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ORIGINAL ARTICLE

Distribution of infectious endogenous retroviruses in mixed-breed and purebred cats

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Abstract

Endogenous retroviruses of domestic cats (ERV-DCs) are members of the genus *Gammaretrovirus* that infect domestic cats (*Felis silvestris catus*). Uniquely, domestic cats harbor replication-competent proviruses such as ERV-DC10 (ERV-DC18) and ERV-DC14 (xenotropic and nonecotropic viruses, respectively). The purpose of this study was to assess invasion by two distinct infectious ERV-DCs, ERV-DC10 and ERV-DC14, in domestic cats. Of a total sample of 1646 cats, 568 animals (34.5%) were positive for ERV-DC10 (heterozygous: 377; homozygous: 191), 68 animals (4.1%) were positive for ERV-DC14 (heterozygous: 67; homozygous: 1), and 10 animals (0.6%) were positive for both ERV-DC10 and ERV-DC14. ERV-DC10 and ERV-DC14 were detected in domestic cats in Japan as well as in Tanzania, Sri Lanka, Vietnam, South Korea and Spain. Breeding cats, including Singapura, Norwegian Forest and Ragdoll cats, showed high frequencies of ERV-DC10 (60–100%). By contrast, ERV-DC14 was detected at low frequency in breeding cats. Our results suggest that ERV-DC10 is widely distributed while ERV-DC14 is maintained in a minor population of cats. Thus, ERV-DC10 and ERV-DC14 have invaded cat populations independently.

Introduction

Endogenous retroviruses (ERVs) are remnants of ancestral retroviral infections that are transmitted vertically from parents to offspring in a Mendelian fashion [1–3]. ERVs are present in the genomes of all vertebrates, making up approximately 6%–10% of the cat, human and mouse genomes [4–6]. Most ERVs are inactive, but some ERVs are replication-competent in several species [7], including mice [8], koalas [9, 10], pigs [11–15], and cats [16, 17].

The domestic cat, *Felis silvestris catus (F.s. catus)*, is one of the most recently evolved members of the Felidae. *F.s. catus* descended from *Felis silvestris lybica* in the Near East approximately 131,000 years ago [18]. The development of domestic breeding cats was a consequence of artificial

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selection imposed by humans [19, 20]. The International Cat Association currently recognizes 71 breeds of domestic cats (http://www.tica.org) while the Cat Fanciers' Association recognizes 42 pedigreed breeds for display in the Championship Class (http://www.cfa.org). Cat breed standards are defined by phenotypic characteristics, and subspecies have diverged both morphologically and behaviorally [21]. Moreover, several morphological characteristics of cat breeds are determined by ERV insertions in KIT loci, which alter coat pigmentation [22]. Endogenous feline leukemia virus (enFeLV) sequences are present in the genomes of domestic cats and wild species of the genus *Felis*, with an estimated frequency of 6–12 copies per haploid genome in domestic cats. Approximately 9–16 distinct autosomal loci were detected per domestic cat examined [23–29]. A previous study analyzed insertional enFeLV polymorphisms among 79 domestic cats, including purebred and nonbreeding cats, and found that enFeLV-GGAG was present in 12 animals (15.2% of cats and 8.2% of chromosomes examined). The presence of enFeLVs in only these felid species suggested that enFeLVs entered the germline in a common ancestor of domestic cats before the lineage radiated (i.e., millions of years ago) [25]. Burmese cats had a higher proportion of homozygous enFeLV sites (77%) than other cats, perhaps due to limited outbreeding during development of the breed. Many enFeLVs are not fixed in different cat breeds due to the presence of heterozygous enFeLV insertional polymorphic sites, implying that associations between enFeLVs and disease may not affect all members of a breed [24]. Associations between enFeLV copy number and the outcomes of exogenous feline leukemia virus infections remain uncertain [24], although it has been established that enFeLV is the counterpart of exogenous feline leukemia virus subgroup B [30, 31].

ERVs of domestic cats (ERV-DCs) are endogenous gammaretroviruses that are classified into three genotypes: genotype I (ERV-DC1, -DC2, -DC3, -DC4, -DC8, -DC14, -DC17, and -DC19), genotype II (ERV-DC7 and -DC16), and genotype III (ERV-DC6, -DC10, and -DC18). ERV-DC10, -DC14 and -DC18 are infectious proviruses [16, 17]. The *env* genes of genotype I proviruses were transduced into feline leukemia viruses, generating a novel interference subgroup called FeLV subgroup D [17]. ERV-DC7 and ERV-DC16 have been found to be homozygous in all Japanese domestic cats tested [17]. These two loci encoded an anti-retroviral factor (Refrex-1) active against FeLV subgroup D [32].

Our previous work [17] showed that ERV-DC10 was broadly detected in Japanese domestic cats (N = 244, 37.7% positive), while ERV-DC14 was only sporadically detected in this cat population (N = 244, 2.5% positive). The ERV-DC18 provirus was identified in only one cat and its siblings. The ERV-DC18 proviral sequences were nearly identical but distinct from ERV-DC10 sequences. Thus, ERV-DC18 was probably generated by mobilization of ERV-DC10 [17]. ERV-DC14 and ERV-DC10 proviruses use different viral receptors, enabling these two ERVs to replicate in different types of cultured cells: while ERV-DC14 broadly infects many cell types, ERV-DC10 has a more limited tropism [16]. A single-nucleotide polymorphism (A280→T) in the ERV-DC 5' long terminal repeat (LTR) may represent the *cis* element influencing ERV-DC basal promoter activity. The ERV-DC A-type LTR (A280) is less prevalent in cat genomes than the T-type LTR (T280), conferring reduced promoter activity based on *in silico* analysis [16]. However, the invasion of these two infectious proviruses in cat lineages and their pathogenesis remain unclear.

