currently unknown; but the spot-on combination of fipronil 8.3%, (S)-methoprene 10%, eprinomectin 0.4% and praziquantel 8.3% (Broadline) was found effective as a preventative treatment for aelurostrongylosis in an experimental setting [EBM grade II].⁹³

Zoonotic risk

C. aerophila has zoonotic potential and sporadic cases of human capillariosis have been described worldwide. The disease manifests as bronchitis with a productive cough, but the presence of haemoptysis and nodular infiltrative lesions in the lung means that pulmonary neoplasia needs to be considered as a differential diagnosis.¹⁷

Paragonimiasis is a food-borne zoonosis acquired by people eating raw crustaceans. Infected cats do not present a risk to people.^{55,97}

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Conflict of interest

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CYTAUXZONOSIS IN CATS

ABCD guidelines on prevention and management

Albert Lloret, Diane Addie, Corine Boucraut-Baralon, Herman Egberink, Tadeusz Frymus, Tim Gruffydd-Jones, Katrin Hartmann, Marian C Horzinek, Margaret J Hosie, Hans Lutz, Fulvio Marsilio, Maria Grazia Pennisi, Alan D Radford, Etienne Thiry, Uwe Truyen and Karin Möstl



Introduction

Cytauxzoonosis has been documented in wild felids such as bobcats, Florida panthers and Texas cougars. The first cases in domestic cats were documented in 1976.¹ For many years, cytauxzoonosis in domestic cats was only reported in North America (south eastern and central states and mid-Atlantic regions) and South America, but in recent years the infection has also been documented in Europe.

Agent properties

Cytauxzoon species are apicomplexan haemoparasites (family *Theileriidae*) of wild and domestic cats, which are transmitted by ticks. Several species have been identified. *Cytauxzoon felis* is the main species, with numerous different strains or genotypes^{2,3} producing infection and severe disease in domestic cats, lions and tigers. Wild cats (bobcats, mountain lions, ocelots, spotted cats and jaguars) in North and South America can act as reservoir or incidental hosts. Recent studies have shown that domestic cats can also harbour subclinical infections and may act as reservoirs.^{4,5} In some endemic areas, the prevalence of subclinical infection in cats may be as high as 30%.⁶ Tick vectors for *C felis* are *Amblyomma americanum* and *Dermacentor variabilis*.⁷⁻⁹

Other species have been identified: *Cytauxzoon manul* in Pallas cats (Mongolia), *Cytauxzoon* spp in Iberian lynx and domestic cats in Spain,¹⁰ and *C* spp in domestic cats in Italy.¹¹ The tick vectors for the European species are still not known, but most likely are *Dermacentor* spp or *Ixodes ricinus*.

Epidemiology

It has been hypothesised that infection in domestic cats involved a species jump from bobcats, in which the prevalence of infection may be high in certain geographic areas.⁸ Disease shows a seasonal incidence from spring to early autumn,^{12,13} associated with peak activity of the tick vectors. There is a significant association between infection and both outdoor access and feral cats in areas where vector ticks are prevalent.¹² No association with gender, breed, age or retroviral status has been found.¹¹

Overview: *Cytauxzoon* species are apicomplexan haemoparasites, which may cause severe disease in domestic cats, as well as lions and tigers. For many years, cytauxzoonosis in domestic cats was only reported in North and South America, but in recent years the infection has also been seen in Europe (Spain, France and Italy).

Infection: *Cytauxzoon felis* is the main species; it occurs as numerous different strains or genotypes and is transmitted via ticks. Therefore, the disease shows a seasonal incidence from spring to early autumn and affects primarily cats with outdoor access in areas where tick vectors are prevalent. Domestic cats may experience subclinical infection and may also act as reservoirs.

Clinical signs: Cytauxzoonosis caused by *C felis* in the USA is an acute or peracute severe febrile disease with non-specific signs. Haemolytic anaemia occurs frequently; in some cats neurological signs may occur in late stages. The *Cytauxzoon* species identified in Europe differ from *C felis* that causes disease in the USA and are probably less virulent. The majority of infected cats have been healthy; in some cases anaemia was found, but disease as it occurs in the USA has not been reported to date.

