GUIDELINE for Blood transfusion in cats

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Key Points

- Blood transfusion in patients with poor prognosis should be avoided.
- Each step of the blood transfusion procedure should be performed under strict hygienic 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 be discarded when there is any suspicion of change in colour or other visible abnormality.
- The worldwide core screening panel for donor cats includes FeLV, FIV, *Bartonella* and feline *haemoplasma* species.
- The most useful, practical, rapid and inexpensive preventive measure is to check the risk profile of donor cats.
- Risk assessment may eliminate the need of 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.

Abstract

Blood transfusion in dogs and cats is more commonly done than in the past and fresh whole blood can be made available to clinicians because it is 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, deals with adverse events caused by infectious agents following transfusions, 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.

The risk of contamination of donor blood is potentially higher in cats because collection is usually obtained using a semi-closed system that requires a multi-step manipulation of syringes and other devices. It is crucial that each step of the procedure is performed under the strictest hygienic conditions in order to prevent bacterial contamination of blood bags. The longer the storage of blood bags, the higher the risk of growing of contaminating bacteria and bacterial endotoxins can cause an immediate febrile reaction or even fatal shock.

To prevent transmission of blood-borne infectious diseases the American College of Veterinary Internal Medicine adopted basic criteria for selecting pathogens to be tested in donor pets. According to that the worldwide core screening panel for donor cats includes feline leukaemia virus, feline immunodeficiency virus, *Bartonella* spp. and feline haemoplasma species. The list should however be adapted to the local epidemiological situation concerning other feline vector-borne pathogens and the best examples are currently in Europe *Cytauxzoon* sp. and *Leishmania infantum*. The most useful, 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. However, blood transfusion can never be considered

totally safe.

Introduction

Over recent decades, small animal transfusion medicine has made significant progress, contributing to the development of emergency medicine and critical care. Blood transfusion in dogs and cats is more commonly done than in the past and fresh whole blood can be made available to clinicians because it is taken from in-house donor cats or “volunteer” feline blood donors. However, the evidence-based benefit of blood transfusion is still lacking in certain cases (Davidow, 2013).

Thanks to the commercial availability of in-house typing kits, gel cross-match systems, and blood-collection closed systems for cats, blood transfusion has become safer and more accessible in feline practice (Rudd, 2013a; Crestani et al., 2018; Goy-Thollot et al., 2019). However, blood transfusion implies a certain amount of risk to the recipient cat and, to some extent, also to the donor cat, subjected to an invasive procedure, usually requiring sedation for blood collection (Spada et al., 2015). These risks always need to be carefully weighed against the achievable benefits; this is especially important now that feline blood components are increasingly available for purchase making transfusions increasingly ‘simple’. Blood transfusion in patients with poor prognosis should be avoided and other treatment options should be considered (Rudd, 2013b). Surprisingly, blood transfusion did not reduce the risk of 30-day mortality in humans in a study performed in a critical care setting (Hérbert et al., 1999).

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 specific guidance is provided elsewhere (BSAVA Scientific Committee, 2000; Helm and Knottenbelt, 2010a, 2010b; Barfield and Adamantos, 2011; Davidow, 2013; Rudd, 2013a, 2013b; Kisielewicz and Self, 2014; Zaremba et al., 2019).

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

Prevention of contamination of donor blood

The blood collecting procedure in cats is of greater risk of contamination when blood is not collected through a closed system, and a multi-step manipulation of syringes and other devices is required, with the help of several assistants. This semi-closed system of collection increases the risk of contamination as about 50 ml of blood is collected from the donor cat using three (20 ml) or five (10 ml) different syringes, each containing the appropriate quantity of anticoagulant obtained from a human blood collection bag (Rudd, 2013b). Usually, a T-connector and a three-way tap connect the 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 (Fig. 1). Finally, blood is transfused through a giving set inserted into another port of the bag when transfusion has to be performed.

