Contamination of live attenuated vaccines with an infectious feline endogenous retrovirus (RD-114 virus)

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Abstract Retroviruses are classified as exogenous and endogenous retroviruses according to the mode of transmission. Endogenous retroviruses (ERVs) are retroviruses which have been integrated into germ-line cells and inherited from parents to offspring. Most ERVs are inactivated by deletions and mutations; however, certain ERVs maintain their infectivity and infect the same host and new hosts as exogenous retroviruses. All domestic cats have infectious ERVs, termed RD-114 virus. Several canine and feline attenuated vaccines are manufactured using RD-114 virus-producing cell lines such as Crandell-Rees feline kidney cells; therefore, it is possible that infectious RD-114 virus contaminates live attenuated vaccines. Recently, Japanese and UK research groups found that several feline and canine vaccines were indeed contaminated with infectious RD-114 virus. This was the first incidence of contamination of ‘infectious’ ERVs in live attenuated vaccines. RD-114 virus replicates efficiently in canine cell lines and primary cells. Therefore, it is possible that RD-114 virus infects dogs following inoculation with contaminated vaccines and induces proliferative diseases and immune suppression, if it adapts to grow efficiently in dogs. In this review, we summarize the incidence of contamination of RD-114 virus in live attenuated vaccines and potential risks of infection with RD-114 virus in dogs.

Endogenous retroviruses (ERVs)

Retroviruses enter host cells via binding of the envelope proteins with the host receptor(s). After entering the cells, viral RNA is reverse-transcribed into DNA and then the DNA is integrated into the host genome to be a provirus. Viral RNA is transcribed from the provirus and the structural and enzymatic proteins of the virus are synthesized from the transcribed viral RNA.

Retroviruses are classified as exogenous and endogenous retroviruses according to the mode of transmission [14]. Usually, exogenous retroviruses infect somatic cells but not germ-line cells, and they are transmitted horizontally by infection via viral particles. On the other hand, ERVs are retroviruses which have been integrated into germ-line cells. ERVs behave like normal genes and are inherited from parents to offspring as Mendelian’s law. ERVs occupy about 10% of mammalian genomes and are mostly inactivated by deletions and mutations [14, 15, 17, 27]. However, a limited number of ERVs maintain their infectivity and infect new hosts as exogenous retroviruses [6].

Exogenous retroviruses are classified into seven genera, i.e., alpharetrovirus, betaretrovirus, gammaretrovirus, deltaretrovirus, epsilonretrovirus, spumaretrovirus and
lentivirus. ERVs are divided into at least three classes I, II and III [14]. Class I ERV is closely related to exogenous counterparts of gammaretrovirus and epsilonretrovirus. Class II and III ERVs are similar to alpharetrovirus and betaretrovirus, and spumavirus, respectively.

**Feline ERVs**

All domestic cats have an infectious ERV, termed RD-114 virus [4, 12, 32]. RD-114 viral genomes have not been detected in large felids, such as lions and pumas [4]. RD-114 virus is closely related to baboon endogenous retrovirus (BaEV) in env region, but is distantly related to BaEV in gag-pol region, and is considered to be a recombinant virus between a feline ERV, termed FeEV, in gag-pol region and BaEV in env region [47]. BaEV is also a recombinant virus between a Papio cynocephalus ERV, termed PcEV, and a simian type D virus [19]. The gag-pol regions of both RD-114 virus and BaEV are closely related to gammaretroviruses (class I ERV), and the env region is closely related to betaretroviruses (class II ERV).

**ERVs and pathogenicity**

Generally, ERVs do not induce diseases in the original hosts. However, there are several incidences where ERVs exhibit pathogenicity; for example, ERVs of AKR mice induce lymphoma in the host [31]. Recently, it was found that replication activity of mouse ERVs was resurrected in infection and induced fibrosarcoma [10, 11].

**Contamination of ERVs in human vaccines**

Mice, pigs, cats, and chickens have infectious ERVs [6, 34]. In previous studies, it was reported that MMR vaccines (measles, mumps and rubella vaccines) and yellow fever vaccines that were propagated in chicken embryos were contaminated with endogenous avian leukosis viruses (ALVs) and endogenous avian retroviruses (EAVs), which originate from chicken embryonic fibroblast substrates [16, 45]. It is unknown whether contaminated endogenous ALV and EAV are infectious ERVs, because these studies only detected the viral RNA, proteins and reverse transcriptase (RT) activities in vaccines using RT-polymerase chain reaction (PCR), immunoblotting and the RT assay, respectively (16, 45). Nevertheless, no evidence was found that these ERVs had infected humans by vaccination [16, 45].

