Table. Overview of results from all Ebola virus RT-PCRs performed during hospitalization of breast-feeding mother of twin babies, Guinea, 2015*

Day after admission	Blood, C_t	Breast milk, C_t	Urine, C_t
1	32.5, glycoprotein	NT	NT
3	33.7, glycoprotein	NT	NT
6	NT	21.6, nucleoprotein	NT
14	40.5, nucleoprotein	27.5, nucleoprotein	NT
18	41.0, glycoprotein	NT	NT
21	40.3, nucleoprotein	32.7, nucleoprotein	NT
25	39.3, nucleoprotein	NT	NT
29	Negative, glycoprotein and nucleoprotein	NT	Negative, glycoprotein and nucleoprotein

*Testing performed by using the Xpert Ebola Assay (GeneXpert Instrument Systems, Cepheid, Sunnyvale, CA, USA). The lowest of the reported glycoprotein and nucleoprotein values are reported. C_t values <20 are highly positive, whereas C_t values >35 are weakly positive. C_t, cycle threshold; NT, not tested.

at least 26 days after EVD symptom onset and demonstrate a case in which a baby was not infected by breast milk from his EBOV-positive mother. However, it should be noted that the woman's breast milk was never tested while she was breast-feeding baby 2.

The literature on EBOV in breast milk of EBOV-positive patients is extremely scarce (*3*). In a previous study from the 2000 Sudan EBOV outbreak in Gulu, Uganda, breast milk from a convalescent-phase patient was sampled 15 days after symptom onset and tested positive for EBOV by RT-PCR and virus culture (*4*). Another study conducted in Guinea during the current outbreak, reported a mother–baby pair in which EVD developed in the baby 14 days after symptom onset in the mother, but breast milk from the mother sampled 17 days after symptom onset was negative by EBOV RT-PCR (*1*).

It is unclear whether infectious virus or defective particles are being secreted in breast milk. C_t values were consistently lower in breast milk than in blood when tested concomitantly, but in this case, breast milk samples were not collected until day 6. Our findings suggest that breast milk is infected by EBOV at a later stage of the disease than blood but then follows the expected replication kinetics observed in venous blood.

Considering the high EVD death rate, until further evidence is found, we recommend that EBOV-positive women stop breast-feeding immediately and that breast-feeding not be resumed until 2 negative RT-PCR tests of the breast milk have been confirmed. This suggestion is in line with the World Health Organization recommendation for testing semen in male EVD survivors (*5*). The public health risk for EBOV to remain in breast milk for at least 26 days after EVD symptom onset and for breast milk to possibly be infectious after a patient has cleared the virus from the blood should also be acknowledged.

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Chronic Infection of Domestic Cats with Feline Morbillivirus, United States

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To the Editor: Feline morbillivirus (FeMV) was first reported in Hong Kong, and mainland China in 2012 (*1*) and has been associated with tubulointerstitial nephritis, the histopathologic correlate of idiopathic chronic kidney disease (CKD); however, this association has not been proven by studies in FeMV-naive animals. In 2013, phylogenetically related strains were found in Japan, indicating broader geographic distribution in Asia (*2*). The lack of complete genome sequences for strains from other regions prevents assessment of the clinical relevance and genetic diversity of FeMV. Classical morbilliviruses, such as measles and canine distemper viruses, have a global distribution, suggesting that FeMV might be present elsewhere in the world (*3*). To confirm the presence of FeMV and assess its genetic diversity and infection patterns in the United States, we collected and analyzed urine samples from domestic cats.

We generated amplicons from 10 (3%) of 327 samples; 3 samples were from cats with CKD and 7 from cats without CKD. Sequencing results confirmed that these 493 bp amplicons correspond to unique strains of FeMV (*1*). FeMV^{US1} is 97% similar in the L gene amplicon sequence to FeMV^{776U} (*1*), whereas FeMV^{US5} is only 85% identical, making it very different to all previously identified FeMVs. We used these sequences to develop a pan-US primer set, priFeMV^{US}panL+ and priFeMV^{US}panL-, to amplify a highly conserved region (460 bp) of the L gene of the US strains (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/4/15-1921-Techapp.pdf). The results of these analyses demonstrated that FeMV is present outside of Asia.

