

A Survey of Feline Ab Group Blood Types in Israel (2007 to 2009)

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ABSTRACT

The feline AB blood group system is the predominant feline blood group system. Feline blood typing is clinically important for prevention of neonatal isoerythrolysis and acute hemolytic transfusion reactions. The distribution of feline blood types varies significantly in different districts and between different cat breeds and is unknown in Israel. This retrospective study was designed to determine the prevalence of feline AB blood group types in cats presented to the Hebrew University Veterinary Teaching Hospital and to the Israeli Animal Blood Bank. The study included 242 cats consisting of 100 ill-cat recipients of blood components and 142 blood donors. The frequency of blood types A, B and AB in the present study was 72.7%, 14.5% and 12.8%, respectively. The frequency of blood type AB is higher than previously published studies. This is especially surprising since most cats in this survey were of mixed breed. This study is the first survey of feline blood types in Israeli cats. The findings warrant a wider survey that will better represent the Israeli cat population, using several typing methods. Nevertheless the present results have important implications in regards to feline transfusion medicine in Israel. Cross matching of donor and recipient blood is imperative because of the high chances of incompatibility.

Key words: transfusion medicine, blood typing, blood groups, blood donors, cross matching, cat

INTRODUCTION

Feline AB system blood types have three distinct phenotypes; A, B, and AB (1). The A and B phenotypes are inherited through simple autosomal Mendelian genetics with the 'a' allele being dominant over the 'b' allele (2, 3). Thus, all blood type-B cats are homozygous for the 'b' allele (genotype b/b), while type-A cats can be either homozygous (genotype a/a) or heterozygous (genotype a/b). Blood type AB is not a result of co-dominance of alleles 'a' and 'b', (4) and despite extensive breeding studies and pedigree analysis, its mode of inheritance is still unclear, although it appears to be recessive to allele 'a' and dominant to allele 'b' (4, 5). An additional blood

group system, the MIK system, has been described, with two phenotypes, MIK(+) and MIK(-) (6).

In the feline AB blood system, the group antigens are determined by sialic acid composition in gangliosides on the erythrocyte's outer membrane. Type-A cats present n-glycolyl-neuraminic acid (NGN), while type-B cats present n-acetyl-neuraminic acid (NAN) in their membranous gangliosides (7, 8). The enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) determines the sugar bound to the red cell by converting NAN to NGN (9). Mutations in type-B cats likely disrupt the gene function of CMAH, leading to a predominance of NAN. The *a* and *b* CMAH alleles described can distinguish type-A and type-B

cats without blood sample collections (9). Blood type-A and -B cats have naturally-occurring isoantibodies against erythrocyte surface antigens that they are negative for. It is the sialic acid moieties, NGN and NAN in the gangliosides that have the antigenic properties in blood types A and B, respectively. These naturally occurring isoantibodies are responsible for complement activation and acute hemolytic transfusion reactions, as well as neonatal isoerythrolysis (NI), due to intravascular or extravascular hemolysis (1). Most (70%) blood type-A cats have low titers of anti-B-type hemagglutinins of the IgM class, as well as hemolysins, consisting of equal amounts of IgG and IgM. A minority (30%) of blood type-A cats receiving blood type-B blood, will have severe hemolytic reactions due to high hemolysins levels (10). In contrast, all blood type-B cats have high titers of both anti-A-type hemagglutinins and hemolysins, mostly of the IgM class, with lesser IgG amounts (11, 12). Thus, transfusion of as little as 1 ml of type-A blood to a blood type-B cat can lead to an acute, potentially fatal transfusion (10). Blood type-AB cats do not possess isoantibodies against either A or B antigens, however, this blood should not be transfused to type-A or -B cats, because it will induce an acute hemolytic transfusion. Type-AB cats should be transfused with AB blood, or if unavailable, washed A-type erythrocytes can be transfused, to avoid as much as possible even a minor reaction (1).