**Contamination of animal vaccines with ‘infectious’ RD-114 virus**

Many live attenuated vaccines for animals are manufactured using feline cell lines which may produce infectious RD-114 virus (Table 1). Therefore, it is possible that infectious RD-114 virus contaminates these vaccines [24]. We developed RT-PCR and realtime RT-PCR to detect RD-114 viral RNA and the LacZ marker rescue assay to detect infectious RD-114 virus in vaccines [37, 49]. When we examined feline live attenuated vaccines purchased in Japan (Vaccines F/g1, F/h2 and F/d3) for the presence of infectious RD-114 virus by the LacZ marker rescue assay, a vaccine manufactured by one company (Vaccine F/g1) was contaminated with infectious RD-114 virus (Table 2) [25]. The Japanese regulatory authority, National Veterinary Assay Laboratory (NVAL), also confirmed this finding independently [30]. In addition, we also confirmed that three products of ‘canine’ live attenuated vaccines purchased in Japan (Vaccines C/a1, C/a2 and C/b3), manufactured using ‘feline’ cell lines, were contaminated with infectious RD-114 virus (Table 2) [25]. The titers of RD-114 viruses in the contaminated vaccines were 1,800, 1,000 and 1.8 50 % tissue culture infective dose (TCID50)/dose, respectively (Table 2). Copy numbers of RD-114 viral RNA were also estimated by real-time RT-PCR. We found that 4.5×10^7, 9.7×10^7 and 8.3×10^6 copy number/dose of RD-114 viral RNAs were present in Vaccines C/a1, C/a2 and C/b3, respectively (Table 2) (unpublished data). Another research group in the University of Glasgow also confirmed that feline and canine live attenuated vaccines purchased in the United Kingdom were contaminated with infectious RD-114 virus using immunoblot analysis and RT assay [25]. In addition, we found that two canine live
attenuated vaccines (Vaccines C/f8 and C/f9) produced using ‘non-feline’ cell lines (Table 1) were contaminated with infectious RD-114 virus (Table 2) [49]. The infectious titers of RD-114 virus in contaminated vaccines were 180 and 10,000 TCID50/dose, respectively and the copy numbers of RD-114 viral RNAs were 2.1 × 10^8 and 5.0 × 10^8 copies/dose respectively (Table 2) [49]. The NVAL also confirmed these findings independently [29].

**Possible contamination routes of RD-114 virus in live attenuated vaccines**

Several feline cell lines such as CRFK cells, MCC cells and FER cells produce infectious RD-114 virus [2, 7, 33, 38, 48]. Therefore, if the vaccine strains of feline and canine viruses are propagated in RD-114 virus-producing feline cell lines, RD-114 virus contaminates live attenuated vaccines (Fig. 1). Moreover, RD-114 virus infects and proliferates efficiently in human, canine and mink cell lines [2, 36, 49, 51]. Therefore, when seed stock viruses are contaminated with infectious RD-114 virus and the vaccines are produced using RD-114 virus-permissive cell lines, RD-114 virus may contaminate live attenuated vaccines, irrespective of the species origin of the cell lines (Fig. 1). Actually, as mentioned above, two canine live attenuated vaccines (Vaccines C/f8 and C/f9) produced using ‘non-feline’ cell lines were contaminated with infectious RD-114 virus (Table 2) [49]. These vaccines contained an attenuated canine parvovirus type 2 (CPV-2) (Table 1) and many CPV-2s have been attenuated using CRFK cells [26]. When we examined CPV-2 stock viruses in an assay laboratory of a Japanese vaccine company for the presence of infectious RD-114 virus, seven out of eighteen CPV-2 vaccine stocks were contaminated with infectious RD-114 virus [50].