In October 2013, we obtained the initial FeMV^{US1}-positive sample from a healthy 4-year-old male domestic shorthair cat (animal 0213). Approximately 15 months later, we obtained a follow-up urine sample from the still healthy cat, performed reverse transcription PCR (RT-PCR), and generated amplicons (online Technical Appendix Figure, panel A). Amplification and sequencing of the hemagglutinin (H) gene from the 2015 sample indicated that it was identical to that from the 2013 sample, suggesting that the cat was chronically infected. We developed a quantitative RT-PCR test by using L gene primers and a real-time probe (online Technical Appendix Table 2). Results indicated stable and comparable virus loads: 9.8×10^4 copies/mL in 2013 versus 7.8×10^4 copies/mL in 2015. This finding corroborates the view that cats can be chronically infected with FeMV and that the virus is persistently shed in urine.

We used primers to generate cDNA from clinical material and then determined the complete genome sequence of FeMV^{US1} (GenBank accession no. KR014147) by using RT-PCR and rapid amplification of cDNA ends. The major morbillivirus surface antigen is the H glycoprotein, and we used pan-FeMV H gene primer sets to detect additional viruses (e.g., FeMV^{US2}) (online Technical Appendix Figure, panel B). An indirect immunofluorescence assay was developed to screen serum samples for FeMV-specific antibodies. Antibodies to FeMV^{US1} were detected in fixed cells expressing FeMV H glycoprotein (positive up to 1:12,800 dilution), and antibodies to FeMV^{US5} were detected in nonpermeabilized cells (positive up to 1:6,400 dilution) (online Technical Appendix Figure 2). This result confirms that H

