Biologicals 39 (2011) 89–93

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Development of a real-time reverse-transcription-PCR method for detection of RD114 virus in canine vaccines

Rie Narushima [a,*], Tomoaki Shimazaki [b], Toshio Takahashi [c]

[a] National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries, 1-15-1 Tokura, Kokubunji, Tokyo 185-8511, Japan
[b] Animal Health Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries, 1-2-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8950, Japan
[c] Department of Veterinary Science, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino-shi, Tokyo 180-8602, Japan

ARTICLE INFO

Article history:
Received 9 June 2010
Received in revised form
9 December 2010
Accepted 18 January 2011

Keywords:
Endogenous retrovirus
RD114 virus
Culturing cells of feline origin
Canine live vaccines
Real-time reverse-transcription-PCR

ABSTRACT

It is known that certain feline cell lines, such as the Crandell-Rees feline kidney cell, produce an RD-114-like virus. As a feline endogenous retrovirus, RD114 virus, exists in the genome of all cats, it can be assumed that contamination with the virus in feline and canine live vaccines manufactured by culturing cells of feline origin occurs.

To detect an infectious RD114 virus *in vitro*, a LacZ marker rescue assay has recently been established. In feline and canine live vaccines approved in Japan, feline cell lines are widely used to produce vaccines, especially those containing canine parvovirus components. The LacZ marker rescue assay detects infectious viral particles, but the real-time reverse-transcription-PCR detects both infectious and defective viruses. The canine live vaccines manufactured in cells of feline origin showed positive results for the *env* gene by the real-time reverse-transcription-PCR, including all of the 8 vaccines produced in feline cell lines that were negative in the LacZ marker rescue assay. In conclusion, the present investigation suggests that the newly developed method has the advantages of shorter time requirements and can be applied as a valuable screening method to detect RD114 viral RNA in vaccines.

1. Introduction

Endogenous retroviruses (ERVs) are retroviruses that have been integrated into germ line cells [1]. At least two ERVs, endogenous FeLV and RD114 virus, are present in cat genome [2]. The RD114 virus is a recombinant comprising a *gag-pol* gene from a gammaretrovirus and an *env* gene from a betaretrovirus [3]. Infectious RD114 virus and RD114-like virus are known to be produced from several feline cell lines, such as CRFK cells derived from feline kidney cells [4], MCC cells derived from feline large granular lymphoma [5], and FER cells derived from feline fetal fibroblast cells [2]. ERVs are not generally pathogenic in their original hosts, although some ERVs induce diseases for new host [6,7]. To detect infectious RD114 virus, a LacZ marker rescue assay has recently been established [8]. Recently Miyazawa et al. [9] found that certain live attenuated vaccines for cats and dogs contained infectious RD114 viruses by means of this method. Most feline and canine live vaccines approved in Japan are produced in feline cell lines, especially those containing canine parvovirus components.

The LacZ marker rescue assay detects infectious viral particles, but the real-time reverse-transcription-PCR detects both infectious and defective viruses. The real-time reverse-transcription-PCR developed can give rapid, sensitive and specific results for the detection of virus infection [10,11]. To detect RD114 virus, several assays [8,12] have been developed; however there has been no report on real-time reverse-transcription-PCR. In addition, the newly developed technique employing minor groove binder (MGB) probes can enlarge the difference in Tm values between the target template and other non-specific nucleic acid amplification products, to obtain high specificity [13]. The MGB probe can also improve hybridization stability and reduce the fluorescence background to obtain higher sensitivity [13]. Therefore, we attempted to develop a real-time reverse-transcription-PCR method to detect RD114 virus in canine live vaccines.

2. Materials and methods

2.1. RNA extraction from vaccines and reverse-transcription reaction

The principal canine live vaccines approved in Japan were submitted in present investigation prior to their expiration. Viral

* Corresponding author. Tel.: +81 42 321 1841; fax: +81 42 321 1769.
E-mail address: narusima@nval.maff.go.jp (R. Narushima).

1045-1056/$36.00
doi:10.1016/j.biologicals.2011.01.004

RNA was extracted from each vaccine using spin columns (QIAamp Viral RNA Mini Kit™, Qiagen, CA, USA) as described by the manufacturer. A final volume of 60 μl of viral RNA was extracted from each vaccine. RNA templates (12 μl) were incubated with 4 μl of a mixture consisting of 2 μl gDNA wipeout buffer and 2 μl RNase free water at 42 °C for 2 min to remove contaminating genomic DNA. Following the incubation, cDNA was synthesized using reverse transcriptase (RT) (Quantitect Reverse Transcription™: Qiagen, CA, USA) at 42 °C for 30 min. The reaction mixture (total 6 μl) consisted of 0.5 μl antisense primer, 5′-gtaccggataagacttggac-3′ (10 pmol/μl), 0.5 μl RNase free H_2O, 4 μl 5 × RT buffer and 1 μl RT was added to DNase treated RNA templates. DNase and RT were inactivated at 95 °C for 3min.