### Table 1 List of cell lines for live attenuated vaccines purchased in Japan

| Virus | Vaccine ID | C/a1 | C/a2 | C/b3 | C/c4 | C/c5 | C/d6 | C/e7 | C/f8 | C/f9 |
|-------|------------|------|------|------|------|------|------|------|------|------|
| Canine distemper virus | A | A | S | S | Unknown | S | C | A | S |
| Canine adenovirus type 2 | P | P | C | C | Unknown | n.a. | C | C | C |
| Canine parvovirus | A | A | C | F (CRFK) | Unknown | F | C | M | M |
| Canine parainfluenza virus | F (CRFK) | F (CRFK) | C | C | Unknown | n.a. | C | n.a. | S |
| Canine coronavirus | F (CRFK) | F (CRFK) | F | n.a. | Unknown | n.a. | F | n.a. | n.a. |

### Table 2 Contamination of live attenuated vaccines purchased in Japan with RD-114 virus

| Vaccine ID | RNA copy number/dose | TCID50/dose |
|------------|----------------------|-------------|
| C/a1 | 4.5 × 10^7 | 1,800 |
| C/a2 | 2.8-9.7 × 10^7 | 1,000 |
| C/b3 | 8.3 × 10^6 | 1.8 |
| C/c4 | BGL | n.t. |
| C/c5 | BGL | n.t. |
| C/d6 | BGL | n.t. |
| C/e7 | BGL | n.t. |
| C/f8 | 2.1 × 10^8 | 180 |
| C/f9 | 5.0 × 10^8 | 10,000 |
| F/g1 | BGL | 1.8 |
| F/h2 | BGL | n.t. |
| F/d3 | BGL | n.t. |

BGL, background level; n.t., not tested

* Codes used to anonymize the vaccines used. Details of codes are described in Table 1

* Copy numbers of RD-114 viral RNA were measured by real-time RT-PCR

* Infectious titers of RD-114 virus were measured by LacZ marker rescue assay and expressed as 50 % tissue culture infectious dose (TCID50)
Potential risks of infection with RD-114 virus in dogs

RD-114 virus efficiently infects canine cell lines and primary cells [36, 51]. It is important to identify viral receptor(s) in predicting viral tropisms and pathogenicity. In human cell lines, it is found that the receptor for RD-114 virus is a sodium-dependent neutral amino acid transporter, termed ASCT [35, 40]. Humans have two types of ASCT molecules, termed ASCT1 and ASCT2 [1, 46]. The homology between ASCT1 and ASCT2 is about 57 % [1, 46]. Both human ASCT1 and ASCT2 function as RD-114 receptor, but the virus utilizes ASCT2 more efficiently than ASCT1 [20]. In humans, ASCT1 is ubiquitously expressed in tissues [1], whereas the expression of ASCT2 is limited in various tissues, and the expression level of ASCT2 also varies among tissues [13, 46]. Recently, we identified canine ASCT1 and ASCT2 as RD-114 virus receptors [50].

RD-114 may infect a variety of tissues in dogs, although the distribution of ASCT1 and ASCT2 in dogs is unknown at present. Actually, in a previous study, X-linked severe combined immunodeficiency was corrected in dogs by intravenous injection of concentrated RD-114-pseudotyped retrovirus vector encoding the interleukin-2 receptor γ chain [44]. These data indicate that RD-114 virus can infect bone marrow cells in dogs. However, Narushima and coworkers [28] at NVAL reported that RD-114 proviral DNA was not found in dogs inoculated with RD-114 virus subcutaneously. Unfortunately, they only investigated RD-114 provirus in quite limited tissues (lymph nodes, spleen and bone marrow) and peripheral blood, and the sensitivity of the one-step PCR to detect RD-114 proviral DNA was obscure. Recently, we also investigated whether RD-114 virus infects dogs by experimental infection. When four dogs were inoculated with high titer of RD-114 virus, RD-114 proviral DNA was detected in blood cells, mesenteric lymph nodes, spleens and testes (Yoshikawa et al., unpublished). In addition, anti-RD-114 antibodies and neutralizing antibodies were detected in the inoculated dogs (Yoshikawa et al., unpublished).

In human cells, human ASCT2 is a functional receptor for pathogenic retroviruses, such as simian retrovirus (SRV) 1, 2, 3, 4 and 5, avian reticuloendotheliosis virus, and duck spleen necrosis virus [35]. It is known that SRV-1, SRV-2 and SRV-3 induce a fatal immunodeficiency in some macaque species (Celebes and rhesus macaques) [21–23]. In a previous study, we confirmed that both SRV-2 and SRV-3 can utilize canine ASCT2 as a receptor ([51], unpublished data). Intriguingly, RD-114 virus has an immunosuppressive domain in transmembrane envelope protein and the amino acid sequence (LQNRRGLDLTDQGGI) of the domain is identical with that of SRV-3 [5, 8]. Therefore, RD-114 virus may induce immunosuppression as well as proliferative diseases such as leukemia/lymphoma, if it adapts to replicate efficiently in dogs.