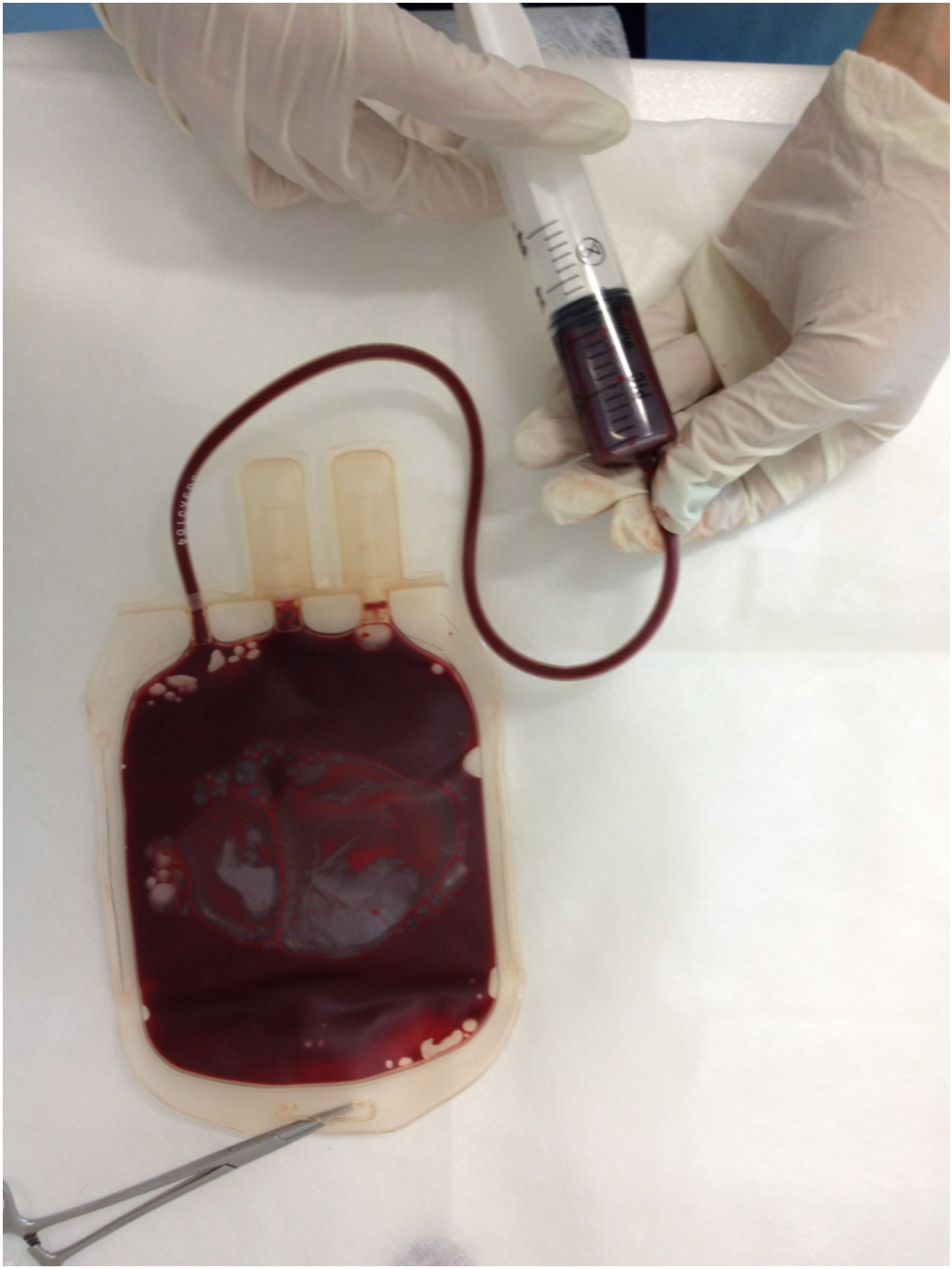

Fig. 1. Transfer of blood collected with syringes into a single, plain blood collection bag through the injection port. Courtesy of Eva

Spada, University of Milan, Milan, Italy.

It is crucial that each step of the procedure is performed under the strictest hygienic conditions, even in an emergency (FECAVA, 2013). The surface carrying the disposable equipment should be sterile 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 always necessary. Closed blood collection equipment is now available for cats (e.g. Futurlab, Italy).

The longer the delay between blood collection and transfusion, the higher the risk of detection of cold-growing bacteria in the collected blood stored at 4°C. In fact, bacterial growth was not found one day after donation of eight blood units collected using a closed system, but one of them was positive for *Serratia marcescens* after 35 days of proper storage (Crestani et al., 2018). Another report described the occurrence of visible colour changes 32 days after the collection by a semi-closed system in a whole blood bag undergoing a daily visual inspection (Stefanetti et al., 2016). Conversely, the control analysis of 489 feline packed red blood cell units did not detect bacterial contamination after one week of storage (Blasi Brugué et al., 2018). However, the blood bag should never be stored once the giving set has been inserted.

