Thieme

Updates on practical *ABC* blood compatibility testing in cats

Avoiding acute hemolytic transfusion reactions and neonatal isoerythrolysis

Neuigkeiten zur praktischen *ABC*-Blutgruppen-Bestimmung bei Katzen

Vermeidung akuter hämolytischer Transfusionsreaktionen und der neonatalen Isoerythrolyse

Authors

Alexandra Kehl[1], Laura Truchet[1], Ines Langbein-Detsch[1], Elisabeth Müller[1], Urs Giger[2]

Institutes

1 Laboklin GmbH & Co. KG, Bad Kissingen, Germany
2 Section of Medical Genetics (PennGen), University of Pennsylvania, Philadelphia, USA

Schlüsselwörter

Katze, Blutgruppe, Kreuzprobe, Alloantikörper, Anämie, feline neonatale Isoerythrolyse

Key words

Feline, blood type, crossmatch testing, alloantibody, anemia, hemolysis of newborn

received 30.08.2019
accepted 21.10.2019

Bibliography

DOI http://dx.doi.org/10.1055/a-1035-9502
Tierarztl Prax Ausg K Kleintiere Heimtiere 2019; 47: 1–11

ISSN 1434–1239

Correspondence address

Alexandra Kehl
Laboklin
Steubenstraße 4
97688 Bad Kissingen
Germany
kehl@laboklin.com

ZUSAMMENFASSUNG

Bis in die 1980er Jahre wurden die Blutgruppen bei Katzen als unwichtig angesehen. Seitdem gab es jedoch viele neue Erkenntnisse. Wir wissen heute: Das wichtigste Blutgruppensystem der Katze ist das *AB*-Blutgruppensystem (umbenannt als *ABC*-Blutgruppensystem) und umfasst die Blutgruppen *A*, *B* und *AB* (heute *C* genannt). Katzen haben natürlich vorkommende Antikörper gegen die Antigene der jeweils anderen Blutgruppe. Insbesondere Katzen mit Blutgruppe *B* haben hohe Titer an natürlich vorkommenden Anti-*A*-Alloantikörpern. Dadurch kann es bei *A*-*B*-inkompatiblen Bluttransfusionen und bei der sog. neonatalen Isoerythrolyse bei Welpen mit Typ *A* und *C* (*AB*) von Muttertieren mit Typ *B* zu Unverträglichkeitsreaktionen kommen. Um dies zu verhindern, ist es essenziell, für Zucht und im Vorfeld von Transfusionen die Blutgruppe und die zugrundeliegende Genetik zu kennen. Klinisch helfen serologische und genetische Tests. Diese Synopsis gibt einen aktuellen Überblick über die Blutgruppen, deren physiologische und genetische Grundlagen, mögliche Unverträglichkeitsreaktionen sowie die verschiedenen Nachweismethoden zu deren Vermeidung.

ABSTRACT

In feline practice, blood groups were considered unimportant until the 1980s. Since then much has been learned. The most important blood group system in cats is the *AB* (renamed here as *ABC*) blood group system consisting of blood types *A*, *B* and *AB* (better referred to as *C*). Type *B* cats have strong anti-*A* alloantibodies potentially leading to incompatibility reactions during *A*-*B* mismatched transfusions or neonatal isoerythrolysis (NI) in type *A* and *C* (*AB*) kittens born to type *B* queens. Acute hemolytic transfusion reactions as well as NI have been clinically well documented in cats. Immunological and genetic tests have been established and blood typing and crossmatching test kits have become commercially available. This review updates the current knowledge of these blood types, their genetics, associated incompatibility reactions, and different diagnostic tools for avoiding such reactions in clinical practice.

▶ **Table 1** Blood type frequencies in non-purpose-bred cats in different European countries based on past surveys of ~100 to over 700 typed cats per country.

▶ **Tab. 1** Blutgruppenhäufigkeit bei Hauskatzen in verschiedenen europäischen Ländern basierend auf Untersuchungen mit ca. 100 bis 700 untersuchten Katzen pro Land.

Country of survey		Blood type frequency (%)			Cats (n)	Reference
		A	*B*	*C (AB)*		
Austria		97	3	0	101	[41]
Denmark		98.1	1.9	0	105	[42]
England	Southeast	67.6	30.5	1.9	105	[10]
	Bristol	79.3	12.2	8.5	82	[9]
France		89.6	10	0.4	231	[43]
Germany		94.1	5.9	0.0	404	[44]
Greece		78.3	20.3	1.4	207	[45]
Hungary		100	0	0	81	[46]
Ireland		84.7	14.6	0.7	137	[47]
Italy		90.7	7.1	2.1	140	[48]
Netherlands		95.8	3.1	1.1	95	[49]
Portugal	Porto	97.3	2.7	0	771	[11]
	Lisbon	97.5	2.1	0.4	55	[50]
	North	89.3	4.4	6.3	159	[51]
Scotland		97.1	2.9	0	70	[49]
Spain	Barcelona	91.1	8.9	0	56	[11]
Switzerland		87.6	8	4.4	1014	[52]
Turkey		72.8	25	2.2	312	[53]