Diagnosis: Diagnosis is usually obtained by *Cytauxzoon* detection in blood smears and/or fine-needle aspirates from the liver, spleen and lymph nodes. PCR assays are able to detect low levels of parasitaemia and may be used for confirmation.

Treatment: Currently a combination of the antiprotozoal drugs atovaquone and azithromycin is the treatment of choice. Concurrent supportive and critical care treatment is extremely important to improve the prognosis. Cats that survive the infection may become chronic carriers for life.

Prevention: Cats with outdoor access in endemic areas should receive effective tick treatment.

A hyperendemic focus may be found within endemic areas, but is likely due to tick exposure of cats rather than cat-to-cat transmission, which has never been proven.^{14,15} In some areas of the USA an increase in cytauxzoonosis diagnoses has been observed in the past decade and it is considered an emerging disease.¹³

In recent years, the infection has also been documented in Europe. Cases have been described in the Iberian lynx (Figure 1)^{10,16,17} and in domestic cats¹⁸ in the south of Spain, and in domestic cats in France.¹⁹ Moreover, a case series was reported in north-eastern Italy (Trieste) and two cases in central Italy.^{11,20} In the Trieste region, samples from domestic and feral cats showed a 23% prevalence of infection, with a higher prevalence in feral cats (30%). *Cytauxzoon* species in the European cases is different from *C felis*, which produces infection and disease in the USA.

Pathogenesis

The life cycle and complex pathogenesis has been well described for this infection.²¹ Vector ticks ingest merozoite-infected red blood cells from the natural reservoir host (bobcat, lynx or domestic cat). The parasite initiates a process of sexual replication (gametogenesis) in the tick gut and salivary glands. This leads to the formation of sporozoites, which are the infective form and can be transmitted if the tick attaches to a domestic cat. Sporozoites infect endothelial-associated mononuclear cells and undergo asexual replication within the macrophages; these, in turn, develop into large structures known as schizonts – large enough to occlude blood vessels, especially in the liver, spleen and lungs. Widespread dissemination of schizonts results in parasitic thrombosis, circulatory impairment, tissue infection and a severe systemic inflammatory response, which can lead to multi-organ dysfunction and failure and death within 3 weeks of infection.²² When schizonts rupture in the circulation, large numbers of merozoites are released, infecting red blood cells and additional mononuclear cells. This is late-stage disease, with erythroparasitaemia (piroplasm structures within red blood cells) which can be readily observed in blood smears and may lead to haemolytic anaemia and erythrophagocytosis.

Recent studies have evaluated systemic and lung immune responses in cats naturally infected with *C felis* based on serum concentrations of cytokines (TNF α , IL-1 β) and serum



In some endemic areas, the prevalence of subclinical infection in cats may be as high as 30%.

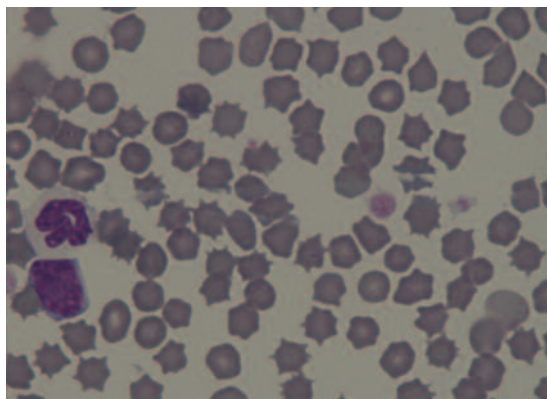


Figure 1 Merozoites within red blood cells in an Iberian lynx from southern Spain. Courtesy of Professor Josep Pastor, Veterinary School of Medicine, Universitat Autònoma de Barcelona, Spain

European Advisory Board on Cat Diseases

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proteins, immunohistochemical expression of several inflammatory mediators and PCR assay for CD18.^{23,24} Both studies demonstrated a marked systemic and lung pro-inflammatory response that can contribute to the pathogenesis of the disease; the response was even more pronounced in cats that died compared with survivors.^{23,24}

Clinical presentation

Cytauxzoonosis (*C felis*) in the USA is typically an acute or peracute severe febrile disease. Clinical signs are non-specific and consist of depression, anorexia, high fever, icterus, dyspnoea, tachycardia, generalised pain and vocalisation. Signs of haemolytic anaemia are frequent (pale mucous membranes, pigmenturia, splenomegaly, hepatomegaly).