Blood type-A is the most common blood type identified in studied cat populations worldwide. Blood type-B is less common and type-AB has been reported to be even less common than type-B blood and in some surveys it was extremely rare (1). In all past surveys, Siamese and related cat breeds were found to have type-A blood, exclusively (1). The proportion of blood types -A and -B in domestic shorthair (DSH) and domestic longhair cats (DLH) dif-

fers markedly between geographic locations (Table 1). In addition, the proportion of feline blood types differs markedly among other breeds (1). Blood type-AB has been reported to occur only in breeds in which blood type-B is relatively common (1). It has been detected in domestic shorthair (DSH) and longhair (DLH) cats as well as in Abyssinian, Birman, British shorthair, Norwegian forest, Persian, Scottish fold, Cornish and Devon Rex, Maine Coon, Manx, Ragdoll, Sphynx, Bengal, Egyptian mau, Siberian, European, and Somali purebred cats. (1,5)

Blood typing of domestic cats has an important role in clinical practice in prevention of severe transfusion reactions from whole blood, packed cells and plasma transfusions. In addition, feline blood typing has an important role in breeding of cats, especially in breeds in which the prevalence of blood type-B is relatively high. Typing is necessary to prevent neonatal isoerythrolysis (NI), which occurs when a blood type-B queen is bred with a blood type-A male, resulting in

Table 1: Prevalence of feline AB blood types of non pedigree cats in different surveys

Blood type prevalence (%)			Country of survey	Number of cats included	Reference (year)	Reference number
A	B	AB				
73.3	26.3	0.4	Australia	1895	Auer & Bell (1981)	4
62.0	36.0	1.6		355	Malik <i>et al.</i> (2005)	17
97	3	0	Austria	101	Giger <i>et al.</i> (1992)	18
97.1	2.9	0	England	477	Holmes (1950)	22
87.1	7.9	5.0	UK	139 ¹	Knottenbelt (1999)	24
54.6	40.1	5.3		207 ²		
100	0	0	Finland	61	Giger <i>et al.</i> (1992)	18
94	6	0	Germany	600	Giger <i>et al.</i> (1992)	18
92.6	6.7	0.7		868	Haarer & Grunbaum (1993)	32
94.1	5.9	0.0	404 ¹			
95.8	3.1	1.1	Holland	95	Giger <i>et al.</i> (1992)	18
88.8	11.2	0	Italy	401	Giger <i>et al.</i> (1992)	18
89.3	4.4	6.3	Portugal	159	Silvestre-Ferreira <i>et al.</i> (2004)	23
78.3	20.3	1.4	Greece	207	Mylonakis <i>et al.</i> (2001)	16
72.8	25.0	2.2	Turkey	312	Gurkan <i>et al.</i> (2005)	19
89.3	1	9.7	Japan	299	Ejima <i>et al.</i> (1986)	27
97.1	2.9	0	Scotland	70	Giger <i>et al.</i> (1992)	18
87.6	8	4.4	Switzerland	1014	Hubler <i>et al.</i> (1993)	31
85.1	14.9	0	France	350	Eyquem <i>et al.</i> (1962)	20
99.7	0.3	0	USA	1072	Giger <i>et al.</i> (1989)	30
98.1	1.7	0.1		3785	Giger <i>et al.</i> (1991)	25
72.7	14.5	12.8	Israel	242	Merbl <i>et al.</i> (2011) ³	

1, non-pedigree cats only; 2, pedigree cats only; 3, this refers to the present survey

acute hemolysis in the kittens, which often leads to considerable mortality. NI is an important factor, among others, in what is known as 'fading kitten syndrome' (13).

The cat population in Israel has changed considerably in the last decades due to newly introduced cat breeds that were extremely rare in the country, by immigrants from the former Soviet Union, as well as commercial importation of cats (I. Aroch, personal communication). A survey of feline blood types in Israel has never been reported. Knowledge of the prevalence of feline blood types is important for management and recruitment of donor cats in veterinary blood banks in the country. Knowledge of the prevalence of feline blood types in the cat population might reduce the chances of transfusion reactions if blood typing and cross matching cannot be made. For example, because all Siamese and related cat-breeds worldwide have only blood type-A, there is no need to determine their blood type if both the donor and the recipient are Siamese-related.

The purpose of this retrospective survey was to determine the prevalence of feline blood types in ill cats presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) and blood donor cats from the Israeli Animal Blood Bank (IABB), and to assess their prevalence in several districts in Israel.

MATERIALS AND METHODS

Animals and study design

The medical records of cats presented to the HUVTH and of donor cats of the IABB from 2007 to 2009, were retrieved. Ill cats were included in the present study if their medical records were complete and their AB system blood type was definitely determined. Data retrieved from the medical records included the geographic district location, blood type, sex, neutering status and breed.