In this study, we assessed the invasion of the infectious proviruses ERV-DC10 and ERV-DC14 in domestic cats. Our results indicated that ERV-DC10 and ERV-DC14 independently invaded domestic cats and that their frequency distributions differed significantly. Furthermore, we investigated the relationships between the regions where domestic cats live and breed with the frequencies of ERV-DC10 and ERV-DC14.

Materials and methods

Samples

Blood samples (N = 955) from mixed-breed cats (N = 939) and purebred cats (N = 16) were voluntarily submitted by veterinarians in Japan [33]. Pure-breeding samples of Japanese domestic cats (N = 516) were provided by the Veterinary Diagnostic Laboratory, Marupi Lifetech. Additionally, we collected blood, tissue and DNA samples from cats in different countries including South Korea (N = 44), Vietnam (N = 20), Sri Lanka (N = 20), Tanzania (N = 60) and Spain (N = 31). Frequencies of ERV-DC14 in Spanish domestic cat samples were detected in our previous study [34]. Details of these samples are shown in Table 1. DNA was extracted from blood and tissue using a DNeasy Blood and Tissue Kit (QIAGEN,

Table 1 Characteristics of samples used in this study

Country	Cat breed	Sample no.	Source of DNA
Japan	Mix	939	Blood
	Purebred	532	Blood
Vietnam	Mix	20	Blood
South Korea	Mix	44	Blood
Sri Lanka	Mix	20	Tissues
Tanzania	Mix	60	Blood
Spain	Mix	31	Blood and tissues

Osaka, Japan), by phenol/chloroform extraction [35], or using DNAzol (Life Technologies Japan, Tokyo, Japan).

Detection of ERV-DC10 and ERV-DC14

For genotyping of ERV-DC10 [17], we performed PCR using the primer pair Fe-122S (5-TGAAGGAAGGAACTTTTC ATGTAGG-3) and Fe-38R (5-CACACATGCTCTAGACAC AATACCC-3) to detect preintegration sites. We performed PCR using the internal primer Fe-36S (5-AACCGCTTGGTA CARTTCATAAGAG-3) to detect ERV-DC10 insertional polymorphic sites. For ERV-DC14, we performed PCR using the primer pair Fe-58S (5-CATTCAGACTTGCAGTTAAGG GACT-3) and Fe-42R (5-CCATAGCAGCTGACTAGTTTG AATG-3) to detect preintegration sites. We performed PCR using the primer Fe-102R (5-GGATGAGATCCTCCCAGG TG-3) to detect ERV-DC14 insertional polymorphisms. PCR was performed using KOD Fx Neo (Toyobo, Osaka, Japan), and the cycling conditions were as follows: 94 °C for 2 min (predenaturation); 30 cycles of 98 °C for 10 s (denaturation), 62 °C for 30 s (annealing), and 68 °C for 1.5 min (extension). PCR cycling conditions using GoTaq polymerase (Promega, Madison, WI, USA) were as follows: 95 °C for 2 min (predenaturation); 30 cycles of 95 °C for 30 s (denaturation), 57 °C for 30 s (annealing), and 72°C for 1.5 min (extension); 72 °C for 5 min. To identify single nucleotide polymorphisms in ERV-DC14 *env*, the ERV-DC14 *env* gene was amplified by PCR, using the primers Fe-510S (5-AAGGAATTGCCA AAGGAGTTCTAA-3) and Fe-42R (5'- CCATAGCAGCTG ACTAGTTTGAATG -3') and KOD Fx Neo polymerase. The detection and genotyping of proviruses by PCR is shown in Fig. 1A and B. The pre-integration site spanned approximately 500 bp, while the insertional polymorphic sites spanned approximately 800 bp and 1.2 kbp in ERV-DC10 and ERV-DC14, respectively. Heterozygosity was assessed in both pre-integration sites and insertional polymorphic sites. However, homozygosity was assessed only in insertional polymorphic sites.

Statistical analysis

Associations between the frequencies of ERV-DC10 or ERV-DC14 and different geographic regions were evaluated by univariable analysis using the chi-square test or Fisher's exact test. *P*-values less than 0.05 were considered statistically significant.

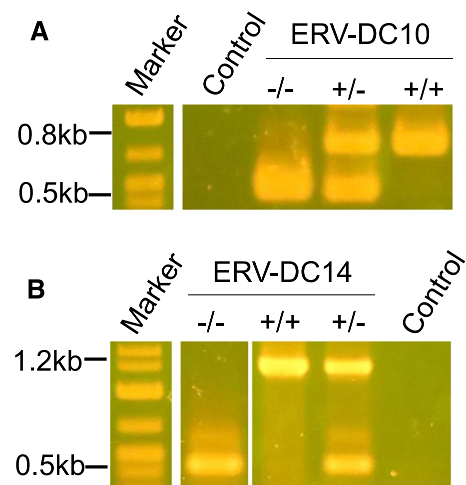

Fig. 1 Genotyping of ERV-DC10 and ERV-DC14 by PCR. (A) ERV-DC10 detection and genotyping. Band sizes of 0.5 kbp and 0.8 kbp represent pre-integration sites and proviral insertional polymorphic sites, respectively. (B) ERV-DC14 detection and genotyping. Band sizes of 0.5 kbp and 1.2 kbp represent pre-integration sites and proviral insertional polymorphic sites, respectively. −/−, no copy of provirus present on either chromosome; +/+, proviral copy present on both chromosomes (homozygous); +/−, proviral copy present on one of two chromosomes (heterozygous)