Feline *ABC* blood types

Geographic and breed frequencies

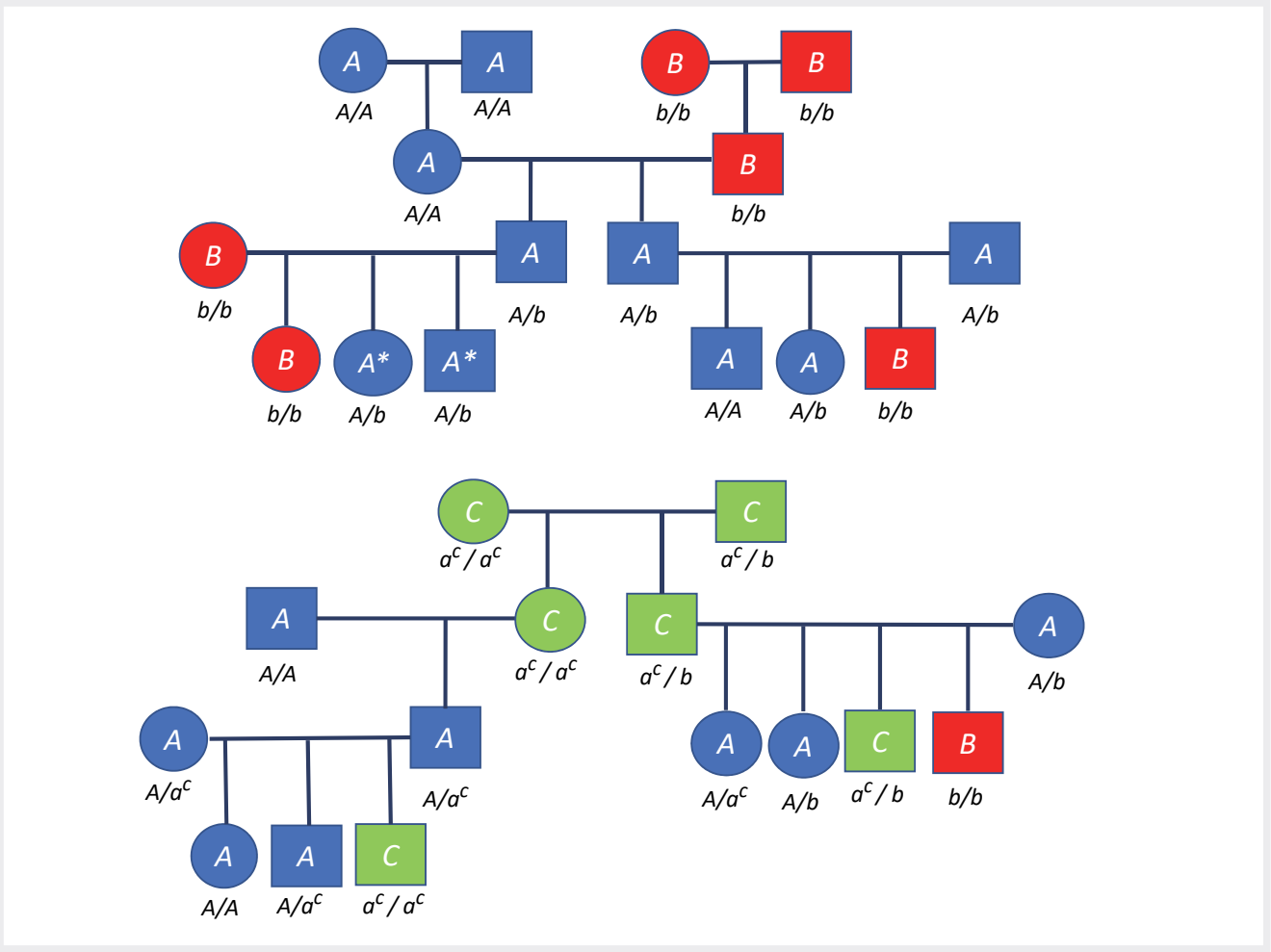
The major blood group system in domestic cats is known as the feline *AB* (possibly better referred to as *ABC*) blood group system with 3 blood types: *A, B,* and *AB* (better referred to as *C*) [1][2][3][4]. While overall type *A* is most common and type *C* is extremely rare, the frequencies of these three blood types vary among breeds and geographic regions based on many surveys (▶ **Table 1**, ▶ **Table 2**). Between 90–100 % of European domestic shorthair cats have type *A* blood, except in the United Kingdom, Greece, Turkey, and Israel, where 75–80 % have type *A* blood. Among purpose-bred cats, the Siamese and closely related breeds seem to have almost exclusively type *A* blood, while in other common breeds, like Abyssinians, Himalayans, and Persians, type *A* frequency ranges between 70–90 % with the remaining being *B* or *C*. In contrast, in British Shorthair, Birman, Rex, and Sphinx as well as the Turkish Angora and Van breeds, the type *B* has a high frequency up to 50 % (▶ **Table 2**). Of course, these estimates may vary greatly locally and among catteries, because breeding regimes may influence the blood type frequencies. Interestingly, type *C* is extremely rare except in Ragdolls [4][5][6][7][8], Turkish Angoras, and non-purpose-bred cats in England, Israel, and the Iberian Peninsula (albeit typing methods may have affected some results) [9][10][11].

In addition to the feline *ABC* blood group system there are others such as the *Mik* blood group with mostly *Mik+* and very rarely *Mik–* cats [12]. Because of these other blood group systems and the

▶ **Table 2** Blood type frequencies (typed by Alvedia kit) in several cat breeds in Germany. Internal retrospective study from Laboklin Germany with overall > 3000 cats. Note these frequencies are similar to those reported previously around the world [2].

▶ **Tab. 2** Blutgruppenhäufigkeit (typisiert mittels Alvedia-Testkit) bei verschiedenen Katzenrassen in Deutschland. Interne retrospektive Studie von Laboklin mit über 3000 Katzen. Die Häufigkeiten entsprechen den Werten, die weltweit in anderen Ländern veröffentlicht wurden [2].

Breed	Cats (n)	Blood type (%)		
		A	*B*	*C (AB)*
Birman	295	88.8	11.2	0
British Shorthair	1128	75.0	24.9	0.1
Devon Rex	70	70.0	30.0	0
Chartreux	134	89.5	10.5	0
Maine Coon	257	96.9	3.1	0
Neva Masquerade	62	95.2	4.8	0
Norwegian Forest	65	98.5	1.5	0
Ragdoll	534	83.8	6.1	10.1
Scottish Fold	59	84.7	15.3	0
Siamese	49	100	0	0
Siberian	167	94.0	5.4	0.6
Thai	258	73.6	26.4	0



▶ **Fig. 1** Two schematic examples of feline blood types in pedigrees. Circles represent females, squares represent males. * Offspring at risk for neonatal isoerythrolysis. Blood type is given in circle/square, and below the genotype with the 2 alleles for each autosome is shown. Source: © A. Kehl.

▶ **Abb. 1** Zwei schematische Beispiele für Stammbäume mit Blutgruppen bei Katzen. Kreise symbolisieren Kätzinnen, Quadrate Kater. * Nachkommen sind gefährdet für neonatale Isoerythrolyse. Die Blutgruppe steht im Kreis bzw. Quadrat, darunter der Genotyp. Quelle: © A. Kehl.

potential for naturally occurring and induced alloantibodies, crossmatching has been recommended by some even for the first transfusion with *A*-*B* matched blood [12][13]. Because little is known about non-ABC feline blood groups, and there are no reagents or test kits currently available for *Mik*, these will not be discussed here further. However, the presence of naturally occurring or induced alloantibodies can be detected by crossmatching. Some clinicians still recommend xenotransfusions of canine blood to anemic cats [14], although it has been shown that canine red blood cells are incompatible in cats and are thus lysed within 4 days [15].

Inheritance of blood types

When assessing the mode of inheritance, it is important to differentiate between phenotype and genotype. Phenotyping for blood types involves demonstrating antigen expression on the erythrocyte membrane, while genotyping reveals the molecular genetic determinants (alleles) at a particular gene locus for each type in a blood group system. Based on extensive breeding records and studies, type *A* is known to be phenotypically dominant over *C* and *B* [16][17]. Furthermore, type *C* is not the product of a regular type *A* to *B* mating, but is separately inherited – hence the term *C* is used here (▶ **Fig. 1**) [3][18]. Cats with type *A* blood have the genotype *A*/*A*, *A*/a^c or *A*/*b*, while only homozygous type *b*/*b* cats express the type *B* antigen on their erythrocytes, and cats with genotype a^c/a^c or a^c/*b* exhibit blood type *C* (▶ **Fig. 1**, ▶ **Table 3**).