Laboratory methods

Blood type was determined in the HUVTH Diagnostic Laboratory from blood collected in ethylenediaminetetraacetic acid (EDTA) tubes using a commercial kit (Gel test feline A+B Typing, DiaMed, Cressier, Switzerland) (14). The test is based on monoclonal anti-A and anti-B antibodies agglutinating sera that are mixed with the tested blood sample in separate test tubes. The kit also includes a negative control containing no antibodies in order to detect auto-agglutina-

tion in the recipient's own blood (Figure 1). In the event of auto-agglutination in the control sample, a second test was performed using donor's erythrocytes, washed three times in saline. For erythrocytes washing, the recipient blood sample was centrifuged, and the plasma separated. One milliliter of saline was added to the packed cells (making approximately a 5% suspension of erythrocytes), gently stirred, resuspended, incubated for five minutes and then the sample was centrifuged at 1000g for five minutes and the supernatant was separated. This procedure was repeated twice (15).

Statistical analysis

The association between blood type and other categorical variables (i.e., sex, breed and cat sub populations [i.e., donors and recipients]) were determined using Pearson's chi-square or Fisher's exact tests. For assessment of prevalence differences between different districts in Israel, cats were divided into eight different districts. In all tests, P value ≤ 0.05 was considered significant. All analyses were performed using statistical

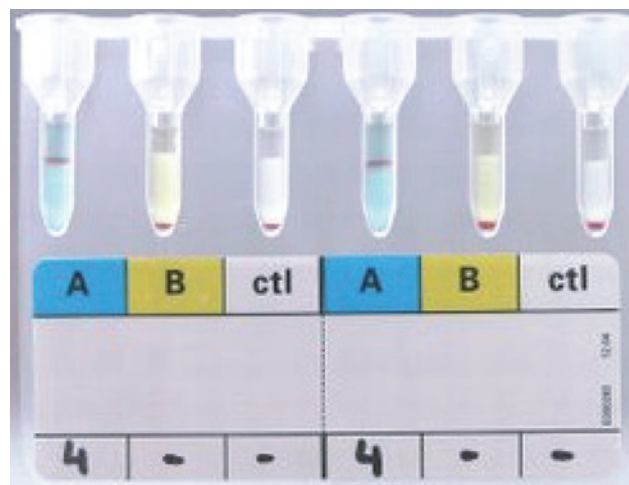


Figure 1: The Gel test feline A+B typing kit (Diamed, Switzerland) used for blood typing in this study.

The blood typing kit includes 6 test tubes used to classify blood types of 2 cats. Note that both blood samples types in this figure are blood type A. Strong agglutination is noted in the test tubes containing anti-A antisera. Agglutination is seen as the marked red line on the gel surface. The test tube containing anti-B antisera did not show agglutination and thus this marked red line is absent. The control test tube has no antibodies and is used to rule out autoagglutination in the recipient's own blood. When agglutination is absent, the erythrocytes sink to the bottom of the test tube as seen in the B and control (ctl) test tubes.

software (SPSS 17, SPSS Inc., Chicago, IL, USA).

RESULTS

This study included 242 cats, of which 100 (41.3% of all cats; 51 males and 49 females) were ill cats that were transfused with blood products, and 142 blood donors (58.7% of all cats; 94 males, 37 females and 11 in which the sex was not recorded). Of 231 cats in which sex was recorded, there were 62.8% males and 37.2% females.

Most cats were DSH, DLH or crosses of these with other breeds (e.g., Persian and Siamese). The remaining cat breeds are presented in table 2. The percentage of DSH, DLH and their crosses (i.e., mixed breed) was significantly higher ($P = 0.0005$) in the donors group [134 mixed breed cats (94.4%) and 8 pure breed

Table 3: Geographic distribution and proportions of blood types of 242 cats included in the present survey

Cat population	District	n (%)	Blood type n (%)			P value
			A	B	AB	
Hospitalized blood recipients	Tel Aviv	42 (42)				0.75
	Rehovot	21 (21)				
	Jerusalem	16 (16)				
	Ramla	9 (9)				
	Ashkelon	4 (4)	75	14	11	
	Petah-Tikwa	4 (4)	(75.0)	(14.0)	(11.0)	
	Hadera	2 (2)				
	Haifa	2 (2)				
All cats	100 (100)					
Blood donors*	Rehovot	82 (60.3)				
	Petah-Tikwa	21 (15.4)				
	Ramla	16 (11.8)	101	21	20	
	Haifa	11 (8.1)	(71.1)	(14.8)	(14.1)	
	Tel Aviv	6 (4.4)				
	All cats	136 (100)				
All cats	Rehovot	103 (44)				
	Tel Aviv	48 (20)				
	Ramla	25 (11)				
	Petah-Tikwa	25 (11)				
	Jerusalem	16 (7)	176	35	31	
	Haifa	13 (5)	(72.7)	(14.5)	(12.8)	
	Ashkelon	4 (2)				
	Hadera	2 (1)				
	All cats	236 (100)				