The same principles must be applied in case of autologous transfusion, a procedure reported in dogs and cats in emergency situations such as haemothorax or haemoperitoneum: in this situation blood is collected from the body cavity using cell salvage devices and transfused after appropriate washing (Tasker, 2013; Kisielewicz and Self, 2014; Cole and Humm, 2019).

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*. *Pseudomonas fluorescens* was detected in a blood bag filled with a semi-closed system after 32 days of storage when a brown-purple darkening was observed (Stefanetti et al., 2016). *Serratia marcescens* was isolated from contaminated feline blood bags and from transfused cats that presented with fever, vomiting, diarrhoea, jaundice and even death (Hohenhaus et al., 1997; Crestani et al., 2018). Fatal endotoxin-related shock occurring soon after blood transfusion is the most dangerous consequence in such cases, and it may be misdiagnosed as an immune-mediated adverse reaction.

Blood bags should be visually inspected before use and be discarded where there is any suspected change in colour or other visible abnormality (Wardrop et al., 2016).

Prevention of transmission of blood-borne infectious diseases

Information on feline blood-borne infectious agents is becoming increasingly available, in particular regarding vector-borne pathogens and infections detected in candidate healthy blood donors (Hackett et al., 2006; Beugnet and Marié, 2009; Vilhena et al., 2013; Persichetti et al., 2018). Since 2005, the American College of Veterinary Internal Medicine (ACVIM) consensus statement on canine and feline blood donor screening for infectious diseases adopted basic criteria for selecting pathogens to be tested in donor pets (Wardrop et al., 2005, 2016). Testing is recommended for pathogens that meet at least three of the following criteria: (1) documented clinical disease obtained in recipients by blood transmission; (2) possibility of subclinical infections (healthy carrier state); (3) possibility of detection using cultivation or PCR from the blood of an infected animal; (4) the disease caused is severe or difficult to clear. However, optimal standards of safety require testing also for pathogens that can be experimentally transmitted with blood inoculation (Wardrop et al., 2016).

The worldwide core screening panel for donor cats (Table 1) therefore includes: feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), *Bartonella* spp. and feline haemoplasmas (Reine, 2004; Wardrop et al., 2005, 2016; Gary et al., 2006), but the list of pathogens to be tested in donor cats should be adapted to the local epidemiological situation and other infectious agents may be investigated in endemic areas (Table 2).

Table 1: List of core pathogens worldwide to be screened for candidate blood donors

PATHOGEN (*)	DIAGNOSTIC TESTS
Feline leukaemia virus (FeLV)	FeLV provirus PCR §
Feline immunodeficiency virus (FIV)	Rapid anti-FIV antibodies test on blood serum/plasma

PATHOGEN (*)	DIAGNOSTIC TESTS
Mycoplasma haemofelis *Candidatus* Mycoplasma haemominutum *Candidatus* Mycoplasma turicensis	blood PCR
Bartonella spp.	anti-*Bartonella* antibodies (IFAT) and/or blood PCR

(*) For more information on these pathogens, see Hosie et al. (2009), Lutz et al. (2009), Pennisi et al. (2013a), Tasker et al. (2018)
Tests for anti-FIV antibodies and FeLV DNA should be confirmed negative at least three 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 a higher risk.

Table 2: List of pathogens to be considered for screening candidate blood donors on the basis of epidemiological information

PATHOGEN	DIAGNOSTIC TESTS
Cytauxzoon spp.	blood PCR in endemic areas
Babesia spp.	blood PCR in endemic areas*
Leishmania infantum	blood PCR and quantitative antibody detection in endemic areas
Ehrlichia spp.	blood PCR in endemic areas*
Anaplasma phagocytophilum	anti-*A. phagocytophilum* antibodies (IFAT) and blood PCR in endemic area
* probably rare and poorly characterised infection of cats in Europe	

For more information concerning these pathogens, see Carli et al. (2012), Hartmann et al. (2013), Pennisi et al. (2013b), Pennisi et al. (2017)

For instance, *Cytauxzoon* sp. post-transfusion infection was recently reported in a cat in Switzerland and the donor cat was found to be a healthy carrier (Nentwig et al., 2018). Additionally, in endemic areas for *Leishmania infantum* a non-negligible percentage of cats are blood-PCR positive and the parasite transmission has been documented by blood transfusion in dogs and humans (Pennisi et al., 2013b).