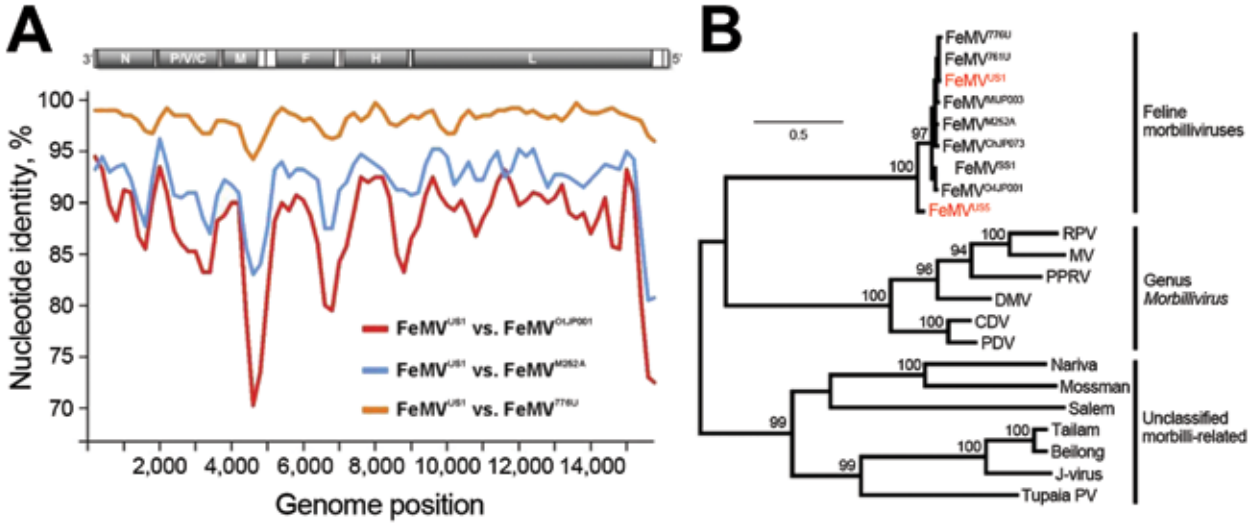

Figure. Phylogenetic analysis of feline morbillivirus (FeMV) whole genomes and hemagglutinin (H) genes collected from cats in the United States. A) Genomic sequence identity of FeMV^{US1}, compared with Asian strains, performed by using SSE V1.2 software (*4*) with a sliding window of 400 and a step size of 40 nt. B) Maximum-likelihood phylogeny of the translated H gene of FeMVs, the genus *Morbillivirus*, sensu strictu, and unclassified morbilli-related viruses was determined by using MEGA5 software (*5*) and applying the Whelan-and-Goldman substitution model and a complete deletion option. Numbers at nodes indicate support of grouping from 1,000 bootstrap replicates. Scale bar indicates substitutions per site.

glycoprotein–specific antibodies are present at high levels concurrent with the longitudinal detection of genomic RNA. A large-scale seroprevalence and cross-neutralization study is ongoing.

We used complete genome and H gene sequences in a comprehensive phylogenetic analysis. FeMV^{US1} is closely related to viruses from Asia, highlighting the global distribution of FeMV (Figure, panel A). Compared with the sequence for the FeMV^{776U} H gene, sequences for FeMV^{US1} and FeMV^{US5} were 98% and 81% similar, and the glycoproteins were 98% and 86% identical. The complete H gene of the most divergent US strain (FeMV^{US5}) clustered phylogenetically in a basal sister relationship with all other viruses from Asia and the United States (Figure, panel B), suggesting a long evolutionary association of FeMV in feline hosts.

Ecologic surveys continue to identify novel viruses that are homologous to known paramyxoviruses in many wildlife species, including bats and rodents (*6*). Investigating closely related viruses in domestic species is warranted, given the substantial number of animals that cohabitate with humans. Switches from natural to unnatural host species can result in enhanced pathogenicity (e.g., receptor switching has caused feline panleukopenia virus to infect dogs as canine parvovirus) (*7*). Given the high degree of antigenic relatedness of morbilliviruses, understanding evolutionary origins and trajectories and conferring cross-protection through immunization are critical. Although no evidence for FeMV transmission to humans or other animals exists, the propensity for noncanonical use of signaling lymphocytic activation molecule 1 F1 (CD150) should be investigated because epizootic transmission of morbilliviruses can occur (*8*).

The detection of FeMV sequences in a clinically healthy animal after 15 months is a novel and surprising observation but is consistent with the known propensity for morbilliviruses to persist in vivo (*9*). All known morbilliviruses cause acute infections, and the typical long-term clinical manifestations occur in the central nervous system, not the urinary system (*1*). These observations should prompt additional research because the prevalence of CKD in cats is high and because CKD decreases the quality of life of affected animals and is the ultimate cause of death for approximately one third of cats (*10*).

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Difficulties in Schistosomiasis Assessment, Corsica, France

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To the Editor: We would like to add some specification and clarification to the discussion regarding the diagnostics and case definitions for urinary schistosomiasis in travelers to Corsica, France (*1–3*). Evidence for a *Schistosoma*

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Chronic Infection of Domestic Cats with Feline Morbillivirus, United States

Technical Appendix

Methods

Urine samples were collected by cystocentesis from 327 cats representing a combination of owned (pet) and unowned (stray) cats, as well as sick and healthy cats. Clinical information (age, sex, breed, body weight, disease status) was recorded for each cat as part of an ongoing large-scale clinical epidemiologic study to determine associations between feline morbillivirus (FeMV) carriage, seroprevalence, and disease status.