Some cats may present or evolve to late-stage disease with neurological signs (ataxia, seizures, nystagmus), hypothermia, moribund state and coma. Many cats die within 1 week of the onset of clinical signs.^{14,25} Veterinarians practising in an endemic area must suspect cytauxzoonosis when faced with any cat with an acute severe disease.

Frequent clinicopathological signs include non-regenerative anaemia, leukopenia with toxic changes, thrombocytopenia, hyperbilirubinaemia, bilirubinuria and an increase in liver enzymes. These changes are associated with erythrophagocytosis and systemic inflammatory response syndrome (SIRS). Coagulation times are usually prolonged due to disseminated intravascular coagulation. Other biochemical abnormalities include hypoalbuminaemia, hyperglycaemia, pre-renal azotaemia, and electrolyte and acid-base disturbances associated with the SIRS state.^{14,25}

Diagnostic imaging reveals non-specific signs consisting of hepatosplenomegaly on abdominal radiography and/or ultrasound, and a pulmonary interstitial-alveolar pattern on thoracic radiography.

Cytauxzoon species infection reported in European cats (Italy, Spain, France) is probably less virulent than *C felis* infection. The majority of infected cats have been healthy, showing only low-level erythroparasitaemia (merozoites within red blood cells) as an incidental finding. In some cats anaemia was described and one cat died after severe disease of a short duration, but no schizont structures were found in tissues, so cytauxzoonosis was not confirmed.

Diagnosis

In clinical practice, diagnosis is usually obtained by identification of *C felis* in blood smears and/or fine-needle aspirates from the liver, spleen and lymph nodes using rapid Romanowsky-type stains.

Observation of schizont-infected myeloid cells on blood and/or tissue smears is the diagnostic test of choice because it confirms acute disease. These are seen as very large (50–250 µm diameter) single cells with an eccentric nucleus containing a single prominent nucleolus. The cytoplasm contains variable numbers of basophilic particles (a few to thousands), which are developing merozoites. These cells may be confused with platelet clumps. The sensitivity of blood smears may be low, so fine-needle aspirates and cytology of liver, spleen, lymph nodes and lungs are indicated if blood smears are not diagnostic in a suspected case.

Observation of merozoites (piroplasms) within red blood cells in thin blood smears prepared with Romanowsky-type stains is supportive of a diagnosis of cytauxzoonosis. However, it does not confirm acute disease as merozoites can be an incidental finding in healthy cats, and may also be observed in cats that have survived acute infection or those with clinical signs of another disease. Piroplasms are usually round to oval structures, 1–2 µm in diameter, with a dark purple eccentric nucleus within a pale blue cytoplasm (signet ring shaped), but in some cases may be more elongated with a bipolar nucleus (Figure 2). One to four merozoites may be observed within individual red blood cells. Sensitivity is not very high, as merozoites appear late in the course of the disease; they are either absent or present in very low numbers in probably more than 50% of cats with acute disease. Blood smears should be performed daily because merozoites can appear over the course of the disease. The distal edges of a blood smear are the best place to look for them.

PCR assays have been developed to confirm the presence of *C felis* and other *Cytauxzoon* species,^{10,11,14} but so far they are not useful as a quick diagnostic tool in practice. It is recommended though that samples from suspected cats are submitted to appropriate laboratories to further confirm the infection. Low levels of parasitaemia can only be detected by PCR assay.⁵ In one clinical trial, parasitaemia was determined by qPCR and at significantly lower levels in surviving cats versus non-surviving cats, so qPCR results might be of prognostic value.²⁶

EBM grades

The ranking system for grading the level of evidence of various statements within the treatment and prevention sections of this article is described on page 574 of this Special Issue.