Results

Frequencies of ERV-DC10 and ERV-DC14 proviruses in Japanese domestic cats

We conducted a large-scale survey to assess the frequencies of ERV-DC10 and ERV-DC14 proviruses in 939 mixed-breed and 532 purebred cats in Japan (including 244 domestic cats from a previous study [17]). Of the 1471 Japanese domestic cats, 482 animals (32.8%) were positive for ERV-DC10, of which 333 (22.6%) were heterozygous and 149 (10.2%) were homozygous. Only 58 Japanese domestic cats (4.0%) were positive for ERV-DC14, of which the majority (3.9%) were heterozygous and one (0.1%) was homozygous (Table 2). Only six of 1471 Japanese domestic cats (0.4%) were positive for both ERV-DC10 and ERV-DC14. A comparison of the frequencies of ERV-DC10 and ERV-DC14 between mixed-breed and purebred Japanese cats showed that the frequency of ERV-DC10 in mixed-breed cats (N = 361, 38.4%) was significantly higher than in purebred cats (N = 121, 22.7%) ($P < 0.0001$) (Fig. 2A). By contrast, ERV-DC14 was detected significantly more frequently in purebred cats (N = 36, 6.8%) than in mixed-breed cats (N = 22, 2.3%) ($P < 0.0001$) (Fig. 2B). Among Japanese domestic cats (N = 6) positive for both ERV-DC10 and ERV-DC14, five animals were mixed-breed cats, and one animal (Scottish Fold, ERV-DC10 homozygous and ERV-DC14 heterozygous) was purebred ($P = 0.248$). These results indicated that ERV-DC10

Table 2 Prevalence of ERV-10 and ERV-DC14 in Japanese domestic cats

Provirus	ERV-DC10				ERV-DC14				Subtotal	Source
	−/− (%)	+/− (%)	+/+ (%)	+ (%)	−/− (%)	+/− (%)	+/+ (%)	+ (%)		
Cat										
Mix	142 (61.2)	70 (30.2)	20 (8.6)	90 (38.8)	227 (97.8)	5 (2.2)	0 (0)	5 (2.2)	232 (100)	[17]
Purebred	10 (83.3)	2 (16.7)	0 (0)	2 (16.7)	11 (91.7)	1 (8.3)	0 (0)	1 (8.3)	12 (100)	
Subtotal	152 (62.3)	72 (29.5)	20 (8.2)	92 (37.7)	238 (97.5)	6 (2.5)	0 (0)	6 (2.5)	244 (100)	
Mix	436 (61.7)	196 (27.7)	75 (10.6)	271 (38.3)	690 (97.6)	16 (2.3)	1 (0.1)	17 (2.4)	707 (100)	This study
Purebred	401 (77.1)	65 (12.5)	54 (10.4)	119 (22.9)	485 (93.3)	35 (6.7)	0 (0)	35 (6.7)	520 (100)	
Subtotal	837 (68.2)	261 (21.3)	129 (10.5)	390 (31.8)	1175 (95.7)	51 (4.2)	1 (0.1)	52 (4.3)	1227 (100)	
Mix	578 (61.6)	266 (28.3)	95 (10.1)	361 (38.4)	917 (97.7)	21 (2.2)	1 (0.1)	22 (2.3)	939 (100)	Combined
Purebred	411 (77.3)	67 (12.6)	54 (10.1)	121 (22.7)	496 (93.2)	36 (6.8)	0 (0)	36 (6.8)	532 (100)	
Total	989 (67.2)	333 (22.6)	149 (10.2)	482 (32.8)	1413 (96.0)	57 (3.9)	1 (0.1)	58 (4.0)	1471 (100)	

+/+, copy present on both chromosomes (homozygous); +/−, copy present on one of two chromosomes (heterozygous); −/−, no copies present (null); +, provirus detected