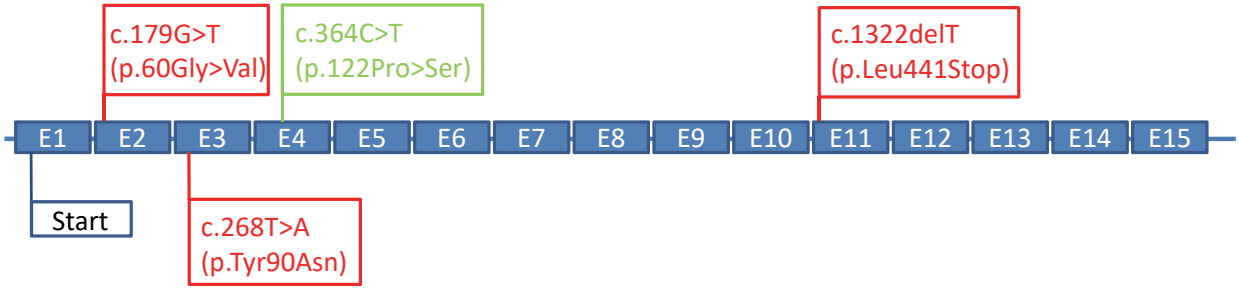
Genetic background

The feline *ABC* blood group system is the first and only system characterized at the biochemical and molecular genetic level in companion animals. The enzyme cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH; EC 1.14.18.2) converts the sialic acid N-acetylneuraminic acid (NeuAc; type *B* antigen) to N-glycolylneuraminic acid (NeuGc; type *A* antigen) [18][19][20]. Many genetic polymorphisms in the DNA, also known as single nucleotide polymorphisms (SNPs) or variants (SNVs, in the past also referred to mutations), have been described in the feline *CMAH* gene sequence (▶ **Fig. 2**). They represent single base changes to deletions and insertions in the *CMAH* gene sequence which can alter

▶ **Table 3** Genotyping scheme for type *A*, type *B*, and type *C* with possible haplotypes at each single nucleotide variant of the feline *CMAH* gene.

▶ **Tab. 3** Genotypisierungsschema für Typ *A*, Typ *B* und Typ *C* mit den möglichen Haplotypen bei den verschiedenen Einzelnukleotid-Varianten des felinen *CMAH*-Gens.

***CMAH* variants**				**Genotype**	**Correlated blood type**
c.179G>T	**c.268T>A**	**c.1322delT**	**c.364C>T**		
GG	*TT*	*TT*	*CC*	*A/A*	*A*
GG	*TA*	*TT*	*CC*	*A/b*	*A*
GT	*TT*	*TT*	*CC*	*A/b*	*A*
GG	*TT*	*T**	*CC*	*A/b*	*A*
GG	*TT*	*TT*	*CT*	*A/a^c^*	*A*
GG	*AA*	*TT*	*CC*	*b/b*	*B*
TT	*TT*	*TT*	*CC*	*b/b*	*B*
GG	*TT*	**	*CC*	*b/b*	*B*
GT	*TA*	*TT*	*CC*	*b/b*	*B*
GG	*TA*	*T**	*CC*	*b/b*	*B*
GG	*TT*	*TT*	*TT*	*a^c^/a^c^*	*C*
GG	*TA*	*TT*	*CT*	*a^c^/b*	*C*
GT	*TT*	*TT*	*CT*	*a^c^/b*	*C*
GG	*TT*	*T**	*CT*	*a^c^/b*	*C*



▶ **Fig. 2** *CMAH* variants (*CMAH* = cytidine monophosphate-N-acetylneuraminic acid hydroxylase), used in the feline *ABC* genotyping scheme which differ from the common (wildtype) *A* allele. Variants in red are for *b* allele. Variants in green are for *a^c^* allele. E = exon. © A. Kehl.

▶ **Abb. 2** *CMAH*-Varianten (*CMAH* = Cytidin-Monophosphat-N-Acetylneuraminsäure-Hydroxylase), die sich vom Wildtyp-*A*-Allel unterscheiden und für die Genotypisierung verwendet werden. Varianten für das Allel *b* sind rot dargestellt, für das Allel *a^c^* grün. E = Exon. © A. Kehl.

the amino acid (3 bases code for an amino acid) and result in defects in protein/enzyme function and stability. Several of these variants are thought to cause a loss or reduction of the regular CMAH activity needed for the type *A* antigen and thereby lead to blood type *B* and *C*, respectively [4][6][20][21].

We recently showed that the *CMAH* variant c.268 T > A (indicates position and base change in gene) results in an intolerable amino acid exchange from tryptophan to asparagine (p.Tyr90Asn), a change from a non-polar aromatic to a polar non-aromatic amino acid, which most likely causes enzyme dysfunction and, thus, type *B*. Indeed, this variant exhibited perfect genotype-phenotype correlation in type *A* and *B* cats [4]. Additionally, the variants c.179 G > T (p.Gly60Val [glycine to valine exchange]) and the nonsense variant c.1322delT (p.Leu441* [leucine to stop codon]) are also detrimental to *CMAH* function and were shown to be associated with the *b* allele [4]. Finally, we and others have recently associated the SNV c.364 C > T (p.Pro122Ser [proline to serine exchange]) with type *C* in Ragdolls and other breeds as well as domestic shorthair cats in Israel [4][6]. Variants and a newly introduced simple genotyping scheme are summarized in ▶ **Fig. 2** and ▶ **Table 3**.

Laboklin surveyed > 2500 purpose-bred pedigreed cats from 31 breeds among which there were 5 breeds with > 100 cats genotyped and a few breeds in the single digits (▶ **Table 4**) [7]. Compared to prior phenotypic blood typing surveys of different breeds, equal or more purpose-bred cats had type *A* blood which may be due to breeders' selection for type *A* cats and breeders' interest in determining, whether their type *A* cats were carriers of the *b* or *a^c^* allele. Overall ~8 % of the purpose-bred cats had genotype *b/b*

▶ **Table 4** Genotype distribution for the feline *ABC* blood group system in different breeds according to an internal Laboklin study with > 2500 cats. While the number of tested cats is small for some breeds these frequencies are similar to those reported in larger surveys [2][4][7].

▶ **Tab. 4** Genotyp-Verteilung innerhalb des *ABC*-Blutgruppensystems bei verschiedenen Katzenrassen in einer internen Studie von Laboklin mit über 2500 Katzen. Für manche Rassen ist die Anzahl der getesteten Katzen zwar gering, aber insgesamt entspricht die Häufigkeit der in größeren Untersuchungen [2][4][7].

Breed	Genotypes (predicted phenotype)						n
	A/A (A)	*A/b (A)*	*b/b (B)*	*A/a^c (A)*	*a^c/b (C)*	*a^c/a^c (C)*	
Abyssinian	25	6	1				32
Bengal	136	2		9		1	148
Birman	55	49	2				106
British Shorthair	128	189	110	2			429
Chartreux	2	3	3				8
Devon Rex	6	9	3				18
Highlander	13	17	8				38
Maine Coon	787	159	10	2			958
Neva Masquerade	4	7	1				12
Norwegian Forest	45	3					48
Persian	11	3	1				15
Ragdoll	90	61	15	24	12	2	204
Savannah	9	1					10
Scottish Fold	6	6	4	1			17
Siberian	17	8	2				27
Somali	6	8	1				15
Thai	4	4		1			6

(type *B* blood) and were homozygous for the missense *A* allele at position 268 or for the deletion at position 1322 or were compound heterozygous. The variant at position 179, responsible for type *B* was found in several purpose-bred and domestic shorthair cats. And the variant at position 364 is the cause of type *C* and was found in type *A* (*A*/*a^c*) and *C* Ragdolls and in several other purpose-bred and domestic shorthair cats [4][6]. This is one of the first examples of a complex trait in cats characterized by different variants in the same gene causing different phenotypes (blood type *A, B,* and *C*).

Phenotypic background

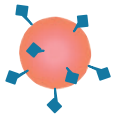
The feline *ABC* blood group system is particularly important, because cats have naturally occurring alloantibodies which can be responsible for acute hemolytic transfusion reactions and NI [1][3][22][23][24][25]. All type *B* cats have very strong naturally occurring anti-*A* alloantibodies (▶ **Fig. 3**). Alloantibodies develop within a few weeks of age and by 3 months are powerful hemolysins and hemagglutinins even after many-fold dilutions of plasma (IgG and IgM titers of 1:32 to 1:2048) [22]. In contrast, type *A* cats have no or only weak anti-*B* alloantibodies (< 1:32). And of course, type *C* cats have neither anti-*A* nor anti-*B* alloantibodies [16][22].