Toxoplasma gondii does not meet the ACVIM criteria (Wardrop et al., 2016) and ABCD does not recommend to test donor cats for *T. gondii* infection. Similarly feline coronavirus (FCoV) is not included in the donor screening panels, however, the presence of antibodies against FCoV in blood products may passively immunize transfused cats. In case of contact with FCoV in the weeks following transfusion, these cats could be exposed to the risk of the antibody dependent enhancement (ADE) of macrophage infection (Takano et al., 2008; Bàlint et al., 2014) if they had been FCoV seronegative prior to transfusion. Although there have been no reports of FIP following blood transfusion, FCoV-antibody negative blood bank donors may be preferred. In reality the ubiquitous nature of FCoV amongst household cats makes this selection criterion too prohibitive for routine blood donor selection and it is not adopted.

Although *Rickettsia felis* and *Rickettsia* spp. 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 (Lappin and Hawley, 2009).

Interestingly, 45% of 31 samples of blood collected from cat donors were retrospectively found positive for parvovirus DNA but the clinical importance of this occurrence is not known (Marenzoni et al., 2018a).

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-specific haemoplasmas but may be relevant for vector-borne infections, some

of which are more common in dogs than in cats (Otranto and Dantas-Torres, 2010). Xenotransfusion is restricted to exceptional circumstances, e.g. emergencies in case of lack of compatible feline blood or oxygen carrier solution (Oron et al., 2017), as it is associated with delayed immune-mediated haemolysis and a very short lifespan of the transfused erythrocytes (Bovens and Gruffydd-Jones, 2013).

Molecular tests have significantly increased the sensitivity and specificity of diagnostic tests for the detection of feline blood-borne agents and their use has increased the safety of blood products. However, the sensitivity of blood PCR is variable according to the method used, with well-designed real-time PCR assays being the most sensitive, and whether the pathogen presence in the blood of healthy carriers is not intermittent. When available, antibody detection tests are useful to better evaluate exposure of donors to blood-borne pathogens.

Healthy cats that test negative for FeLV p27 antigenaemia can still harbour FeLV provirus integrated in their DNA, which means their blood can transmit FeLV infection to transfused cats with a high risk for persistent FeLV infection and FeLV associated disease in the recipient cat (Lutz et al., 2009; Nesina et al., 2015). Blood bank donors should therefore be tested for FeLV provirus using real-time PCR (Hofmann-Lehmann et al., 2008). In life-threatening emergency situations, transfusions from donors can be screened using rapid FeLV antigen tests, but owners should be informed about the risk for the transfused cats and also those in the same household.

The screening of blood donors is also affected by costs. In human medicine, individual blood units are usually tested for several pathogens of major concern (e.g. HIV, HBV, HCV, *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 risk assessment related to travels, sexual behaviour or 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 (Table 3). This questionnaire can be presented together with the informed consent form for blood donation. The ideal low-risk profile of a donor cat is an adult cat (more than three years old in order to reduce the risk for *Bartonella* bacteraemia), strictly indoor since birth, living since being a kitten in the same single-cat household (full history directly available from the guardian), regularly vaccinated and treated against fleas and ticks, with no history of travelling or vector-borne diseases. Heartworm prevention in endemic areas is also indicated, although the blood from infected cats is not infectious following transfusion (Lee and Atkins, 2010).

Table 3. Risk profile form for candidate blood donors

Edit

OWNER:…… CAT'S NAME:……..……. BREED:……. GENDER: M / F NEUTERED: AGE:…… CIRCLE THE CORRECT ANSWER	**IF ALL ANSWERS ARE IN THE RIGHT-HAND COLUMN, THE CAT HAS A LOW-RISK PROFILE FOR TRANSMISSION OF INFECTIOUS AGENTS BY BLOOD.**		
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

OWNER:…… CAT'S NAME:…… BREED:…… GENDER: M / F NEUTERED: □ AGE:…… CIRCLE THE CORRECT ANSWER	IF ALL ANSWERS ARE IN THE RIGHT-HAND COLUMN, THE CAT HAS A LOW-RISK PROFILE FOR TRANSMISSION OF INFECTIOUS AGENTS BY BLOOD.		
Do you regularly use anti-flea products?	No	Don't know	Yes
Has your cat been vaccinated?	No	Don't know	Yes
When your cat was vaccinated last?			
Is your cat eating less than usual?	Yes	Don't know	No
Have you recently seen any unusual behaviour in your cat?	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

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

Risk should be reassessed prior to each transfusion. Risk assessment may eliminate the need of repeating 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 risk of acquiring the infection. The ABCD does not recommend the use of closed colony donors, which are specifically bred for blood banks, as it would be preferable for cats' welfare to live in a more natural environment.