* Geographic origin was determined in 136 of 142 donor cats

Table 2: Proportions of breeds of 242 Israeli cats included in the present survey

Cat breed	Number of cats	Proportion (%)
DSH ¹ , DLH ² and mixed	213	88
Persian	15	6.2
Russian blue	5	2.1
Himalayan	3	1.2
Siberian	2	0.8
Scottish fold	1	0.4
Angora	1	0.4
Brandon exotic	1	0.4
Siamese	1	0.4
Total	242	100

1, domestic shorthair; 2 domestic longhair

(0.06%) vs. 79 (79%) and 21 (21%), respectively] compared to the ill cats group.

Most cats in the present study ($n = 176$, 72.7%) had blood type-A. Blood types -B and -AB were recorded in 35 (14.5%) and 31 (12.8%) cats, respectively. There was no difference ($P = 0.75$) in the proportion of blood types between blood donors and recipients (Table 3) or between males and females ($P = 0.2$, data not shown). No acute hemolytic transfusion reactions or other reactions were recorded throughout the study period, although donor and recipient blood were cross-matched only in a small number of cases.

The present study included a small population of pure breed cats. The small numbers precluded a comparative analysis of breed distribution except in the Persian and Russian blue cats. All other purebred animals were considered a single group. There was a significant ($P = 0.01$) difference in

the proportions of blood types between all pure-breed cats combined compared to mixed-breed cats. Blood type-A was recorded in 69% and 96.6% of the mixed-breed (i.e., DSH, DLH and crosses) cats and pure-breed cats, respectively. Blood type-B was recorded in 16% and 3.4% of mixed-breed and pure-breed cats, respectively. Blood type-AB was recorded in 14.5% and 0% of mixed-breed and pure-breed cats, respectively. (Table 4) Persians and Russian blue cats all had blood type-A in this study. Persian cats had a significantly ($P=0.0114$) higher proportion of blood type-A compared to the DSH/DLH/mixed breed group (15/15, 100% vs. 148/213, 69%, respectively). There was no association between gender and blood type distribution.

The geographic location was recorded in 236 of 242 cats. Most cats were from the Rehovot and Tel Aviv districts (44% and 20%, respectively, Table 4). There were significant ($P = 0.02$) differences in the proportions of feline AB system blood types in different districts in Israel (Table 5). The proportion of blood type-B was significantly higher in Petah-Tikwa (32%) than in the Tel Aviv and Rehovot districts (8.3% and 9.7%, respectively). However, blood type-A was the most frequent in all the districts represented in this study.

Table 4: Feline AB-group blood types prevalence in mixed- and pure- breed cats in Israel

Blood type	Mixed breeds ¹		Pure breeds	
	Number of cats	% of cats	Number of cats	% of cats
A	148	69.5	28	96.6
AB	31	14.5	0	0
B	34	16	1	3.4
Total	213	100	29	100

1, Domestic shorthair and longhair and their crosses

Table 5: Distribution of feline AB blood types in different districts of Israeli cats

Blood type District	A n (%)	B n (%)	AB n (%)	Total n (%)	<i>P value</i> ¹
Rehovot	77 (74.8)	10 (9.7)	16 (15.5)	103 (100)	0.02
Tel-Aviv	35 (72.9)	4 (8.3)	9 (18.8)	48 (100)	
Ramla	20 (80.0)	3 (12.0)	2 (8.0)	25 (100)	
Petah-Tikwa	15 (60.0)	8 (32.0)	2 (8.0)	25 (100)	
Other regions	23 (65.7)	10 (28.6)	2 (5.7)	35 (100)	
All cats	170 (72.0)	35 (14.8)	31 (13.1)	236 (100)	

1, Analysis showed that there are significant differences in AB blood types proportions between districts

DISCUSSION

The study included two different populations, donors, and ill cat recipients. Mixed breeds (i.e., DSH and DLH) which could be considered as 'local' breeds were significantly more common in the donor population compared to the transfused cat population. Because most donors originated from a small area in Rehovot, this might have had an influence on the prevalence of blood types in the study, as this population was probably genetically related. In contrast, a genetic link among the recipient cats is highly unlikely. The latter were mainly from Tel Aviv and Rehovot due to the HUVTH location, in proximity to these cities.