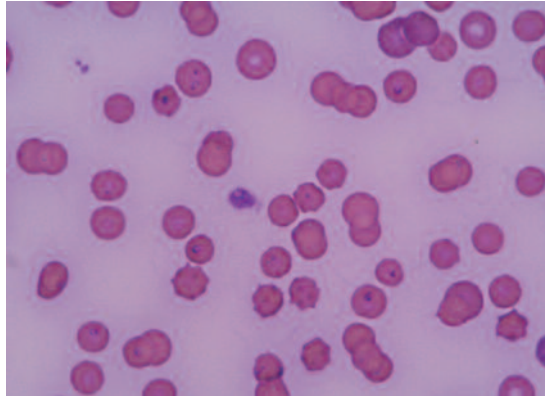


Figure 2 Merozoites within red blood cells in a cat from Trieste (Italy). Courtesy of Dr Erika Carli and Dr Laja Solano-Gallego, Clinica Veterinaria Privata San Marco, Padova, Italy

Cat-to-cat transmission of cytauxzoonosis has never been proven.



Treatment

Historically, cytauxzoonosis has been considered a fatal disease, with mortality approaching 100%. With the recent advances in treatment and/or differences in strain pathogenicity, this is no longer true, although the prognosis remains guarded in some cats.^{27,28}

Supportive and critical care treatment (intensive fluid and oxygen therapy, anti-thrombotic therapies such as unfractionated heparin 200 U/kg SC q8h, blood products, antibiotics, analgesics) is extremely important to keep the cat alive while the antiprotozoal drugs and immune system do their work. Many cats deteriorate during the first days and often die, but if they survive, a gradual improvement is seen over the ensuing days.²⁶

A variety of antiprotozoal drugs have been used in case reports or experimental studies (diminazene, imidocarb dipropionate, thiacetarsamide sodium, tetracycline, parvaquone, buparvaquone) but efficacy has not been proven [EBM grade IV].^{27–29}

Imidocarb had been the drug of choice for many years, although it was not known if it provided any advantage over supportive care alone. However, an open-label randomised prospective clinical trial demonstrated better survival rates (60% versus 26%) with the combination of atovaquone (15 mg/kg PO q8h) and azithromycin (10 mg/kg PO q 24h) compared with imidocarb (3.5 mg/kg IM once) in 80 cats with acute disease.²⁶ Mortality was high (41/80 cats). Most cats died during the first 3 days after presentation, only three cats dying after the third day of treatment. Supportive treatment was the same in all cats, comprising fluid therapy and heparin. This study suggests that this antiprotozoal combination plus supportive treatment is the current approach of choice [EBM grade I].²⁶ In some cats, a nasoesophageal tube may be needed to administer drugs and enteral feeding.

Cats surviving the acute infection may become chronic carriers for life, with piroplasms within the red blood cells. These cats act as reservoirs and may transmit the infection through tick vectors.

A recent study failed to demonstrate efficacy of diminazene at higher doses (4 mg/kg IM) for 5 consecutive days in eliminating or reducing the parasite burden in chronic carrier cats. Moreover, multiple adverse effects appeared, so this treatment is not recommended [EBM grade III].³⁰

Prevention

There is no currently vaccine against *C felis*, although preliminary studies are being conducted.³¹

Prevention is based on living indoors or use of effective tick treatment in cats with outdoor access. Efficacy of an acaricide collar (imidacloprid 10% plus flumethrin 4.5%) for prevention of *C felis* transmission has been proven in a controlled prospective clinical trial. Two groups of cats (with and without a collar) were exposed to ticks (*A americanum*) infected with *C felis*. No cats with a collar, versus 90% of the cats with no collar, were infected [EBM grade II].³²

Testing for the presence of *Cytauxzoon* species in feline blood donors is advised. Although inoculation of merozoites within red blood cells in a blood transfusion does not lead to the development of schizont structures and disease, cats can become chronic carriers and an infection reservoir.