Blood incompatibility reactions

Neonatal isoerythrolysis

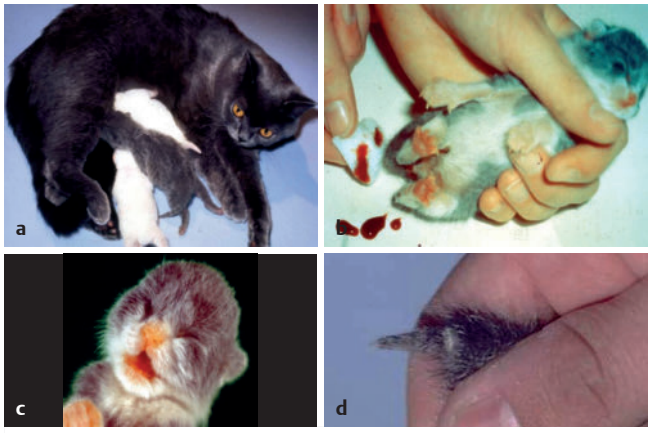
Neonatal isoerythrolysis only occurs in type *A* and *C* neonates born to type *B* queens [18]. Furthermore, type *A* and *C* kittens are born healthy as the complete feline placenta does not permit any transfer of alloantibodies [2][26]. All maternal antibodies including the ones against infectious diseases and type *A* red blood cells (RBCs) are exclusively transferred via colostrum and milk from the queen. This occurs only within the first 12–16 hours after birth [26]. Thereafter, the stomach of the offspring produces acids that destroy proteins, and the gut's junctions between enterocytes are closed preventing any further transfer of immunoglobulins or other proteins after the first day of life [26][27].

In purpose-bred catteries NI can occur in primiparus and multiparus type *B* queens bred to type *A* and *C* tom cats which represents a major but preventable cause of kitten mortality and fading kitten syndrome [27]. Type *A* and *C* kittens, born healthy, can develop NI within hours following ingestion of colostrum or milk during the first few hours of life. This may present as sudden death without any other clinical signs. Other affected kittens develop severe pigmenturia due to massive hemoglobulinuria and then die during the first week of life (▶ **Fig. 4**; kittens can be readily stimulated to urinate when touching the urogenital area with a wet cotton ball). A few may survive and develop anemia and icterus within days and later possibly a tail tip necrosis within 2 weeks, presumably due to

Phenotypes	*A*	*B*	*C*
Genotypes	*A/A, A/b, A/a^c*	*b/b*	*a^c/a^c, a^c/b*
Red blood cell surface antigen			
Plasma alloantibodies			No alloantibodies

▶ **Fig. 3** The major *ABC* blood group system in domestic cats: blood types, genotypes, and alloantibodies (high anti-*A* alloantibody titers in type *B* cats and low to non-existent anti-*B* antibody titers in type *A* cats). © L. Truchet.

▶ **Abb. 3** Das *ABC*-Blutgruppensystem bei der Katze: Blutgruppe, Genotyp, Alloantikörper (hoher Titer an Anti-*A*-Alloantikörpern bei Katzen mit Typ *B* und geringe bis fehlender Anti-*B*-Alloantikörper-Titer bei Katzen mit Typ *A*). © L. Truchet.



▶ **Fig. 4** Type *A* or *C* kittens in a British Shorthair litter (a) showing clinical signs of neonatal isoerythrolysis in form of pigmenturia due to hemoglobinuria (b), icterus (c) and tail tip necrosis (d) after nursing from a type *B* queen (a). © U. Giger.

▶ **Abb. 4** Katzenwelpen mit Typ *A* oder *C* eines Wurfs einer Britisch-Kurzhaar-Katze (a), die klinische Symptome der neonatalen Isoerythrolyse in Form von Pigmenturie aufgrund einer Hämoglobinurie (b), Ikterus (c) und Nekrose der Schwanzspitze (d) nach Säugen bei einer Kätzin mit Typ *B* (a). © U. Giger.

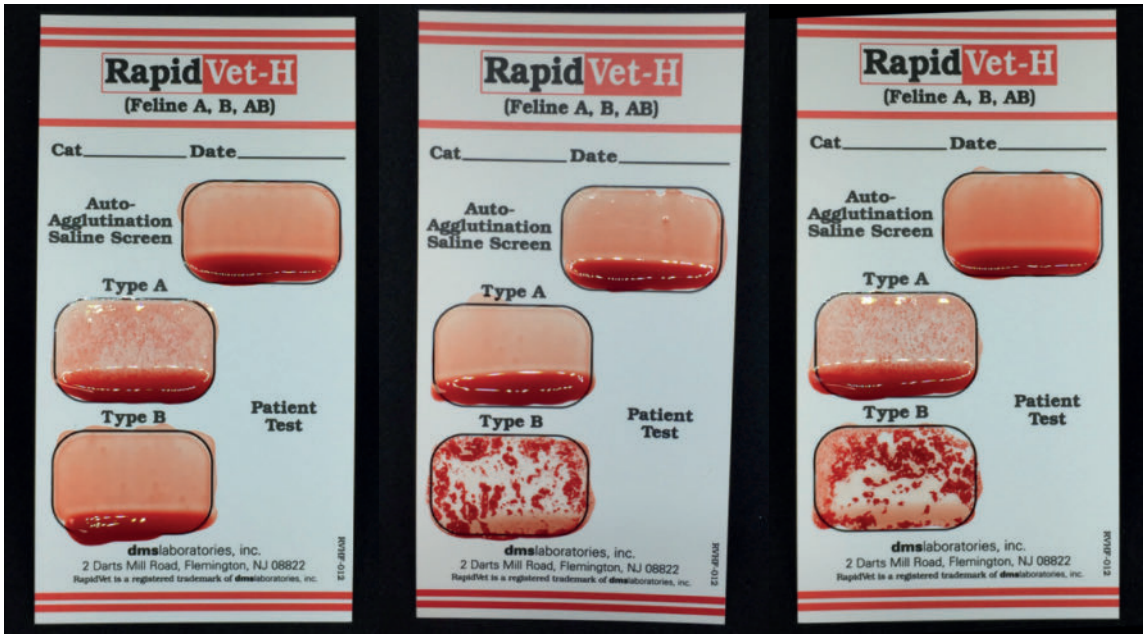
cold agglutinins (▶ **Fig. 4**) [26][27][28][29][30]. Because the intestinal tract can already be impermeable at birth, preventing transfer of immunoglobulins to the newborn, some at-risk kittens may not develop NI [26][27]. Thus, not all type *A* and *C* kittens born to type *B* queens will develop NI. In contrast, due to the low prevalence of anti-*B* antibodies in type *A* queens, type *B* kittens born to type *A* queens do not develop any clinical signs of NI.

While clinically affected newborn kittens typically cannot really be successfully managed, NI can be prevented by prospective typing of breeding cats and avoiding matings between type *B* queens and type *A* and *C* tom cats. Alternatively, newborn kittens with type *A* and *C* from type *B* queens must be strictly separated from the type *B* queen at birth for the first 16–24 hours and fed feline milk-replacer or may be raised by a lactating type *A* queen for the first day [2][26][27]. When giving milk replacer one might consider to supplement with feline plasma from a type *A* cat either orally or parenterally. However, this has not been found to be needed in well maintained catteries but may be useful in catteries with infectious diseases.