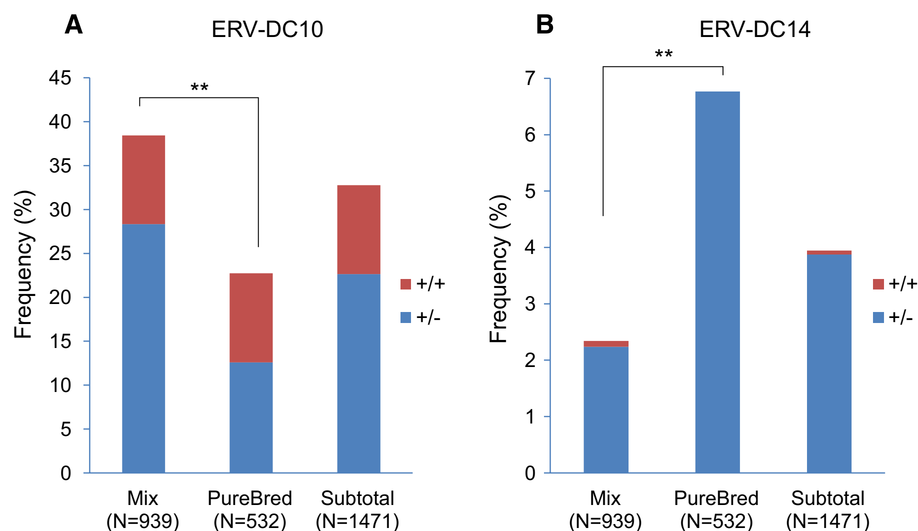

Fig. 2 Prevalence of infectious ERV-DCs among domestic cats in Japan. (A) Prevalence of ERV-DC10 in Japanese domestic cats. (B) Prevalence of ERV-DC14 in Japanese domestic cats. Japanese domestic cats were divided into two groups, consisting of mixed (N = 942) and purebred (N = 532) animals. +/+, copy present on both chromosome (homozygous); +/−, copy present on one of two chromosomes (heterozygous). Differences between frequencies of ERV-DC10 and ERV-DC14 in different regions were analyzed using the chi-square test or Fisher's exact test; **, $P < 0.0001$

was detected at higher frequency than ERV-DC14 in Japanese domestic cats. ERV-DC10 was detected at significantly higher frequency in mixed-breed cats than in purebred cats. Conversely, the frequency of ERV-DC14 was significantly higher in purebred cats than in mixed-breed cats.

Frequencies of ERV-DC10 and ERV-DC14 proviruses in different cat breeds

To better understand the invasion of ERV-DC10 and ERV-DC14 in domestic cats, we further analyzed the frequencies of these two proviruses in purebred Japanese domestic cats. In total, 532 samples classified into 21 purebred cat breeds were analyzed. The frequencies of ERV-DC10 in 16 different purebred cats are shown in Figure 3 and were as follows: Singapura (N = 10, positive = 100%), Tonkinese (N = 5, positive = 40%), Himalayan (N = 16, positive = 12.5%), Norwegian Forest (N = 22, positive = 63.6%), Russian Blue (N = 43, positive = 4.6%), Scottish Fold (N = 110, positive = 25.5%), Abyssinian (N = 33, positive = 27.3%), Persian (N = 12, positive = 16.6%), American Curl (N = 10, positive = 30%), American Shorthair (N = 132, positive = 3.1%), Long-tailed Chinchilla (N = 26, positive = 11.6%), Maine Coon (N = 16, positive = 6.3%), Munchkin (N =

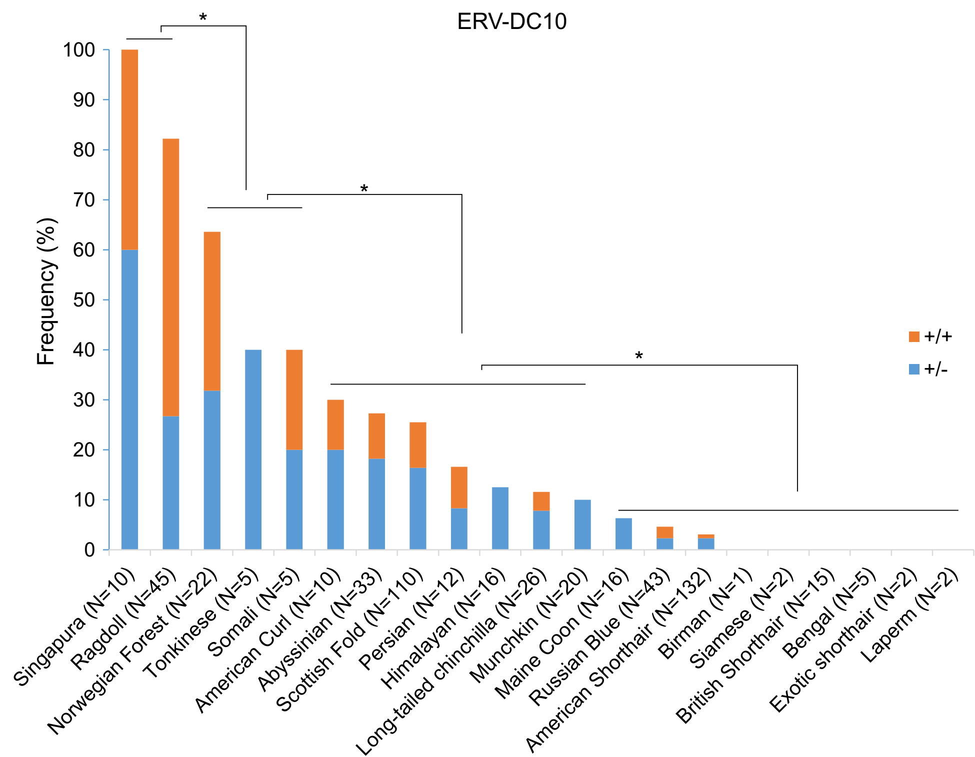

Fig. 3 Comparison of ERV-DC10 prevalence among cat breeds. A total of 21 cat breeds investigated by ERV-DC10 genotyping are shown. +/+, proviral copy present on both chromosomes (homozygous); +/−, proviral copy present on one of two chromosomes (heterozygous). Numbers on the *x*-axis indicate sample sizes for each purebred cat. *, $P < 0.05$ (Fisher's exact test)