2.2. Primers and probe design

Sequence data of the *env* gene of the RD114 virus was obtained from GenBank (accession No; AB559882). The specificity of the primers and probe for the RD114 virus used in this protocol was evaluated by performing a BLAST search, and no significant matches were found to sequences other than that for the RD114 virus. Specific primers and the TaqMan-MGB fluorescent probe were designed with Primer Express Software Version 3.0 (Applied Biosystems, Tokyo, Japan). The probe was labeled with the fluorescent reporter FAM at the 5′ end; with the non-fluorescent quencher at the 3′ end. The sequences of the primers and probe were as follows: sense primer, 5′-ccattcctgccattgatcatta-3′; antisense primer, 5′-ggtgattcccagtccagctagt-3′; probe, 5′-FAM-tacatagacctaaacgagctgt-3′MGB. The amplified fragment was 83 bp in length.

2.3. Real-time PCR

The reaction was conducted in a total volume of 20 μl, consisting of 10 μl master mix (TaqMan Gene Expression Master Mix™, Applied Biosystems, Tokyo, Japan), 1.8 μl forward primer (10 pmol/μl), 1.8 μl reverse primer (10 pmol/μl), 1.0 μl TaqMan probe(5 pmol/μl), 4.4 μl distilled water and 1 μl each template. Real-time PCR was performed in a 96-well format using the StepOnePlus™ system (Applied Biosystems, Tokyo, Japan) and employed the following thermal cycler

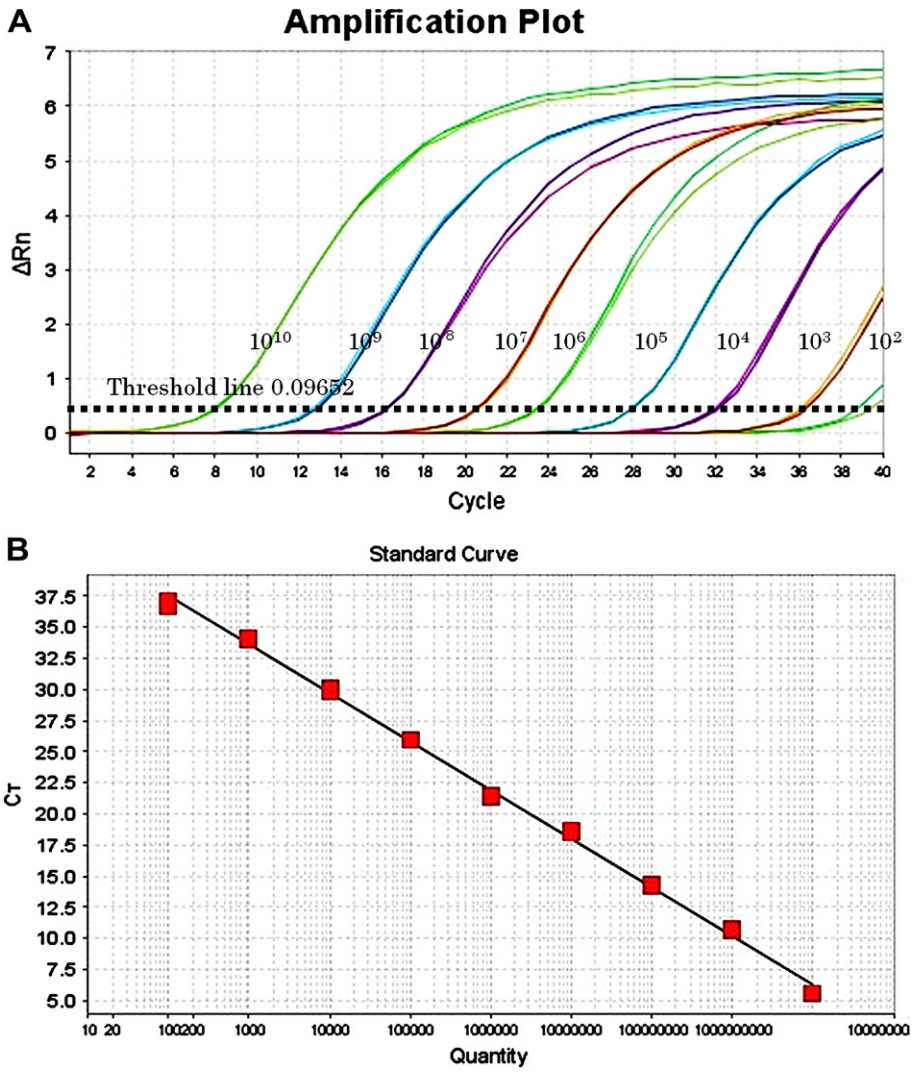

Fig. 1. Quantitative analysis. A – Amplification profile for detection of the *env* gene of the RD114 virus. From left to right, the amplification contained 10^{10}, 10^9, 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2 and 10^1 copies of cDNA, respectively. The threshold for detection was determined as 10^2 copies with the Ct value under 36.8. B – Standard curve created by analysis of known copies of the target *env* gene. Reactions with copy numbers of target gene from 10^{10} to 10^1 were used to create the standard curve. The standard curve was plotted by the log concentration of copy numbers against Ct values ($R^2 = 0.998$).