Acute hemolytic transfusion reactions

Even the first feline whole blood or packed RBC transfusion can result in life-threatening acute hemolytic transfusion reactions if donor-recipient mismatches between type *B* and type *A* or type *C* occur. Aside experimental studies several clinical case reports have also been published [1][23][24][25]. While the normal lifespan of transfused *A-B* matched RBCs is ~70–75 days (half-life ~35 days) [3], mismatched blood transfusions last only hours to a few days and thus are ineffective [3]. Some recipients have been shown to transiently change their blood type due to an *A-B* mismatched transfusion [24][25]. Moreover, feline patients receiving mismatched blood develop no or inadequate rises in hematocrit, hemolyzed plasma, hemoglobulinuria, and often icterus and/or death. Clinically, cats receiving *A-B* mismatched transfusions do not improve but can become more lethargic, hypotensive, and bradycardic [1]. As little as 2 ml of *A-B* mismatched incompatible blood has been shown to cause a fatal acute hemolytic transfusion reaction [1].

There is no universal type for feline blood donor cats. While type *A* blood transfused to type *B* recipients has been reported to cause severe and even life-threatening reactions [1][3][18][23][24][25], type *B* blood administered to type *A* recipients is similarly ineffective and may cause a severe acute hemolytic transfusion reaction mostly due to the anti-*A* alloantibodies in the transfused *B*



▶ **Fig. 5** Card agglutination technique; RapidVet-H by DMS laboratories results: Blood type is identified by gross agglutination in the labelled well. One drop of buffer and blood are added to each well and mixed well before reading for the presence of any agglutination in either the Type A or Type B well. Blood type *A* (left), type *B* (middle), and type *C* (right) where gross agglutination is present in both Type *A* and Type *B* well. Note in the presence of autoagglutination it is recommended to first wash the blood with physiological saline as autoagglutination could interfere with test results. © U. Giger.

▶ **Abb. 5** Agglutinationstechnik; Ergebnisse des Tests RapidVet-H von DMS: Die Blutgruppe wird durch makroskopisch sichtbare Agglutination im jeweils markierten Bereich identifiziert. Jeweils ein Tropfen Puffer und Blut werden auf die Testfelder gegeben und vermischt. Anschließend erfolgt eine Überprüfung der Testfelder für Typ *A* und *B* auf Agglutination. Blutgruppe *A* (links), Blutgruppe *B* (Mitte) und Blutgruppe *C* (rechts) mit Agglutination in beiden Probenfeldern. Im Fall einer Autoagglutination muss das Blut zuvor mit physiologischer Kochsalzlösung gewaschen werden, da sonst Interferenzen auftreten können. © U. Giger.

blood [18]. However, it seems unlikely that rare *B* donors will be used for a transfusion to a type *A* recipient. It is, therefore, crucial to type each feline recipient and donor according to the *ABC* blood group system prior to the first transfusion or, if typing is unavailable, crossmatch donor and anemic patient [2].

Similarly, there is *in vitro* and *in vivo* evidence of rapid destruction of transfused canine RBCs when given to cats. Indeed, xenotransfusions (i. e. canine blood given to cats) appear to result in severe intravascular hemolysis and complete lysis of all canine RBCs within 4 days [15]. Moreover, generally incompatible major and minor crossmatch results between canine and feline blood are observed. Due to the presence of naturally occurring cross-species alloantibodies [15][31], any blood crossmatch between dog and cat will show incompatibilities. Hence, xenotransfusions (and for that matter blood transfusion across species) as well as *A-B* mismatched transfusions are ineffective and detrimental and should and can be avoided in cats. Therefore, xenotransfusion is strongly discouraged.

Current feline typing tools to assure blood compatibility

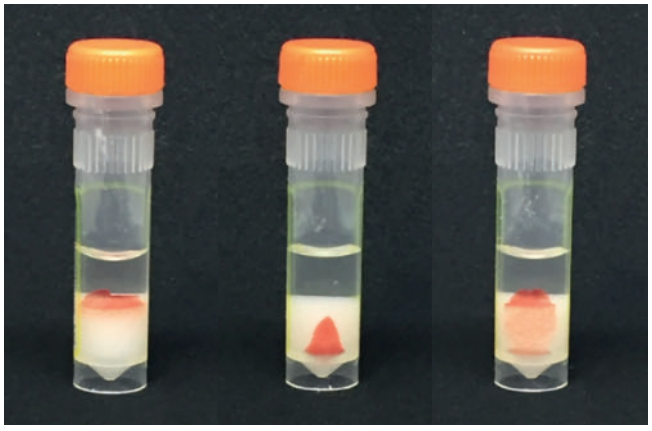
Nowadays, typing cats for the *ABC* blood group system can be readily accomplished in clinics and/or veterinary diagnostic laboratories. There are currently 2 different approaches:

1. Phenotypical blood typing of RBCs detects the *A* and/or *B* antigens on the RBC surface by immunological methods; and is performed using typing kits or at veterinary laboratories.
2. Genotyping is based on identifying specific SNVs for the *CMAH* gene by polymerase chain reaction (PCR), which can be performed by a few specialized veterinary diagnostic laboratories.

While immunological typing is generally sufficient when typing recipient and donor cats in practice, genotyping is preferred or performed in combination with immunological typing to assure the detection of the recessive alleles *b* and a^c in type *A* and *C* cats when breeding cats [7]. Similarly, regular blood typing methods are complemented by genotyping assays to assure accuracy in blood typing in humans for several blood groups [32][33]. Finally, crossmatching may be recommended prior to transfusing untyped and even *A-B* matched cats due to the potential presence of other naturally occurring alloantibodies and particularly for repeated transfusions > 4 days after the first transfusion [12][13][34].

Immunological blood typing kits (Alvedia, DMS, Abaxis/QuickVet)

Current immunological blood typing kits utilize anticoagulated (mostly ethylenediaminetetraacetic acid [EDTA] but also citrate) blood (fresh or kept refrigerated for up to 1 week) and monoclonal