20, positive = 10%), Ragdoll (N = 45, positive = 82.2%), and Somali (N = 5, positive = 40%). ERV-DC10 was not detected in any of the remaining six purebred cats: British Shorthair (N = 15), Birman (N = 1), Siamese (N = 2), Bengal (N = 5), Exotic Shorthair (N = 2), and Laperm (N = 2). Only seven cat breeds (Fig. 4) were positive for ERV-DC14 and all were heterozygous: Himalayan (N = 16, positive = 18.7%), Scottish Fold (N = 110, positive = 6.4%), British Shorthair (N = 15, positive = 6.7%), Persian (N = 12, positive = 16.6%), American Curl (N = 10, positive = 10.0%), American Shorthair (N = 132, positive = 15.3%), and Exotic Shorthair (N =2, positive = 100%). Cat breeds could be classified into four breeding groups whose frequencies of ERV-D10 differed significantly from one another ($P < 0.05$) (Fig. 3). Group I (82.2–100% positive) included Singapura and Ragdoll cats. Group II (40.0–63.6% positive) included Norwegian Forest, Tonkinese, and Somali cats. Group III (10–30% positive) included American Curl, Abyssinian, Scottish Fold, Persian, Himalayan, Long-tailed Chinchilla, and Munchkin cats. Group IV (0.0–6.3% positive) included Maine Coon, Russian Blue, American Shorthair, Birman, Siamese, British Shorthair, Bengal, Exotic Shorthair and Laperm cats. We also classified cat breeds into two groups based on their significantly different frequencies of ERV-DC14 provirus ($P < 0.05$) (Fig. 4). Group I (10–100% positive) included Exotic Shorthair, Himalayan, Persian, American Shorthair, and American Curl cats. Group II (0.0–6.7% positive) included British Shorthair, Scottish Fold, Tonkinese, Somali, Singapura, Siamese, Russian Blue, Ragdoll, Norwegian Forest, Munchkin, Maine Coon, Long-tailed Chinchilla, LaPerm, Birman, Bengal, and Abyssinian cats. Overall, the data indicated that ERV-DC10 was widely distributed while ERV-DC14 was present at lower frequencies in breeding cats in Japan.

Previous studies investigated the places and regions where cat breeds were originally established [36, 37]. We analyzed associations between the origin of purebred cats and the presence of ERV-DC10 and ERV-DC14 proviruses. The purebred cats used in this study were classified into four origin groups: Asia, Europe, Middle East and North America [37]. As shown in Figure 5, the average frequency of ERV-DC10 in Asia, Europe, Middle East and North America was 35%, 22%, 21% and 18.3%, respectively. The average frequency of ERV-DC14 in Asia, Europe, Middle East and North America was 0%, 6.4%, 8.3% and 12.5%, respectively. There were no statistically significant associations between the frequencies of the two proviruses and geographic origin. These results suggest that the distribution of ERV-DC10 and ERV-DC14 proviruses in breeding cats is similar regardless of the geographic origin of the breed.

Frequencies of ERV-DC10 and ERV-DC14 proviruses in domestic cats in different countries

Next, we investigated the frequencies of ERV-DC10 and ERV-DC14 proviruses in domestic cats in different

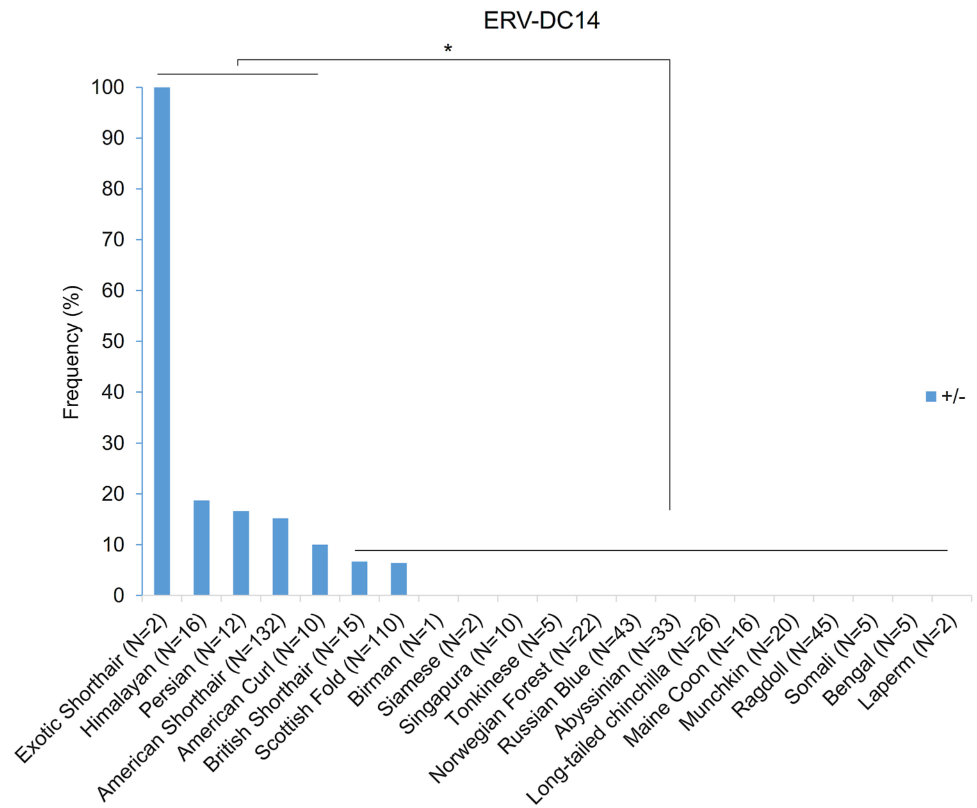

Fig. 4 Comparison of ERV-DC14 prevalence among cat breeds. A total of 21 cat breeds investigated for ERV-DC14 are shown. +/−, copy present on one of two chromosomes (heterozygous). Numbers on the *x*-axis indicate sample sizes for each purebred cat. *, $P < 0.05$ (Fisher's exact test)