Two primers sets were used to screen each cDNA sample by PCR, a semi-nested primer set designed to amplify a conserved region of the L gene in respiroviruses, morbilliviruses, and henipaviruses (RMH) and either priFeMV$^{US1\text{-}L}$, priFeMV$^{US5\text{-}L}$, or priFeMV$^{panUS\text{-}L}$, which amplify a conserved region of the FeMV L gene (Technical Appendix Table 1). A third primer set, feACTβ, was used to amplify a region of feline β-actin, a ubiquitous cell maintenance gene, as a positive control (Technical Appendix Table 1). PCR was performed for each primer set using *Taq* DNA Polymerase (Thermo Fisher Scientific, Grand Island, NY, USA) according to manufacturer's instructions. Reaction mixtures (50 μL) containing 2 μL of cDNA and 1 μL (0.2 μmol/L final concentration) of each primer, were denatured at 94°C for 2 minutes, followed by 40 cycles of 94°C for 15 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. For the RMH primer set, 2 μL of the first PCR reaction was used as a template for a nested PCR using the same conditions as before and using 1 μL each (0.2 μmol/L final concentration) of RMH F2 and R primers. Samples (8 μL) were separated by 1% (w/v) DNA agarose gel electrophoresis to verify the presence of correct sized amplicons. Correct sized amplicons (positive samples) were either purified using QIAquick PCR purification kit (QIAGEN, Hilden, Germany) or were gel-extracted and purified using QIAquick gel extraction kit (QIAGEN); the positive samples were then sequenced (Genewiz, Inc., South Plainfield, NJ, USA) with the same primers (1.67 μmol/L final concentration) used to amplify the target region

Total RNA extractions from urine used for cDNA synthesis were performed in a clean room, using dedicated pipettes, reverse transcriptase, primers, and plasticware. PCRs were set up using different pipettes, dedicated DNA-dependent DNA polymerases and primers. Reverse-transcriptase–negative controls were always included to demonstrate that amplicons were not attributable to contamination. No tube that might contain an FeMV amplicon was ever opened in the clean room. All DNA gel electrophoresis was performed in a separate laboratory on a different floor. Even though we do not present the data in this manuscript, the sequences of all FeMV samples obtained are unique, further demonstrating specificity.

Technical Appendix Table 1. Sequences of FeMV-specific primers sets used to generate amplicons from cDNA after reverse transcription

Gene	Name	Sequence (5′→3′)	Amplicon
L gene	RMH F1	TCITTCTTTAGAACITTYGGNCAYCC	610 bp
	RMH R	CTCATTTTGTAIGTCATYTTNGCRAA	
	RMH F2	GCCATATTTTGTGGAATAATHATHAAYGG	493 bp
	RMH R	CTCATTTTGTAIGTCATYTTNGCRAA	
	US1+	TATCTGGAGAAGGGTTAACATCTG	405 bp
	US1–	TCAGCTATTACTTGACAAGCCCTC	
	US5+	ATCATGCATCCGCTGTAATTAG	357 bp
	US5–	AGACTATATGAGAGATTGAACTC	
	panUS+	ATGTTTTATGCCATTAAGTC	460 bp
	panUS–	GTTGAGAATTATCTTTAGGTACAC	
	US1-rt+	GCCAGAGAGAATTGAGTCTATATC	103 bp
	US1-rt–	TGGYTTACCATTGAACAAGACTTTG	
	US1-rtPr	FAM-CAACAAAAATCGCTTGGCTAATGACCCYAA-BHQ1	Real-time probe
HA gene	US1+	AGGATTTAGTATTTAGAAGAGG	2,077 bp
	US1–	GTACTTCCGGGTATAGAATATC	
	US5+	GCAATACTATCCTATACACATG	2,155 bp
	US5–	AGATAACTATAGGACTGTTGAG	
	panUS+	GGTCAAGGAACATATAGTAG	619 bp
	panUS–	GTATAATTGTAAGGTGGTATT	