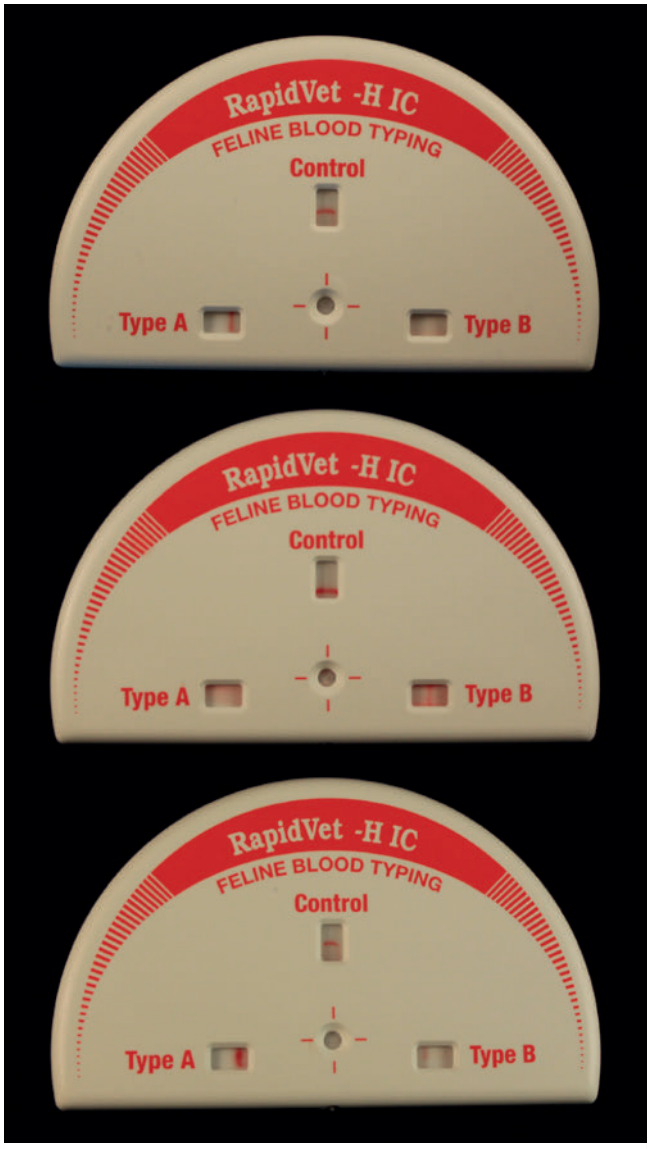


▶ **Fig. 6** Gel tube assay by DMS laboratories; blood typing results: Blood type *A* (left) stays on top of the gel, type *B* (middle) drops to the bottom of the gel, and type *C* (right) is a combination of both. © U. Giger.

▶ **Abb. 6** Ergebnisse des Gelsäulentests von DMS: Blut der Gruppe *A* (links) bleibt auf dem Gel, Blut der Gruppe *B* (Mitte) sinkt im Gel ab und bei Blut der Gruppe *C* (rechts) zeigen sich beide Zustände. © U. Giger.

▶ **Fig. 7** Immunochromatographic strip technique by Alvedia; blood typing results: Type *A* (top), type *B* (middle), and type *C* (bottom). © U. Giger.

▶ **Abb. 7** Ergebnisse des immunchromatografischen Tests von Alvedia: Blutgruppe A (oben), Blutgruppe *B* (Mitte) und Blutgruppe *C* (unten). © U. Giger.



▶ **Fig. 8** Immunochromatographic strip method by DMS laboratories; blood typing results: Type A (top), type *B* (middle) and type *C* (bottom). © U. Giger.

▶ **Abb. 8** Ergebnisse des immunchromatografischen Tests von DMS: Blutgruppe *A* (oben), Blutgruppe *B* (Mitte) und Blutgruppe *C* (unten). © U. Giger.

anti-*A* and anti-*B* alloantibodies (or lectin of *Triticum vulgaris*) with agglutination or immunochromatographic binding assays. It is important to exclude autoagglutination prior to running any kit assay as macroscopic autoagglutination can interfere with results (and may look like a type *C* cat on a card test). In the presence of macroscopic autoagglutination seen in blood tube or by microscopic examination of a blood smear, the anticoagulated blood should be washed 3 times with physiological saline. Briefly, a small amount of blood or packed RBCs are mixed with 4–10 times as much physiological saline and then centrifuged to remove the supernatant; this is repeated 2 more times. The immunochromatographic strips from Alvedia and DMS are less affected by autoagglutination, but with severe agglutination insufficient RBCs are able to move up the strip. Hence it is advisable to check first for macroscopic autoagglutination and if present to wash the blood. If the autoagglutination resolves or becomes very weak, typing and crossmatching can be performed.

▶ **Table 5** Possible CMAH genotypes of kittens depending on genotype of parents. * Kittens with this blood type born to type *B* queens are at risk of neonatal isoerythrolysis.

▶ **Tab. 5** Mögliche resultierende CMAH-Genotypen abhängig vom Genotyp der Elterntiere. * Katzenwelpen sind gefährdet für die neonatale Isoerythrolyse, wenn das Muttertier Blutgruppe *B* hat.

Parent 1		Parent 2		Kittens	
Blood type	**Genotype**	**Blood type**	**Genotype**	**Blood type**	**Genotypes**
A	*A/A*	*A*	*A/A*	*A*	*A/A*
	A/A		*A/b*	*A*	*A/A, A/b*
	A/A		*A/a*^{c}	*A*	*A/A, A/a*^{c}
	A/b		*A/b*	*A, B*	*A/A, A/b, b/b*
	A/b		*A/a*^{c}	*A, C*	*A/A, A/b, A/a*^{c}, *a*^{c}*/b*
	A/a^c		*A/a*^{c}	*A, C*	*A/A, A/a*^{c}, *a*^{c}*/a*^{c}
B	*b/b*	*B*	*b/b*	*B*	*b/b*
A	*A/A*	*B*	*b/b*	*A**	*A/b*
	A/b		*b/b*	*A**, *B*	*A/b, b/b*
	A/a^c		*b/b*	*A**, *C**	*A/b, a*^{c}*/b*
A	*A/A*	*C (AB)*	*a*^{c}*/a*^{c}	*A*	*A/a*^{c}
	A/A		*a*^{c}*/b*	*A*	*A/a*^{c}, *A/b*
	A/b		*a*^{c}*/a*^{c}	*A, C*	*A/a*^{c}, *a*^{c}*/b*
	A/b		*a*^{c}*/b*	*A, B, C*	*A/a*^{c}, *a*^{c}*/b, b/b*
	A/a^c		*a*^{c}*/a*^{c}	*A, C*	*A/a*^{c}, *a*^{c}*/a*^{c}
	A/a^c		*a*^{c}*/b*	*A, C*	*A/a*^{c}, *a*^{c}*/a*^{c}, *a*^{c}*/b*
B	*b/b*	*C (AB)*	*a*^{c}*/a*^{c}	*C**	*a*^{c}*/b*
	b/b		*a*^{c}*/b*	*B, C**	*a*^{c}*/b, b/b*
C (AB)	*a*^{c}*/a*^{c}	*C (AB)*	*a*^{c}*/a*^{c}	*C*	*a*^{c}*/a*^{c}
	a^c*/a*^{c}		*a*^{c}*/b*	*C*	*a*^{c}*/a*^{c}, *a*^{c}*/b*
	a^c*/b*		*a*^{c}*/b*	*B, C*	*a*^{c}*/a*^{c}, *a*^{c}*/b, b/b*

Because type *B* cats are uncommon in most geographic regions and breeds, and type *C* cats are extremely rare (except Ragdolls and specific regions), it is worth back typing cats identified as *B* or *C* by an established veterinary laboratory and trained personnel to confirm typing results. Back typing refers to the detection of anti-*A* alloantibodies in the plasma of type *B* cats and hence is like a major crossmatch.

The card agglutination technique was developed through DMS laboratories, Inc (Flemington, NJ, USA) > 20 years ago (▶ **Fig. 5**). This method is reliable, although some type *C* cats may be difficult to type [35][36][37][38]. A gel column assay was produced by DiaMed (Cressier, Switzerland) and is now similarly offered as a gel tube assay by DMS (▶ **Fig. 6**).