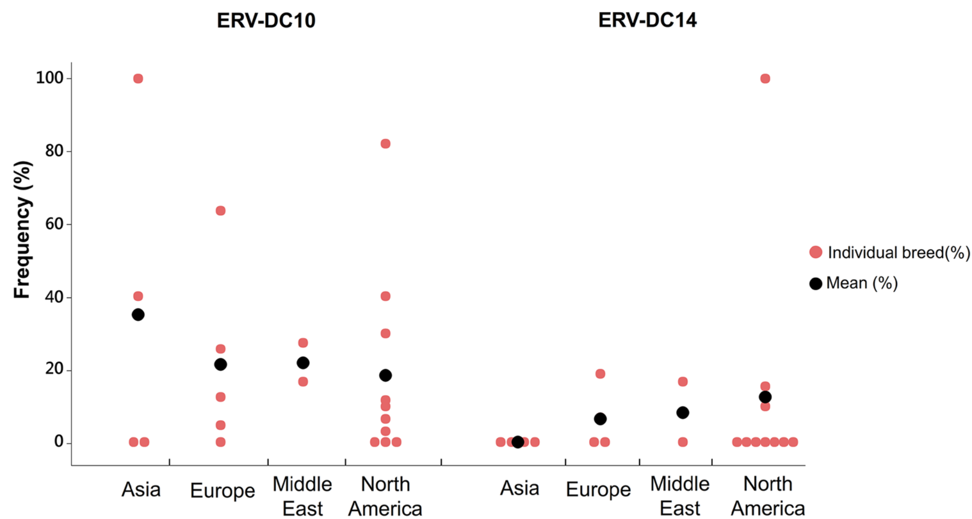

Fig. 5 Prevalence of ERV-DC10 and ERV-DC14 among purebred cats of different origins. Based on the origins of different cat breeds described previously [37], we classified purebred cat samples into four regions. Four breeds were assigned to Asia (Birman, Siamese, Singapura, and Tonkinese). Five breeds were assigned to Europe (British Shorthair, Norwegian Forest, Russian Blue, Scottish Fold and Himalayan). Ten breeds were assigned to North America (Ragdoll, Munchkin, American Curl, American Shorthair, Bengal, Exotic Shorthair, Laperm, Long-tailed Chinchilla, and Maine Coon). Two breeds were assigned to the Middle East (Abyssinian and Persian). Red circles indicate the frequency of each pure breed. Black circles indicate the mean prevalence for each breed's origin with 95% confidence intervals. Differences between frequencies of ERV-DC10 and ERV-DC14 in different regions were analyzed using Fisher's exact test

countries to determine whether or not their frequencies are similar to those of Japanese domestic cats. Domestic cats from South Korea (N = 44), Sri Lanka (N = 20), Vietnam (N = 20), Tanzania (N = 60) and Spain (N = 31) were tested for ERV-DC10 and ERV-DC14. As shown in Figure 6A, frequencies of ERV-DC10 ranged from 19.4% to 66.7 % in domestic cats from five countries other than Japan. Frequencies of ERV-DC10 were highest in Tanzanian domestic cats (66.7%) and lowest in Spanish domestic cats (19.4%). Among Asian domestic cats (Japan, South Korea, Vietnam, and Sri Lanka), frequencies of ERV-DC10 were highest in Sri Lankan cats (57.9%) and did not differ significantly in cats from Japan, South Korea and Vietnam (32.8%, 45.5% and 40.8%, respectively). ERV-DC14 was detected at similar frequencies in domestic cats from South Korea, Sri Lanka, Vietnam and Tanzania (4.5%, 5.0%, 6.7% and 5.0%, respectively) and with similar frequency to domestic cats in Japan (Fig. 6B). Domestic cats in Spain showed somewhat higher frequencies of ERV-DC14 (9.7%) [34], although this difference was not statistically significant. Notably, one Spanish domestic cat and two South Korean domestic cats were positive for both ERV-DC10 and ERV-DC14. These results also indicated that ERV-DC10 and ERV-DC14 were broadly distributed in domestic cats from different countries, and that these two infectious proviruses independently invaded domestic cat populations.

Frequencies of ERV-DC10 and ERV-DC14 proviruses in domestic cats used in this study

In summary, we assessed the presence of ERV-DC10 and ERV-DC14 in 1646 domestic cats from six different countries, including Japan, Vietnam, Sri Lanka, South Korea, Tanzania and Spain. Of these animals, 568 cats were positive for ERV-DC10 (34.5%), and 68 cats were positive for ERV-DC14 (4.1%) (Table 3). Only 10 cats were double-positive for ERV-DC10 and ERV-DC14 (0.6%). Notably, ERV-DC14 homozygosity was observed in only one of 68 ERV-DC14-positive Japanese domestic cats. These results indicated that ERV-DC10 was more frequently detected in domestic cats compared with ERV-DC14. ERV-DC14 appeared to be maintained in a minor population. Thus, these two infectious proviruses may have independently invaded domestic cat populations.