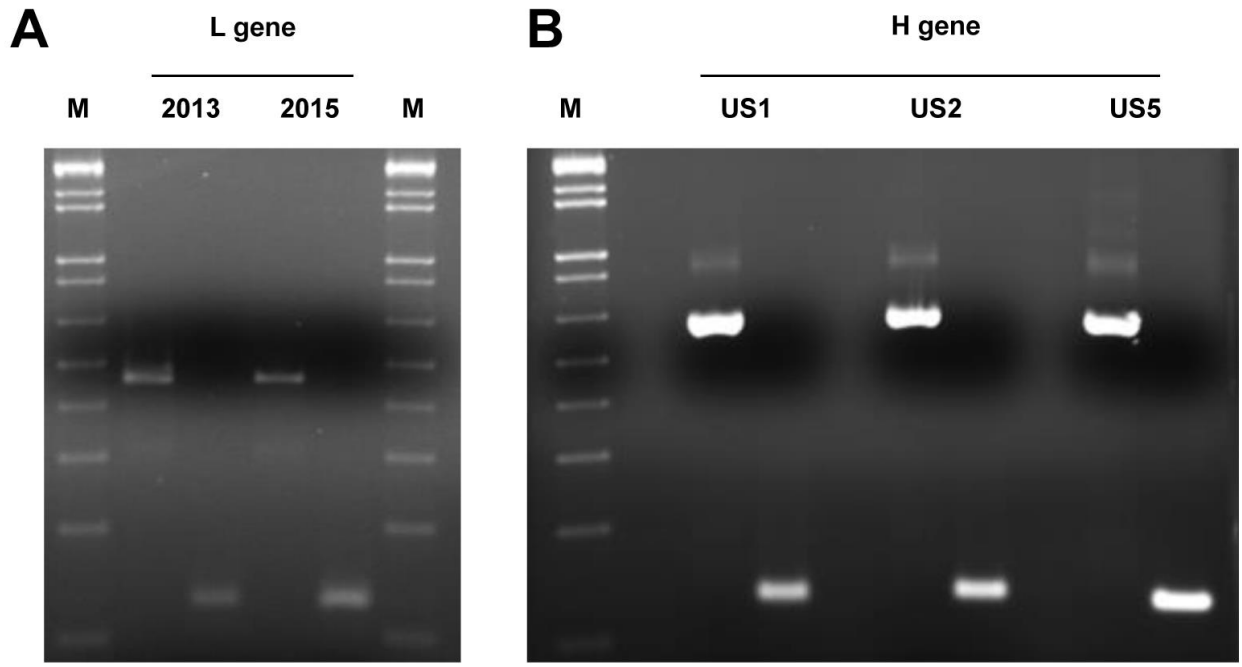
*RMH, respiroviruses, morbilliviruses, and henipaviruses.

Technical Appendix Table 2. Primers used for the reverse transcription and amplification of the complete genome of feline morbillivirus (FeMV) from total RNA in 11 amplicons