Prognosis

The prognosis for cats with cytauxzoonosis in the USA should be considered guarded to fair, if proper intensive care is provided and atovaquone is available. It has been suggested that different *C felis* strains may vary in pathogenicity, as some cats have survived after not receiving antiprotozoal drugs.^{2,27,33} It is recommended that cats are treated in well-equipped hospitals where the best supportive treatment can be provided.

Veterinarians practising in an endemic area must suspect cytauxzoonosis when faced with any cat with an acute severe disease.



Cytauxzoon infection in Europe reportedly has a good prognosis: so far, only cats with subclinical infection or signs of mild disease (anaemia, diarrhoea), possibly unrelated to the infection, have been documented.^{11,20}

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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KEY POINTS

- ❖ Cytauxzoonosis has been reported worldwide, both in domestic and wild cat species.
- ❖ The parasite is transmitted via ticks, and the prevalence of infection is higher in cats with outdoor access and in feral cats.
- ❖ In the USA, cytauxzoonosis is typically an acute or peracute, severe febrile disease. Non-regenerative haemolytic anaemia is often present, as are neurological signs, followed by death in nearly 100% of cases.
- ❖ Cats infected with *Cytauxzoon* spp have been reported in southern Europe, but clinical signs in those cats were mild and possibly unrelated to the infection.
- ❖ In practice, diagnosis is often based on blood smears and/or fine-needle aspirates from the liver, spleen and lymph nodes using rapid Romanowsky-type stains.
- ❖ PCR assays have been developed to confirm the presence of *C felis* and *Cytauxzoon* species, but are not useful for a quick diagnosis in practice.
- ❖ Current treatment of choice is a combination of atovaquone (15 mg/kg PO q8h) and azithromycin (10 mg/kg PO q24h), as well as fluids, heparin and supportive care.
- ❖ Surviving cats may become chronic carriers.
- ❖ Prevention is based on living indoors or use of effective tick treatment in cats with outdoor access.



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HEPATOZOONOSIS IN CATS

ABCD guidelines on prevention and management



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Overview: Hepatozoonosis of domestic cats has been reported in several countries, mainly as a subclinical infection.

Disease agent: Infection has been described mostly in areas where canine infection is present and, in recent years, *Hepatozoon felis* has been identified as a distinct species by molecular techniques. The vector for feline hepatozoonosis remains unknown and the pathogenesis has not been elucidated.

Infection in cats: Feline hepatozoonosis is mainly a subclinical infection and few cases have been reported with clinical signs. The diagnosis of hepatozoonosis in cats can be made by observation of parasite gamonts in blood smears, parasite meronts in muscles by histopathology, and detection of parasite DNA in blood and tissue by PCR.

Disease management: The treatment of choice is not known, but single cases have been treated with doxycycline or oxytetracycline and primaquine. Although the mode of transmission and the type of vector is not known, preventive treatment against blood-sucking vectors (fleas and ticks) is advised.

Agent properties

Hepatozoon species are apicomplexan parasites (family *Hepatozoidae*) with a blood-sucking arthropod final host and a vertebrate intermediate host.¹ In general, the agent is acquired by ingestion of the infected arthropod (eg, *Rhipicephalus sanguineus* in *H canis* and *H americanum* infection of dogs), but meat eating and hunting are also routes of infection (*H americanum*), as is transplacental transmission (*H canis*).²

More than 340 species of *Hepatozoon* have been described, not only in mammals but also in amphibians, reptiles, birds and marsupials. The first report in a domestic cat dates from 1908 when the parasite was named *Leucocytozoon felis domestici*.³ Later it was reclassified in the genus *Hepatozoon* species⁴ as a result of similarities with the species infecting dogs and wild canids. For some time, reports of the infection in cats referred to *Hepatozoon* species or *Hepatozoon*-like species.