Previous surveys conducted in several countries, and this study detected a prevalence to blood type-A (1, 3, 16-23). The proportion of type-A blood is relatively low in Israel and those of blood types-B and -AB are relatively high as compared to the other population studies. If these results are representative of blood type prevalence in the Israeli cat population they are remarkably different from other cat populations studied as the proportion of blood type-AB is the highest described to date in the literature (Table 1). In a United States report of 1072 mixed breed (i.e., DSH and DLH cats) 99.7% had blood type-A, while the prevalence of blood type-B was only 0.03%, and none of these cats had blood type-AB (3). Studies conducted in England ($n = 139$), Greece ($n = 207$) and Australia ($n = 187$) described a lower prevalence of blood type-A, and higher prevalence of blood type-B (7.9%, 20.3% and 26.3%, respectively) as well as a blood type-AB (5%, 1.4% and 0.4%, respectively) (16, 17, 21). The prevalence of blood type-AB has been reported to be higher in breeds in which the prevalence of blood type-B is relatively high, which are mainly certain pure breed cats (e.g., Cornish and Devon Rex, Persian and British shorthair) (24).

When the blood types distribution in mixed breed cats (i.e., DSH, DLH and their crosses) was compared to that of the pure breed cats (Persian and Russian blue cats excluded), the mixed breeds had a lower proportion of blood type-A, and higher proportion of blood types -B and -AB. These results differ from previous studies (15, 16, 20). Moreover, in the pure breed cats no blood type-AB cats were identified. It should be noted though, that

this group included Siamese cats that have been shown to be blood type-A in all previous studies (25, 26).

There are several possible explanations for the unexpectedly high proportion of blood type-AB in Israeli cats. Most Israeli cats, whether indoor or outdoor cats, are DSH and DLH and their crosses with other breeds. Many are actually stray cats, adopted by their owners. Israel is a relatively small country and is separated from its neighboring countries either by natural geographic barriers (i.e., the Negev desert in the south, the Jordan River in the east and the Mediterranean Sea in the west) or man-built barriers (i.e., fences and mine fields in the northern borders with Lebanon and Syria). These barriers prevent movement of stray cats into and out of Israel. In a sense, the Israeli cat population is isolated from other domestic cats in the neighboring countries. These circumstances have been present for decades and may have led to inbreeding in Israeli cats over the years, especially since the northern borders were fortified. Israel's situation is somewhat similar to that of an island, such as Japan, in which a survey of cats also revealed a high prevalence of blood type-AB (27). Genetic drift of both these two rather isolated populations, combined with inbreeding, potentially might explain these similar results. In addition, most cats in the present study originated from Central Israel, a fact that might have even increased this effect. Third, introduction of pure breed cats in Israel over the last decade probably had little effect on the local stray cat population, because pure breed cats, especially pedigree cats, are mostly indoor cats and are not bred with local DSH/DLH cats.

Another possible explanation for these results is a high rate of false results due to consistently erroneous interpretation of blood typing results in the HUVTH laboratory, leading to over representation of blood type-AB in our results. We believe that this is extremely unlikely for several reasons. Firstly, typing was only done by two experienced laboratory technicians, who are familiar with the typing procedure. Secondly, this particular commercial kit has been previously used and evaluated and has been reported to be reliable in a study comparing several different typing methods in cats (14). Thirdly, ill cats included in the present study were transfused with blood products, based on the present typing results, while cross-matching was performed in a limited number of cases. No acute transfusion reactions or other reactions were observed in any of these cats following blood component therapy. Typing errors would have led to some acute