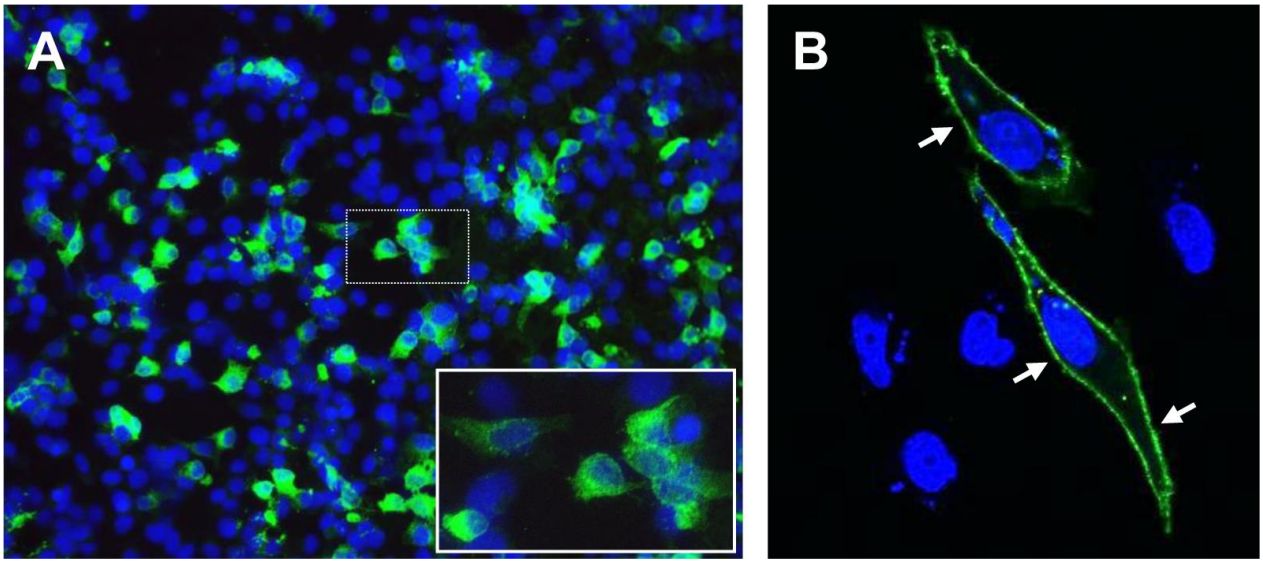
Amplicon (bp)	cDNA synthesis	cDNA primer sequence (5′→3′)	Forward primer	Sequence (5′→3′)	Reverse primer	Sequence (5′→3′)	Kit*
Leader (382)	priAdaptor-dT17	GAC TCG AGT CGA CAT CGA TTT TTT TTT TTT TT	priAdaptor-dT17	GAC TCG AGT CGA CAT CGA TTT TTT TTT TTT TTT TT	priFeMV^{US1} 347–	AAT TGA CCT GGA GAT TCG AC	P
N (820)	priFeMV^{US1} 3,541–	GAC ACC ATG AAG AAG TAG ATA C	priFeMV^{776} 38+	TGT GAC CTA TTC TAA CGA CAA G	priFeMV^{776} 858–	CAA TAT CAC AAA TCA TTT CAG C	T
N-P (2,344)	priFeMV^{US1} 754+	CTG AGA TTG AGC AAA GGA TGG C	priFeMV^{US1} 754+	CTG AGA TTG AGC AAA GGA TGG C	priFeMV^{US1} 3,098–	CTG ATG GTT GAT GTG CTT GCA TG	P
P-M (465)	priFeMV^{US1} 3,541–	GAC ACC ATG AAG AAG TAG ATA C	priFeMV^{776} 3,076+	CAA GAA CGA AAC ATC TGC AAT C	priFeMV^{US1} 3,541–	GAC ACC ATG AAG AAG TAG ATA C	T
M (1,516)	priFeMV^{US1} 754+	CTG AGA TTG AGC AAA GGA TGG C	priFeMV^{US1} 3,293+	CAT GCA TTA TAG GTT GTA ATT G	priFeMV^{US1} 4,809–	CTG AGT TAG ACA GGC CCT AGA C	P
F-H (4,216)	priFeMV^{US1} 4,697+	GAT TCA TTA AAG TTA GAT TCT TG	priFeMV^{US1} 4,697+	GAT TCA TTA AAG TTA GAT TCT TG	priFeMV^{US1} 8,913–	GTA CTT CCG GGT ATA GAA TAT C	P
L (1,553)	priFeMV^{US1} 4,697+	GAT TCA TTA AAG TTA GAT TCT TG	priFeMV^{US1} 8,744+	GCC ATT TTC AAT TTA ATA AGC TG	priFeMV^{US1} 10,297–	ACT CAT TTC TGA CAG GTG AC	P
L (1,908)	priFeMV^{776} 7,007+	CAA AGA TTC TAG CCG GTA C	priFeMV^{US1} 10,261+	TGT TTT ATG CCA TTA AGT CTA G	priFeMV^{776} 12,169–	CCC TTA GTA GTG TCA AGC ATA C	P
L (1,754)	priFeMV^{776} 7,007+	CAA AGA TTC TAG CCG GTA C	priFeMV^{776} 12,118+	ACT TAA TAA TCC CTA GAG CAG	priFeMV^{US1} 13,872–	GGA TTT GTT CTC CTC TCA TTA TC	P
L (2,159)	priFeMV^{US1}	GAG ATC CTT	priFeMV^{M252A}	GGA TGC TTA	priFeMV^{M252A}	CCA GAC AAA	Q