An immunochromatographic strip technique to type cats was developed by Alvedia (Limonest, France; ▶ **Fig. 7**) [37][38][39][40] and is available as a single kit, multi-test lab assay or in combination with a crossmatch test. This immunochromatographic strip technique uses binding of erythrocytes of the *A* or *B* blood type to monoclonal anti-*A* or anti-*B* alloantibodies at a specific location on the strip to form a red band with RBCs. A similar immunochromatographic strip method is now also produced by DMS (▶ **Fig. 8**). Furthermore, Abaxis (Zoetis, Parsippany, USA) and QuickVet (Zoetis, Farum, Denmark) are offering the same typing cartridge technique for cats with their coagulation instruments. Finally, the plasma from type *B* cats containing naturally occurring anti-*A* antibodies and the lectin from *Triticum vulgaris* cells can be used by veterinary laboratories to detect type *B* and *A* antigens, respectively, although most veterinary laboratories also use commercial kits for feline typing [18][19][35][37].

In conclusion, typing kits offer rapid accurate determinations of the 3 blood types in the feline *ABC* blood group system.

Genotypic blood typing – original versus new improved genotyping scheme

Genotyping cats for the *ABC* blood group system offers advantages over phenotypic/immunological methods, as these tests have the potential to detect recessive (hidden) alleles, such as different *b* and *a*^{c} alleles. This information is needed when breeding cats from breeds with type *B* and *C* cats to avoid NI. Although the *CMAH* gene, which is responsible for the types *A, B,* and *C*, was sequenced more than a decade ago [6][20], genotyping of type *B* and *C* cats has proven to be inaccurate until recently.

Based on Laboklin's recent genomic analyses, specific SNVs have been determined to cause types *B* and *C* in purpose-bred cats. We have found an excellent correlation between phenotype and genotype, when screening many purpose-bred cats [4][7]. Based on this

finding, we have developed a specific novel genotyping scheme for the detection of the common alleles *A*, *b*, and a^c since the end of 2017. A panel of 4 SNVs is included to assess genotypes (▶ **Fig. 2**, ▶ **Table 3**). While this system has been found to be accurate, it is possible that in the future new genetic variants will be found in different cat populations (e. g. in different breeds as well as non-purpose-bred cats and geographic regions) which can be readily added to the current genotyping scheme.

Laboklin's recent geno- and phenotyping surveys [4][7] showed the superiority of the new genotyping scheme (SNVs c.179 G > T, c.268 T > A, c.364 C > T and c.1322delT) over the original scheme (SNVs c.142 G > A and Δ-53) [6][20]. Type *C* cats with the genotypes a^c/a^c and a^c/b can now also be accurately detected. Additionally, the *B* type caused by either SNVs c.179 G > T or c.1322delT alone or as a compound heterozygote with the SNV c.268 T > A can be identified by the improved scheme. Moreover, no genotyping-phenotyping discordances have been observed in purpose-bred cats. The new SNV panel also demonstrates its strength in detecting the "breed-related" SNVs such as c.179 G > T, c.364 C > T, and c.1322delT in additional breeds.

For this genotyping assay, cheek swabs are adequate, and no blood is necessary, which is a major advantage for breeders testing their cats and kittens directly. The obtained swabs/brushes (plastic not wood handle) can easily be shipped in an envelope. Genotyping for the *ABC* blood group system is recommended to breeders of cats of breeds with type *B* and *C* cats to avoid and/or predict litters with NI. In ▶ **Table 5**, possible matings with different geno- and phenotypes and outcomes are summarized.

CONCLUSION FOR PRACTICE

In conclusion, immunohematological blood typing for the *ABC* blood group system is readily available as in-practice kits as well as in veterinary diagnostic laboratories. It is recommended to type every donor and recipient cat prior to any transfusion including the first. Only *A*-*B* matched transfusions are safe, and type *C* cats should receive (preferably) crossmatched type *A* packed RBCs or blood, if no type *C* blood is available. Due to the presence of other alloantibodies, some have recommended crossmatching in addition to *AB* typing even for the first transfusion. Breeding cats should also be typed to avoid mating of a type *B* queen with a type *A* or *C* tom cat and occurence of NI. In order to predict blood types in offspring the genotyping technique with the new improved scheme is recommended.

Acknowledgements

The authors would like to thank the staff at Labogen, Laboklin, Bad Kissingen, Germany for their routine testing and commenting on our results.

Disclosures

Alexandra Kehl, Laura Truchet, Ines Langbein-Detsch and Elisabeth Müller are employed by Laboklin which offers blood typing and blood compatibility testing. A patent «Verfahren und Vorrichtung zur Bestimmung der Blutgruppe einer Katze im AB-Blutgruppensystem» (no. 10 2017 124 998.2) on the molecular genetic markers and panel testing has been granted. Urs Giger is the director of PennGen at the University of Pennsylvania which is a not-for-profit laboratory offering special blood typing and compatibility testing and is supported by the National Institutes of Health (OD 010939). He has been a scientific advisor to Alvedia, DMS, and Laboklin.

References

[1] Auer L, Bell K, Coates S. Blood transfusion reactions in the cat. J Am Vet Med Assoc 1982; 180 (7): 729–730

[2] Giger U. Blood typing and crossmatching to ensure blood compatibility. In: Bonagura JD, Twedt DC, eds. Kirk's Current Veterinary Therapy. XV ed. Missouri: St. Louis; 2014: 143

[3] Giger U, Bücheler J. Transfusion of type-A and type-B blood to cats. J Am Vet Med Assoc 1991; 198 (3): 411–418

[4] Kehl A, Heimberger K, Langbein-Detsch I et al. Molecular characterization of blood type A, B, and C (AB) in domestic cats and a CMAH genotyping scheme. PLoS One 2018; 13 (9): e0204287

[5] Spada E, Miglio A, Proverbio D et al. Signalment and blood types in cats being evaluated as blood donors at two italian university blood banks. Vet Med Int 2014; 2014: 704836

[6] Gandolfi B, Grahn RA, Gustafson NA et al. A novel variant in CMAH is associated with blood type AB in Ragdoll cats. PLoS One 2016; 11 (5): e0154973

[7] Kehl A, Mueller E, Giger U. CMAH genotyping survey for blood types A, B and C (AB) in purpose-bred cats. Anim Genet 2019; 50 (3): 303–306

[8] Proverbio D, Spada E, Perego R et al. Assessment of blood types of Ragdoll cats for transfusion purposes. Vet Clin Pathol 2013; 42 (2): 157–162

[9] Tasker S, Barker EN, Day MJ et al. Feline blood genotyping versus phenotyping, and detection of non-AB blood type incompatibilities in UK cats. J Small Anim Pract 2014; 55 (4): 185–189

[10] Forcada Y, Guitian J, Gibson G. Frequencies of feline blood types at a referral hospital in the south east of England. J Small Anim Pract 2007; 48 (10): 570–573

[11] Vieira SM, Ferreira RRF, de Matos AJ et al. Distribution of feline AB blood types: a review of frequencies and its implications in the Iberian Peninsula. JFMS Open Rep 2017; 3 (2): 2055116917727693

[12] Weinstein NM, Blais MC, Harris K et al. A newly recognized blood group in domestic shorthair cats: the Mik red cell antigen. J Vet Intern Med 2007; 21 (2): 287–292

[13] McClosky ME, Cimino Brown D, Weinstein NM et al. Prevalence of naturally occurring non-AB blood type incompatibilities in cats and influence of crossmatch on transfusion outcomes. J Vet Intern Med 2018; 32 (6): 1934–1942