If no feline blood is available from a blood bank, veterinary practitioners should try to be able to rely on an adequate number of pre-selected potential donors evaluated as low-risk cats and negative for blood-borne pathogens of interest. Based on epidemiological data on blood-borne pathogens, outdoor cats should never be candidated as potential donors (Hackett et al., 2006; Ravagnan et al., 2017; Persichetti et al., 2018). 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 (Wardrop et al., 2016).

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 of the donor health. Moreover, only in-house tests can be used for testing donors in emergency cases, which implies they will be screened only for retroviral infections following a physical examination, CBC, biochemical profile, and urinalysis. Records of the donor and recipient cats should be taken and an EDTA blood sample from the donor (the tube and sample taken for CBC may be used) should be kept, stored frozen at -20 °C, for possible further investigations.

National guidelines for companion animal blood transfusions are in force in some European countries, such as Germany and Italy, that allow lower safety levels than those proposed by ABCD (Ministero della Salute della Repubblica Italiana, 2008, 2016; Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011). In fact, they both require testing for FIV and FeLV but these investigations can be performed by in-house tests only, precluding the identification of cats with non-progressive FeLV infection (Hofmann-Lehmann et al., 2008; Lutz et al., 2009; Masucci et al., 2010; Marenzoni et al., 2018b). Haemoplasma PCR testing is required in Germany before the first donation and again when ectoparasites are found, as well as other diagnostic tests in case of suspicion of other infections (e.g. *A.*

phagocytophilum, L. infantum, Bartonella spp., *E. canis, Neorickettsia risticii* (USA), *Cytauxzoon felis* (USA)) (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011). Conversely, in Italy only *M. haemofelis* infection testing is additionally required and the detection can be based only on blood smear cytology, whose sensitivity is really poor for detecting healthy carriers (Masucci et al., 2010; Ministero della Salute della Repubblica Italiana, 2008, 2016; Tasker et al., 2018). It is however remarkable that German guidelines exclude from donation free-roaming cats and cats in contact with them, as well as cats from countries where different risks of blood-transmitted infections exist.

Other use of blood products in practice

The topical application of blood serum is empirically used as anticollagenolytic treatment in the medical management of deep corneal ulcers (Hartley, 2010; Mitchell, 2013; Fig. 2) or some corneal surgery in human patients (Sul et al., 2018).