More recently, with the use of molecular techniques, *H felis* was identified as a distinct and predominant species in cat infections;^{5,6} however, there is also evidence that *H canis* can infect cats.^{7–9}

Epidemiology

Feline hepatozoonosis has been reported in several countries worldwide, including India, South Africa, Nigeria, the USA, Brazil, Israel, Spain, France and Portugal.^{3,6,10–16} The prevalence of infection varies depending on the geographical area, cat life style and type of samples tested. Two studies showed a high prevalence of infection in Israel. In one study, meronts were found in the myocardium of 36% of cats examined post mortem.¹⁴ In a more recent study, *Hepatozoon* DNA was found in blood samples of 36% of cats tested.⁹ In Spain, in studies using blood PCR, prevalence rates were much lower, but varied depending on the study populations: 0.6% in domestic cats, 16% in a colony of feral cats and 4% in a group of privately owned cats visiting a referral hospital.^{5,6,17} Two recent studies in Portugal found *H felis* DNA in blood samples in 15.6% of randomly sampled cats and 8.6% of owned and shelter cats.^{16,18}

A significant association between infection and outdoor access has been reported, but no association with gender or age has been observed.⁹ There are conflicting observations as regards retroviral status; one study

European Advisory Board on Cat Diseases

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found no association between feline immunodeficiency virus (FIV) infection and *H felis* infection,⁹ while other studies found a significant association between FIV and feline leukaemia virus (FeLV) infection and hepatozoonosis in cats.^{15,17,19}

The route of transmission has not been fully elucidated yet, but the association with outdoor access suggests transmission by some ubiquitous vectors such as the common flea, mites or ticks, or predation as in other species. The arthropod vectors of *H felis* remain unknown, but recently *H felis* DNA was detected in ticks (*R sanguineus*) in Turkey and Portugal.^{20,21} Transplacental transmission of *H felis* has been suggested and could represent an important route of infection.⁹

Pathogenesis

There have been no published studies on the pathogenesis of infection in cats. Two forms of the parasite have been found in the cat: intracellular gamonts in neutrophils and monocytes, and meronts in several tissues. *H felis* usually produces an infection of myocardial and skeletal muscles.^{14,15} The infection does not lead to significant inflammatory reaction around the parasite meronts, so the cat rarely develops clinical signs.^{9,14,15} The presence of meronts has been observed in many other tissues as well as skeletal muscle and myocardium; for example, lung, liver, pancreas, bone marrow, lymph node and placenta, as well as in amniotic fluid.⁹

The level of parasitaemia is low, with fewer than 1% of neutrophils and monocytes containing *H felis* gamonts.¹⁹ Some studies have shown no correlation between the presence of gamonts in blood smears and meronts in muscle tissues.^{7,14,19}

Clinical presentation

Feline hepatozoonosis caused by *H felis* is mostly subclinical; a high proportion of cats appear to be infected with no overt clinical signs.⁹

The scant clinical information on the disease that exists is based on three case reports describing systemic disease; liver and/or kidney disease were present and *Hepatozoon*-like parasites were demonstrated in liver or blood.^{11,12,22} The remaining reported cases were infected cats with no clinical signs. In a retrospective study of seven cats with *Hepatozoon* species detected in

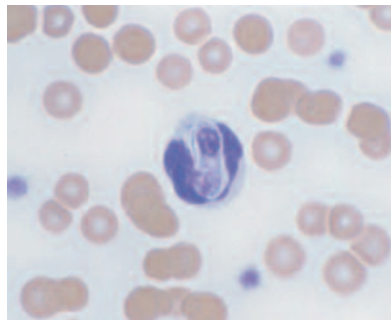


Figure 1 *Hepatozoon felis* gamont within a neutrophil in a cat blood smear. Courtesy of Professor Gad Baneth, School of Veterinary Medicine, Hebrew University, Jerusalem, Israel

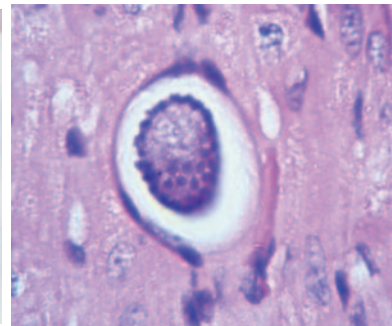


Figure 2 *Hepatozoon felis* meront within myocardial muscle in a cat. Courtesy of Professor Gad Baneth, School of Veterinary Medicine, Hebrew University, Jerusalem, Israel