Amplicon (bp)	cDNA synthesis	cDNA primer sequence (5′→3′)	Forward primer	Sequence (5′→3′)	Reverse primer	Sequence (5′→3′)	Kit*
Trailer (410)	10,232+ priFeMV^{US1} 15,525+	CTG TGG ATT TAG GAT AGA GTG TGA TTA TCC ATC	13,689+ priFeMV^{US1} 15,675+	TTT ATC TGA TC CTT CAA CAG TTA GTC GAG CCC G	16,028– priAdaptor-dT17	GAA AGC TAT AGG GAC TCG AGT CGA CAT CGA TTT TTT TTT TTT TTT TT	P

*P, Phusion High-Fidelity DNA-Dependent DNA Polymerase (New England Biolabs, Ipswich, MA, USA); T, Taq DNA-Dependent DNA Polymerase (Thermo Fisher Scientific, Grand Island, NY, USA); Q, Q5 High-Fidelity DNA-Dependent DNA Polymerase (New England Biolabs).



Technical Appendix Figure 1. Detection of feline morbillivirus (FeMV) genomic RNA in urine samples and FeMV-specific antibodies in serum from domestic cats. A) Longitudinal samples from 2013 and 2015 were collected from animal 0213, total RNA was isolated, cDNA was generated by reverse transcription, and partial L gene amplicons (460 bp) were obtained using priFeMV^{US}panL (+/-). B) Generation of partial HA gene amplicons (619 bp) from cDNA produced from total RNA isolated from urine samples of animals 0213 (US1), 0382 (US2), and 0122 (US5). Feline β-actin amplicons (127 bp) were generated as positive controls, and a 1 kb Plus Ladder (Thermo Fisher Scientific, Grand Island, NY, USA) was used for size markers. Reverse transcriptase (RT)–negative controls were routinely included to demonstrate specificity and ensure that amplicons were not obtained from cross-contaminating DNA (data not shown).



Technical Appendix Figure 2. Detection of feline morbillivirus (FeMV)–specific antibodies in serum from domestic cats. Vero cells were transfected with a plasmid expressing the hemagglutinin glycoprotein of $FeMV^{US1}$ (A) or $FeMV^{US5}$ (B). Cells were fixed and permeabilized (A) or nonpermeabilized (B), and indirect immunofluorescence was performed to detect the glycoproteins in polyclonal serum (1:800 dilution) from animal 0213 (A) and animal 0122 (B). Bound antibodies were detected by using Alexa Fluor 488 goat anti-cat secondary immunoglobulin G (Thermo Fisher Scientific, Grand Island, NY, USA). Analogous to the hemagglutinin glycoprotein in other morbilliviruses, the FeMV hemagglutinin glycoprotein localized to the endoplasmic reticulum and Golgi apparatus, and the protein was exclusively detected at the outer surface membrane in nonpermeabilized cells (indicated by arrows). Nuclei were counterstained with 4',6-diamidino-2-phenylindole, and fluorescence was visualized with confocal scanning laser microscopy by using a Leica SP5 Acousto-Optical Beam Splitter system (Leica Microsystems, Inc., Buffalo Grove, IL, USA).