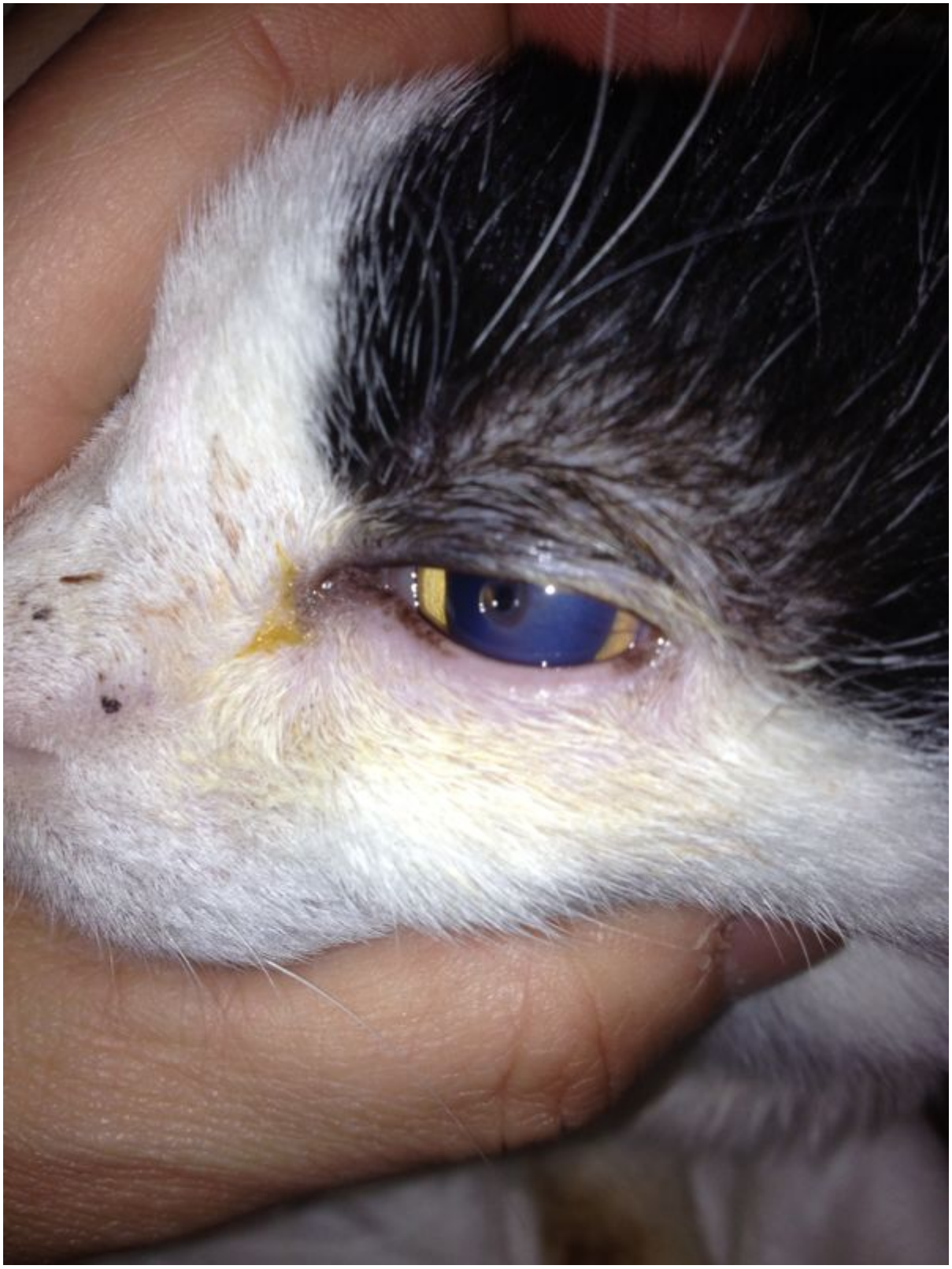

Fig. 2. Deep (stromal) corneal ulcer. Courtesy of Maria Grazia Pennisi, University of Messina, Messina, Italy.

The autologous preparation is cheap and easy to administer in practice, but this must be performed under strict aseptic conditions 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 hours) 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 the impracticality 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 in the ocular mucosa and damaged corneal tissue (Mitchell, 2013). In case of homologous serum, the donor should be carefully selected respecting the same criteria as used for blood transfusion.

Autologous platelet-rich plasma is increasingly used for treating orthopaedic conditions in veterinary practice, including cats. Bacterial contamination during the preparation of the concentrate may occur and this risk must be reduced by strict hygiene (Hoareau et al., 2014).

Homologous or heterologous corneal grafting is among the grafting techniques used to treat, by keratoplasty, very deep or full-thickness corneal defects such as feline corneal sequestrum (Laguna et al., 2015). Corneal tissue is obtained from donors euthanized for reasons other than systemic infection or neoplasia and used fresh (preserved at 4°C for a maximum of 48°C) or frozen (stored at -20°C up to one year). There is still limited information about this promising technique but the risk of transmission of pathogens from the homologous corneal tissue might concern viruses with specific ocular tropism such as feline herpes virus 1 (FHV-1), and feline calicivirus (FCV), but also FCoV, FeLV, and FIV as lymphocytes and macrophages can be found in healthy corneal epithelium and stroma (Herring et al., 2001; Stiles and Pogranichniy, 2008). Moreover, pathogenic bacteria (*Staphylococcus, Bacillus,* and *Clostridium*) were isolated from corneal tissue stored at -20°C less than one year and decontamination protocols should be validated to avoid this risk (Costa et al., 2016).

Conclusion

Blood transfusion can be a life-saving treatment with a crucial impact on anaesthetic and surgical possibilities or intensive care, but it never can be considered totally safe. The development of infectious diseases in recipient cats is an iatrogenic risk that must be minimised by the highest level of care for good 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 removed entirely. The most cost-effective action is to reduce this risk by the pre-selection of low-risk donors.

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