Concluding remarks

Japanese and UK research groups and the NVAL confirmed that several feline and canine vaccines were
contaminated with infectious RD-114 virus. This was the first incidence of contamination of ‘infectious’ ERVs in live attenuated vaccines. Quite importantly, RD-114 virus grew efficiently in cells of dogs which are vaccinees. In future studies, it is necessary to examine the expression profiles of canine ASCT1 and ASCT2 in dogs and determine the principal target of RD-114 virus by experimental infection with high doses of RD-114 virus. Because RD-114 virus does not have any oncogenes, RD-114 virus does not induce acute/subacute proliferative diseases such as fibrosarcoma in dogs. Therefore, after experimental infection of dogs with RD-114 virus, it is necessary to monitor infected dogs for a long period. Even if RD-114 virus does not proliferate in dogs after experimental infection, we cannot dismiss the risk of infection with RD-114 virus in dogs completely. Canine attenuated vaccines are inoculated in several million dogs per year around the world, and RD-114 virus may mutate and acquire more infectivity/productivity in dogs. Therefore, although the risks posed by RD-114 virus are still unclear at present, it is desirable to develop the means to produce RD-114 virus-free vaccines and exclude RD-114 virus-contaminated vaccines.

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References

1. Arriza JL, Kavanaugh MP, Fairman WA, Wu YN, Murdoch GH, North RA, Amara SG (1993) Cloning and expression of a human neutral amino acid transporter with structural similarity to the glutamate transporter gene family. J Biol Chem 268:15329–15332
2. Baumann JG, Günsburg WH, Salmons B (1998) CrFK feline kidney cells produce an RD114-like endogenous virus that can package murine leukemia virus-based vectors. J Virol 72:7685–7687
3. Benveniste RE, Callahan R, Sherr CJ, Chapman V, Todaro GJ (1977) Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: conservation of virogene sequences in related rodent species. J Virol 21:849–862
4. Benveniste RE, Todaro GJ (1974) Evolution of C-type viral genes: inheritance of exogenously acquired viral genes. Nature 252:456–459
5. Blaise S, Mangeney M, Heidmann T (2001) The envelope of Mason-Pfizer monkey virus has immunosuppressive properties. J Gen Virol 82:1597–1600
6. Boeke JD, Stoye JP (1997) Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In: Coffin JM, Hughes SH, Varmus HE (eds) Retroviruses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 343–435
7. Cheney CM, Rojko JL, Kociba GJ, Wellman ML, Di Bartola SP, Rezanka LJ, Forman L, Mathes LE (1990) A feline large granular lymphoma and its derived cell line. In Vitro Cell Dev Biol 26:455–463
8. Cianciolo GJ, Copeland TD, Orozlan S, Snyderman R (1985) Inhibition of lymphocyte proliferation by a synthetic peptide homologous to retroviral envelope proteins. Science 230:453–455
9. Cui J, Tachedjian G, Tachedjian M, Holmes EC, Zhang S, Wang LF (2012) Identification of diverse groups of endogenous gammaretroviruses in mega- and microbats. J Gen Virol 93:2037–2045
10. Denner J (2007) Transspecies transmissions of retroviruses: new cases. Virology 369:229–233
11. Fiebig U, Hartmann MG, Bannert N, Kurth R, Denner J (2006) Transspecies transmission of the endogenous koala retrovirus. J Virol 80:5651–5654
12. Fischinger PJ, Peebles PT, Nomura S, Haapala DK (1973) Isolation of RD-114-like oncornavirus from a cat cell line. J Virol 11:978–985
13. Green BJ, Lee CS, Rasko JE (2004) Biodistribution of the RD114/mammalian type D retrovirus receptor, RDR. J Gene Med 6:249–259
14. Gifford R, Tristem M (2003) The evolution, distribution and diversity of endogenous retroviruses. Virus Genes 26:291–315
15. Griffiths DJ (2001) Endogenous retroviruses and the human genome sequence. Gen Biol 2:1017.1–1017.5