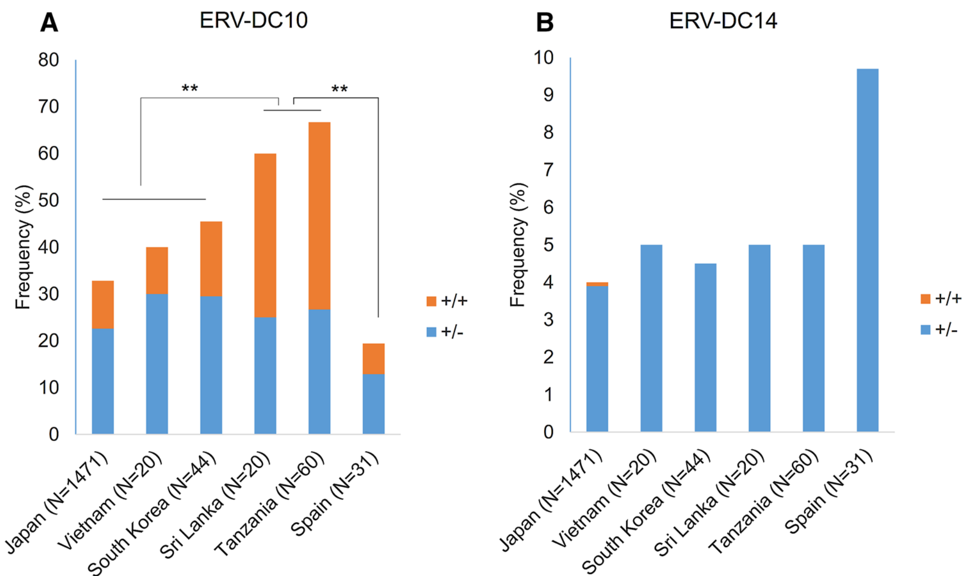

Fig. 6 Prevalence of two infectious ERV-DCs in purebred cats of different countries. Frequencies of ERV-DC10 (A) and ERV-DC14 (B) in domestic cats in different countries. +/+, copy present on both chromosomes (homozygous); +/−, copy present on one of two chromosomes (heterozygous). Differences between frequencies of ERV-DC10 and ERV-DC14 in different regions were analyzed using the chi-square test or Fisher's exact test; *, $P < 0.05$; **, $P < 0.0001$

Table 3 Frequencies of ERV-10 and ERV-DC14 in domestic cats

Provirus	ERV-DC10 (+)	ERV-DC14 (+)	No. of cats positive for both proviruses
−/−	1078 (65.5)	1578 (95.9%)	10 (0.6%)
+/−	377 (22.9%)	67 (4.0%)	
+/+	191 (11.6%)	1 (0.1%)	
Total (+)	568 (34.5%)	68 (4.1%)	
Total	1646 (100%)	1646 (100%)	

+, provirus detected; +/−, heterozygous (copy present on one of two chromosomes); +/+, homozygous (copy present on both chromosomes)

Discussion

In this study, we assessed the frequencies of two infectious ERVs (ERV-DC10 and ERV-DC14) in domestic cats of different breeds and different geographic origins. The prevalence of ERV-DC10 in mixed-breed cats was significantly higher than that in purebred cats in Japan, while the opposite was true for ERV-DC14 (Fig. 2). This difference may relate to the different breeding strategies for mixed and purebred cats.

The frequencies of ERV-DC10 and ERV-DC14 were not associated with the geographic origins of the cats. However, these frequencies appeared to be associated with specific breeds of cats, as shown in Figs. 3 and 4. Frequencies of ERV-DC14 were highest in Exotic Shorthair (100%), Himalayan (18.7%) and American Shorthair (15.3%) cats. Exotic Shorthair and American Shorthair cats originated in the US but now cluster in Western Europe [38]. Moreover, Himalayan cats represent hybrids between UK and US cats [37]. Thus, ERV-DC14 may have originated from European wildcats.

ERV-DC10 was detected at high frequencies in Singapura (100%), Ragdoll (82.2%), Norwegian Forest (63.6%), and Somali (40%) cats (Fig. 2). Singapura cats are descended from a few populations in Asia from Asian wildcat ancestors, while Ragdoll cats are a crossbreed between US and UK cats [37]. Norwegian Forest cats are considered feral cats that were naturally selected [37]. Our results indicated that ERV-DC10 may have first originated in Asian and African wildcats.

Our results indicated that ERV-DC10 and ERV-DC14 proviruses were similarly distributed among purebred cats of four origins (Asia, Europe, Middle East and North America) (Fig. 5). This result was not unexpected, since after the establishment of cat breeds, they spread all over the world.

The frequencies of ERV-DC10 and ERV-DC14 in domestic cats in different countries differed within mixed breeding populations. While ERV-DC10 showed the highest and lowest frequencies in Tanzanian and Spanish domestic cats, respectively, frequencies of this provirus were broadly similar among Asian cats (Japan, South Korea and Vietnam). However, the frequency of ERV-DC10 was also high in Sri Lankan domestic cats, which resemble Tanzanian cats (Fig. 6A). This result suggests that Asian and African domestic cats may harbor ERV-DC10 more frequently than domestic cats in Europe. In contrast, ERV-DC14 frequencies were similar among countries (4.0 to 9.7%) but were lowest in Asian and African countries (Fig. 6B). We observed ERV-DC14 homozygosity in only one of 1646 domestic cats, while ERV-DC14 heterozygosity was observed in 67 domestic cats.