transfusion reactions. If type-B blood or plasma is erroneously transfused to a blood type-A cat, both an acute minor reaction (i.e. anti-A type isoantibodies lead to hemolysis of recipient erythrocytes) and a major reaction (i.e. anti-B type isoantibodies lead to hemolysis of the donor erythrocytes) occur. The same is true if type-A blood is erroneously transfused to a blood type-B cat (15, 28, 29). There is every reason to believe that type-AB blood transfused to a type-B cat will lead to an acute major reaction and hemolysis, because type-AB erythrocytes agglutinate with both type-A and type-B sera (15), although the severity of such a reaction will depend on the antibody titer in the type-B cat and the percentage of the erythrocytes in the donor that present NGN on their surface. A previous study in Turkey has estimated that the transfusion of type-A or type-AB blood to type-B cats carries a potential risk of transfusion reactions that are acute and severe in 6.4% and mild clinical reactions in 85.9% of recipients (19). In such a case, the major reaction is severe and potentially fatal. Even in cases when type-A blood is transfused to a blood type-AB cat, although type-AB cats have no naturally occurring isoantibodies, a significant minor reaction might occur, because such antibodies adhere to the surface of the donor's erythrocytes. Thus, since no acute transfusion reactions were presently recorded, we conclude that no major typing errors had occurred, although the possibility that minor reactions did occur that have led to decreased life span of the donated erythrocytes later on should not be overlooked, because follow-up of the recipients was limited to their hospitalization period. The possibility that the same blood typing error has consistently occurred in all cases, both for the recipient and its donor cat, is highly unlikely. However, the present surprising and unprecedented results warrant a future wider survey with representation of different geographic regions in Israel, using several blood typing methods and a genetic molecular study of Israeli blood type-AB cats.

A previous study in U.S. that included Persian cats (3) reported a blood type-B prevalence of 25% in this breed, whereas all Persian cats had blood type-A in this study. The origin of the Persian cat population in Israel is uncertain and cats that are considered as Persians might actually include crosses of Persians with DLH cats. This is in contrast to the U.S. where cats that were classified as Persians in similar surveys were likely to be pedigree cats of pure ancestry. This difference between the present results and previous findings warrants further studies. The number of Russian blue cats in

this study was limited, however, in agreement with previous studies, all had blood type-A (25, 26).

The present study has shown that there is significant blood type distribution difference between cats from different districts within Israel, even when districts are near to each other. For example, in Petah-Tikwa, the proportion of blood type-B was 32% and was significantly higher than in the Tel Aviv and Rehovot populations, with proportions of 8.3% and 9.7%, respectively. These districts are within a 50 km radius and the results suggest that one cannot extrapolate the distribution of feline blood types of cats between districts.

The present results highlight the need to type cats prior to transfusion of blood components and since these results differ substantially from the literature, extrapolation from other studies would be unwise. When blood typing is impossible, cross matching should be done before transfusion. If typing or cross matching is not done, our results show that the risk of an acute, potentially life-threatening transfusion reaction should be seriously considered against the potential benefit of the transfusion. Based on the present findings, the probability of transfusing a wrong blood type in Israel, when both the donor's and recipient's blood types are unknown, is 43.4%, which is extremely high. Based on our study, the risk of erroneously transfusing type-A blood to a type-B recipient cat in Israel is 14.5%. This means that there is a 14.5% chance of inducing an acute severe, potentially fatal hemolytic reaction. If a blood type-AB cat receives type-A blood, the minor reaction should also be considered, although such latter reactions have never been reported previously in the veterinary literature. In Israel, our results suggest that the probability of erroneously transfusing type-A blood to a blood type-AB cat is 12.8%. Nevertheless, one should bear in mind that transfusion of the wrong blood type, even if it results in a minor adverse transfusion reaction, might prove to be with serious deleterious consequences when administered to an ill, anemic cat. It might complicate the recipient's condition and adversely affect morbidity and mortality.

Since routine blood typing of donor and recipient cats at the HUVTH has begun only from mid 2007, the number of cats in this study is limited. The majority of the present donor cats is from a specific area in Rehovot, and comprised mainly of DSH and DLH cats. Since there were no significant blood type prevalence differences between donor and recipient cats, and these two populations are genetically un-

related, the present AB-blood group prevalence cannot be a sole result of inbreeding within the donor population.

Nonetheless, due to the limited number of cats, the present study should be regarded as a preliminary survey of blood types in Israeli cats. The results need to be verified by larger numbers of cats, different methodologies (i.e. different commercial reagents, back-typing, especially of type-AB cats and genetic analysis) and additional as well as a larger number of geographic regions within Israel.

In conclusion, this is the first survey of feline blood types in Israel. The present results particularly show an unexpectedly high proportion of blood type-AB, which is the highest ever recorded in any previous survey worldwide. This is surprising, especially because most cats presently included were DSH, DLH and mixed breed, in which the proportion of blood types-B and -AB is usually low. There were variations in the blood type distribution among different districts. The present results warrant performing a future wider survey, that should include cats from all parts of Israel, and blood typing should be done using several different methods.

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