[14] Bovens C, Gruffydd-Jones T. Xenotransfusion with canine blood in the feline species: review of the literature. J Feline Med Surg 2013; 15 (2): 62–67

[15] Euler CC, Raj K, Mizukami K et al. Xenotransfusion of anemic cats with blood compatibility issues: pre- and posttransfusion laboratory diagnostic and crossmatching studies. Vet Clin Pathol 2016; 45 (2): 244–253

[16] Griot-Wenk ME, Callan MB, Casal ML et al. Blood type AB in the feline AB blood group system. Am J Vet Res 1996; 57 (10): 1438–1442

[17] Giger U, Bucheler J, Patterson DF. Frequency and inheritance of A and B blood types in feline breeds of the United States. J Hered 1991; 82 (1): 15–20

[18] Griot-Wenk ME, Giger U. Feline transfusion medicine. Blood types and their clinical importance. Vet Clin North Am Small Anim Pract 1995; 25 (6): 1305–1322

[19] Griot-Wenk M, Pahlsson P, Chisholm-Chait A et al. Biochemical characterization of the feline AB blood group system. Anim Genet 1993; 24 (6): 401–407

[20] Bighignoli B, Niini T, Grahn RA et al. Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group. BMC Genet 2007; 8: 27

[21] Omi T, Nakazawa S, Udagawa C et al. Molecular characterization of the cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) gene associated with the feline AB blood group system. PLoS One 2016; 11 (10): e0165000

[22] Bücheler J, Giger U. Alloantibodies against A and B blood types in cats. Vet Immunol Immunopathol 1993; 38 (3–4): 283–295

[23] Giger U, Akol KG. Acute hemolytic transfusion reaction in an Abyssinian cat with blood type B. J Vet Intern Med 1990; 4 (6): 315–316

[24] Niggemeier A, Haberstroh HF, Nelson VE et al. An accidental transfusion of a type A kitten with type B blood causes a transient switch from blood type A to B. J Vet Intern Med 2000; 14 (2): 214–216

[25] Koenig A, Maglaras Ryan C, Giger U. Acute hemolytic reaction due to A-B mismatched transfusion on a cat: transient AB blood type and blood compatibility testing results. J Vet Emerg Crit Care (in press)

[26] Casal ML, Jezyk PF, Giger U. Transfer of colostral antibodies from queens to their kittens. Am J Vet Res 1996; 57 (11): 1653–1658

[27] Giger U, Casal ML. Feline colostrum – friend or foe: maternal antibodies in queens and kittens. J Reprod Fertil Suppl 1997; 51: 313–316

[28] Hubler M, Kaelin S, Hagen A et al. Feline neonatal isoerythrolysis in two litters. J Small Anim Pract 1987; 28 (9): 833–838

[29] Silvestre-Ferreira AC, Pastor J. Feline neonatal isoerythrolysis and the importance of feline blood types. Vet Med Int 2010; 2010: 753726

[30] Axnér E. A questionnaire on survival of kittens depending on the blood groups of the parents. J Feline Med Surg 2014; 16 (10): 781–787

[31] Priolo V, Masucci M, Spada E et al. Naturally occurring antibodies in cats against dog erythrocyte antigens and vice versa. J Feline Med Surg 2018; 20 (8): 690–695

[32] Reid ME, Denomme GA. DNA-based methods in the immunohematology reference laboratory. Transfus Apher Sci 2011; 44 (1): 65–72

[33] El-Sayed YS, Mohamed OI, Ashry KM et al. Using species-specific repeat and PCR-RFLP in typing of DNA derived from blood of human and animal species. Forensic Sci Med Pathol 2010; 6 (3): 158–164

[34] Sylvane B, Prittie J, Hohenhaus AE, Tozier E. Effect of cross-match on packed cell volume after transfusion of packed red blood cells in transfusion-naïve anemic cats. J Vet Intern Med 2018; 32 (3): 1077–1083

[35] Stieger K, Palos H, Giger U. Comparison of various blood-typing methods for the feline AB blood group system. Am J Vet Res 2005; 66 (8): 1393–1399

[36] Seth M, Jackson KV, Winzelberg S et al. Comparison of gel column, card, and cartridge techniques for dog erythrocyte antigen 1.1 blood typing. Am J Vet Res 2012; 73 (2): 213–219

[37] Seth M, Jackson KV, Giger U. Comparison of five blood-typing methods for the feline AB blood group system. Am J Vet Res 2011; 72 (2): 203–209

[38] Spada E, Proverbio D, Baggiani L et al. Evaluation of an immunochromatographic test for feline AB system blood typing. J Vet Emerg Crit Care (San Antonio) 2016; 26 (1): 137–141

[39] Hourani L, Weingart C, Kohn B. Evaluation of a novel feline AB blood typing device. J Feline Med Surg 2014; 16 (10): 826–831

[40] Proverbio D, Spada E, Baggiani L et al. Assessment of a gel column technique for feline blood typing. Vet Res Commun 2009; 33 Suppl 1: 201–203

[41] Leidinger J, Leidinger E, Giger U. Verteilung und Bedeutung der Blutgruppen A und B bei Haus- und Rassekatzen in Oesterreich. Wien Tierärztl Monatsschr 1993; 80: 10–14

[42] Jensen AL, Olesen AB, Arnbjerg J. Distribution of feline blood types detected in the Copenhagen area of Denmark. Acta Vet Scand 1994; 35 (2): 121–124

[43] Barrot A, Buttin R, Linsart A et al. Frequency of feline blood types in non-pedigree cats in France. Rev Med Vet 2017; 168: 235–240

[44] Haarer M, Grünbaum EG. [Blood group typing in the cat]. Tierarztl Prax 1993; 21 (4): 339–343

[45] Mylonakis ME, Koutinas AF, Saridomichelakis M et al. Determination of the prevalence of blood types in the non-pedigree feline population in Greece. Vet Rec 2001; 149 (7): 213–214

[46] Bagdi N, Magdus M, Leidinger E et al. Frequencies of feline blood types in Hungary. Acta Vet Hung 2001; 49 (4): 369–375

[47] Juvet F, Brennan S, Mooney CT. Assessment of feline blood for transfusion purposes in the Dublin area of Ireland. Vet Rec 2011; 168 (13): 352

[48] Proverbio D, Spada E, Baggiani L et al. Comparison of gel column agglutination with monoclonal antibodies and card agglutination methods for assessing the feline AB group system and a frequency study of feline blood types in northern Italy. Vet Clin Pathol 2011; 40 (1): 32–39

[49] Giger U, Gorman N, Hubler M et al. Frequencies of feline A and B blood types in Europe. Anim Genet 1992; 23: 12

[50] Marques C, Ferreira M, Gomes JF et al. Frequency of blood type A, B, and AB in 515 domestic shorthair cats from the Lisbon area. Vet Clin Pathol 2011; 40 (2): 185–187

[51] Silvestre-Ferreira AC, Pastor J et al. Frequencies of feline blood types in northern Portugal. Vet Clin Pathol 2004; 33 (4): 240–243

[52] Hubler M, Arnold S, Casal M et al. Die Blutgruppenverteilung bei den Hauskatzen in der Schweiz. Schweiz Arch Tierheilk 1993; 135: 231–235

[53] Arikan S, Gurkan M, Ozaytekin E et al. Frequencies of blood type A, B and AB in non-pedigree domestic cats in Turkey. J Small Anim Pract 2006; 47 (1): 10–13