Endogenous Jaagsiekte sheep retrovirus (enJSRV) has been shown to be highly active and more abundant in female sheep than in males because this enJSRV plays a role in trophoblast development [39]. In our study, we found an equal distribution of the two infectious ERVs examined (ERV-DC10 and ERV-DC14) in male and female cats. Thus, gender does not appear to affect invasion by these two ERVs (data not shown), although it remains unknown whether ERV-DC activity may be gender-dependent.

Although most ERVs appear to represent junk DNA, a few ERVs have been co-opted by their hosts to gain a variety of physiological functions through "ERV domestication" [40]. For example, some are antiviral factors [8, 41] or have placenta formation ability [42]. enFeLVs are counterparts of exogenous FeLV subgroup B [30, 43] and produce recombinant viruses with altered biological activity and pathogenicity [44, 45]. enFeLVs have been identified in wild species of the genus *Felis* closely related to domestic cats but were not detected in other lineages within the Felidae [23–25]. Two ERV-DC loci, including ERV-DC7 and ERV-DC16, are fixed in domestic cats and encode antiretroviral factors against FeLV subgroup D and ERV-DC genotype I. Our recent study showed that ERV-DC7 was fixed in European wildcats but that ERV-DC16 was unfixed. Thus, levels of antiretroviral activity against FeLV-D and ERV-DC14 differed slightly between these two cat groups [34]. It remains unknown whether the presence of ERV-DC10 and ERV-DC14 in the cat genome is harmful to the host. However, the existence of any replication-competent retrovirus in the genome poses a potential risk. In this study, the infectivity of at least three full-length ERV-DC10 proviral clones and six full-length ERV-DC14 proviral clones from mixed-breed and purebred cats was assessed. All ERV-DC10 and ERV-DC14 proviral clones assessed were infectious (data not shown). Thus, replication-competent ERV-DC10 and ERV-DC14 in the domestic cat genome may be mobile and interact with other exogenous retroviruses to generate new recombinant viruses. A similar pattern was observed in mice. The genomes of several mouse strains (e.g., AKR, C58, and HRS) carried endogenous ecotropic murine leukemia viruses (E-MLVs) called *Emvs*, most of which can produce infectious viruses during leukemogenesis when *Emv*-derived E-MLVs establish a chronic infection [8, 46, 47].

The process of cat domestication remains controversial. Domestic cats are thought to have originated from African wildcats in the Middle East (*Felis silvestris lybica*) [18]. Ancestral retroviruses, including ERVs, can be valuable tools for understanding the domestication process. enFeLV insertional polymorphisms in different wildcats, breeding cats, and non-breeding cats have suggested a potential scenario for cat domestication [23–25], while a similar result using RD114 virus sequences has suggested a map of cat migration [48]. Although insertional polymorphisms of

the infectious proviruses ERV-DC10 and ERV-DC14 were not suggestive of any particular route of cat domestication, our study ruled out the hypothesis that distinct cat ancestors harbored these two ERV-DC loci (e.g., European wildcats may harbor ERV-DC14 [34], while Asian and African wildcats may harbor ERV-DC10; data not shown). In addition, ERV-DC14 homozygosity was detected in only one of 68 ERV-DC14-positive domestic cats, while ERV-DC10 homozygosity and heterozygosity were detected in 191 and 377 domestic cats, respectively. Based on these results, we propose the hypothesis that ERV-DC14 homozygosity (and potentially heterozygosity) affects embryogenesis and is deleterious in domestic cats. This hypothesis agrees with a previous study in which it was found that ERV-DC14 broadly infects many species, while ERV-DC10 produced only limited infection in a subset of tested cells. In other words, the infectivity of ERV-DC14 is nonecotropic, whereas that of ERV-DC10 (ERV-DC18) appears to be xenotropic [16]. Thus, screening for ERV-DC14 in purebred and mixed-breed cats may have important implications for cat reproduction.

This issue needs to be considered further in future studies. In veterinary clinics, production of induced pluripotent stem cells, blood transfusion and bone marrow transplantation would be more safely accomplished using cat donors that are free of these two infectious ERVs. The relationships between these infectious ERVs (ERV-DC10 and ERV-DC14) and disease in domestic cats remain to be elucidated.

Conclusions

In summary, two infectious endogenous retroviruses (ERV-DC10 and ERV-DC14) were not fixed in cat populations, unlike ERV-DC7 or ERV-DC16 [17]. However, these ERVs still showed high frequencies in domestic cats. These two infectious proviruses have potential to induce disease in cats and to recombine with other feline exogenous retroviruses to generate new recombinant exogenous retroviruses. The existence of these two infectious ERVs in expanded purebred populations poses risks to the host, especially with respect to treatment through transplantation methods (e.g., bone marrow transplantation). Moreover, investigations of ERV-DC10 and ERV-DC14 may help in understanding feline evolution. This study provides useful information for improving our understanding of the pathogenicity of infectious ERVs as well as of cat evolution.

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Compliance with ethical standards

Animal studies were conducted according to the guidelines for the care and use of laboratory animals of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. All experiments were approved by the Genetic Modification Safety Committee of Yamaguchi University.

Conflict of interest All authors declare that they have no conflicts of interest.

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