16. Hussain AI, Johnson JA, Da Silva Freire M, Heneine W (2003) Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigation of transmission to vaccine recipients. J Virol 77:1105–1111
17. International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921
18. Lieber MM, Sherr CJ, Todaro GJ, Benveniste RE, Callahan R, Coon HG (1975) Isolation from the Asian mouse Mus caroli of an endogenous type C virus related to infectious primate type C viruses. Proc Natl Acad Sci USA 72:2315–2319
19. Mang R, Goudsmit J, van der Kuyl AC (1999) Novel endogenous type C retroviruses in baboons: complete sequence, providing evidence for baboon endogenous virus gag-pol ancestry. J Virol 73:7021–7026
20. Marin M, Lavillette D, Kelly SM, Kabat D (2003) N-linked glycosylation and sequence changes in a critical negative control region of the ASCT1 and ASCT2 neutral amino acid transporters determine their retroviral receptor functions. J Virol 77:2936–2945
21. Marx PA, Bryant ML, Osborn KG, Maul DH, Lerche NW, Lowenstein LJ, Kugle JD, Zaiess CP, Henrickson RV, Shiigi SM, Wilson BJ, Malley A, Olson LC, McNulty WP, Arthur LO, Gilden RV, Barker CS, Hunter E, Munn RJ, Heidecker G, Gardner MB (1985) Isolation of a new serotype of simian acquired immune deficiency syndrome type D retrovirus from Celebes black macaques (Macaca nigra) with immune deficiency and retroperitoneal fibromatosis. J Virol 56:571–578
22. Marx PA, Maul DH, Osborn KG, Lerche NW, Moody P, Lowenstein LJ, Henrickson RV, Arthur LO, Gilden RV, Gravel LV, London WT, Seever JL, Levy AJ, Munn JR, Gardner MB (1984) Simian AIDS: isolation of a type D retrovirus and transmission of the disease. Science 223:1083–1086
23. Marx PA, Pedersen NC, Lerche NW, Osborn KG, Lowenstein LJ, Lackner AA, Maul DH, Kwang HS, Kugle JD, Zaiess CP, Sharpe V, Spinner AP, Allison AC, Gardner MB (1986) Prevention of simian acquired immune deficiency syndrome with a formalin-inactivated type D retrovirus vaccine. J Virol 60:431–435
24. Miyazawa T (2010) Endogenous retroviruses as potential hazards for vaccines. Biologicals 38:371–376
25. Miyazawa T, Yoshikawa R, Golder M, Okada M, Stewart H, Palmarini M (2010) Isolation of an infectious endogenous
retrovirus in a proportion of live attenuated vaccines for pets. J Virol 84:3690–3694
26. Mochizuki M, San Gabriel MC, Nakatani H, Yoshida M, Haramawa R (1993) Comparison of polymerase chain reaction with virus isolation and haemagglutination assays for the detection of canine parvoviruses in faecal specimens. Res Vet Sci 55:60–63
27. Mouse Genome Sequencing Consortium (2002) Initial sequencing and comparative analysis of the mouse genome. Nature 420:860–921
28. Narushima R, Horiiuchi N, Usui T, Ogawa T, Takahashi T, Shimazaki T (2011) Experimental infection of dogs with a feline endogenous retrovirus RD-114. Acta Vet Scand 53:3
29. Narushima R, Shimazaki T, Takahashi T (2011) Development of a real-time reverse-transcription-PCR method for detection of RD114 virus in canine vaccines. Biologicals 39:89–93
30. Narusima R, Usui T, Ogawa T, Shimazaki T (2010) Detection of infectious RD114 virus in feline live multivalent vaccines and provisional safety evaluation in cats. J Jpn Vet Med Assoc 63:630–633 [in Japanese with English summary]
31. Nowinski RC, Hays EF (1978) Oncogenicity of AKR endogenous leukemia viruses. J Virol 27:13–18
32. Okabe H, Gilden RV, Hatanaka M (1973) RD 114 virus-specific sequences in feline cellular RNA: detection and characterization. J Virol 12:984–994
33. Okada M, Yoshikawa R, Shojima T, Baba K, Miyazawa T (2011) Susceptibility and production of a feline endogenous retrovirus (RD-114 virus) in various feline cell lines. Virus Res 155:268–273
34. Patience C, Takeuchi Y, Weiss RA (1997) Infection of human cells by an endogenous retrovirus of pigs. Nat Med 3:282–286