***H felis* gamonts are less prominent and so are easily missed compared with the larger *H canis* gamonts in dogs.**



blood smears, diverse clinical signs (lethargy, fever, weakness, lymphadenopathy) and clinicopathological abnormalities (anaemia and thrombocytopenia) were described.¹⁹ However, all seven cats were suffering from other diseases, which could explain the clinical signs. Four of the cats were co-infected with

retrovirus and two with haemotropic mycoplasmas, suggesting that the clinicopathological abnormalities were not associated with *Hepatozoon* infection. Interestingly, five of the cats had clinicopathological abnormalities suggesting muscular damage (elevated levels of creatine kinase and lactate dehydrogenase).¹⁹

Observation of *H felis* gamonts in a feline blood smear might be a sign of immunosuppression, which is why retrovirus testing and investigations for other co-infections and diseases is indicated. In an epidemiological study in Barcelona, Spain, four cats that tested positive for *H felis* were sick (attributed to other diseases) and one had leishmaniosis,⁶ suggesting that immunosuppression and/or concurrent disease could be risk factors for *Hepatozoon* infection.

Diagnosis

In clinical practice, diagnosis is usually based on the observation of *Hepatozoon* gamonts in the cytoplasm of neutrophils and monocytes in blood smears stained with Diff-Quik or May-Grunwald Giemsa methods. *H felis* gamonts have an ellipsoidal shape and are 10.5 x 4.7 µm in size (Figure 1). They are less prominent and so are easily missed compared with the larger *H canis* gamonts in dogs.

Several studies have shown that blood smears have low sensitivity for diagnosis of *Hepatozoon* infection compared with PCR detection of DNA. In one study in Thailand, 32% of 300 cats were PCR positive but gamonts were observed in blood smears in only 0.7% of cats.⁷ Similarly, in a study in Israel, none of the cats with meronts in the myocardium tested positive when blood smears were examined.¹⁴ Therefore, blood PCR should be considered the diagnostic test of choice for confirming *Hepatozoon* infection when blood smears do not show parasites and is the best tool for prevalence and epidemiological studies. However, positive DNA results should be interpreted in the light of the

EBM grades

The ranking system for grading the level of evidence of a statement within the treatment section of this article is described on page 574 of this Special Issue.

clinical picture, as it is most likely that clinical signs are associated with another infectious agent. A quantitative PCR test has been developed to improve the sensitivity of detection.⁸

Meronts (round to oval parasites surrounded by a thick membrane, and measuring 39 x 34.5 µm) in skeletal muscle (Figure 2) might be detected in cats in which muscle biopsies are obtained during investigations of muscle pain or polymyositis, but this scenario has not been reported so far. Meronts in skeletal and myocardial muscle might also be detected as incidental or unexpected findings at necropsy of cats in endemic areas.

Treatment

There have been no prospective controlled studies on the treatment of feline hepatozoonosis and so all information is based on a few historical case reports. Doxycycline was used in one case with no clear results,¹² while a combination of oxytetracycline and primaquine in another case led to a successful outcome [EBM grade IV].¹¹ Treatment with drugs that are frequently used in canine hepatozoonosis has not been reported in cats.

Prevention

No clear recommendations on the prevention of infection can be made, as the routes of transmission in cats remain unknown. It is likely that, as in dogs, transmission is related to blood-sucking vectors, as well as the consumption of meat and the transplacental route. Therefore, preventive treatment against external parasites (fleas, ticks, others) is strongly advised in any cat, especially one with outdoor access.

KEY POINTS

- ❖ Hepatozoonosis in cats has been reported in many countries, mainly as a subclinical infection or an incidental finding.
- ❖ Ingestion of infected vectors (ticks, fleas) or meat, and transplacental transmission seem to be the most common routes of infection.
- ❖ *H felis* is the predominant species in cats, although *H canis* can also infect cats.
- ❖ Observation of *H felis* gamonts in a cat may be a sign of immunosuppression.
- ❖ Blood smears have low diagnostic sensitivity; blood PCR is the diagnostic method of choice.
- ❖ No evidence-based treatment protocol exists for cats.
- ❖ Preventive treatment against ticks and fleas is recommended.



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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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