35. Rasko JE, Battini JL, Gottschalk RJ, Mazo I, Miller AD (1999) The RD114/simian type D retrovirus receptor is a neutral amino acid transporter. Proc Natl Acad Sci USA 96:2129–2134
36. Roth MG, Srinivas RV, Compans RW (1983) Basolateral maturation of retroviruses in polarized epithelial cells. J Virol 45:1065–1073
37. Sakaguchi S, Okada M, Shojima T, Baba K, Miyazawa T (2008) Establishment of a LacZ marker rescue assay to detect infectious RD114 virus. J Vet Med Sci 70:785–790
38. Shimode S, Yoshikawa R, Hoshino S, Nakaya Y, Sakaguchi S, Kobayashi T, Miyazawa T (2012) Sequence comparison of three infectious molecular clones of RD-114 virus. Virus Genes 45:393–397
39. Simmons GS, Young PR, Hanger JJ, Jones K, Clarke D, McKee JJ, Meers J (2012) Prevalence of koala retrovirus in geographically diverse populations in Australia. Aust Vet J 90:404–409
40. Tailor CS, Nouri A, Zhao Y, Takeuchi Y, Kabat D (1999) A sodium-dependent neutral-amino-acid transporter mediates infections of feline and baboon endogenous retroviruses and simian type D retroviruses. J Virol 73:4470–4474
41. Tarlinton RE, Meers J, Young PR (2006) Retroviral invasion of the koala genome. Nature 442:79–81
42. Tarlinton R, Meers J, Hanger J, Young P (2005) Real-time reverse transcriptase PCR for the endogenous koala retrovirus reveals an association between plasma viral load and neoplastic disease in koalas. J Gen Virol 86:783–787
43. Tarlinton R, Meers J, Young P (2008) Biology and evolution of the endogenous koala retrovirus. Cell Mol Life Sci 65:3413–3421
44. Ting-De Ravin SS, Kennedy DR, Naumann M, Kennedy JS, Choi U, Hartnett BJ, Linton GF, Whiting-Theobald NL, Moore PF, Verna W, Malech HL, Felsburg PJ (2006) Correction of canine X-linked severe combined immunodeficiency by in vivo retroviral gene therapy. Blood 107:3091–3097
45. Tsang SX, Switzer WM, Shanmugam V, Johnson JA, Goldsmith C, Wright A, Fadly A, Thea D, Jaffe H, Folks TM, Heneine W (1999) Evidence of avian leukemia virus subgroup E and endogenous avian virus in mesaeles and mumps vaccines derived from chicken cells: investigation of transmission to vaccine recipients. J Virol 73:5843–5851
46. Utsunomiya-Tate N, Endou H, Kanai Y (1996) Cloning and functional characterization of a system ASC-like Na+-dependent neutral amino acid transporter. J Biol Chem 271:14883–14890
47. Van der Kuyl AC, Dekker JT, Goudsmitt J (1999) Discovery of a new endogenous type C retrovirus (FcEV) in cats: evidence for RD-114 being an FcEV<sup>env</sup>BaEV<sup>pol</sup>/BaEV endogenous virus BaEV<sup>env</sup> recombinant. J Virol 73:7994–8002
48. Yoshikawa R, Sato E, Iigunishi T, Miyazawa T (2010) Characterization of RD-114 virus isolated from a commercial canine vaccine manufactured using CRFK cells. J Clin Microbiol 48:3366–3369
49. Yoshikawa R, Sato E, Miyazawa T (2009) Contamination of infectious RD-114 virus in vaccines produced using non-feline cell lines. Biologicals 39:33–37
50. Yoshikawa R, Sato E, Miyazawa T (2012) Presence of infectious RD-114 virus in a proportion of canine parvovirus isolates. J Vet Med Sci 74:347–350
51. Yoshikawa R, Yasuda J, Kobayashi T, Miyazawa T (2012) Canine ASCT1 and ASCT2 are functional receptors for RD-114 virus in dogs. J Gen Virol 93:603–607
52. Young GR, Eksmond U, Salcedo R, Alexopoulou L, Stoye JP, Kassiotis G (2012) Resurrection of endogenous retroviruses in antibody-deficient mice. Nature 491:774–778
53. Yu P, Lübben W, Slomka H, Gebler J, Konert M, Cai C, Wiegand L, Kaufmann A, Nain M, Quintanilla-Martinez L, Bettio S, Schnierle B, Kolesnikova L, Becker S, Schnare M, Bauer S (2012) Nucleic acid-sensing Toll-like receptors are essential for the control of endogenous retrovirus viremia and ERV-induced tumors. Immunity 37:867–879