Journal of Virological Methods 196 (2014) 1–6

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Use of recombinant nucleocapsid proteins for serological diagnosis of feline coronavirus infection by three immunochromatographic tests

Tomomi Takano, Yuka Ishihara, Masafumi Matsuoka, Shoko Yokota, Yukie Matsuoka-Kobayashi, Tomoyoshi Doki, Tsutomu Hohdatsu *

Laboratory of Veterinary Infectious Disease, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

Article history:
Received 25 July 2013
Received in revised form
24 September 2013
Accepted 1 October 2013
Available online 26 October 2013

Keywords:
Feline infectious peritonitis
Feline coronavirus
Serological test
Immunochromatographic test

A B S T R A C T

Three types of immunochromatographic assays (ICAs) were designed to detect anti-feline coronavirus (FCoV) antibodies. Recombinant FCoV nucleocapsid protein (rNP) was used as a conjugate or test line in all 3 ICA kits ($_{CJ}$IgG/$_{T}$NP, $_{CJ}$NP/$_{T}$NP, and $_{CJ}$NP/$_{T}$PA). All three ICA kits were capable of detecting anti-FCoV antibodies; however, non-specific positive reactions of anti-FCoV antibody-negative plasma samples with the test line were observed in 2 ICA kits ($_{CJ}$IgG/$_{T}$NP and $_{CJ}$NP/$_{T}$NP), in which rNP was used as the test line. On the other hand, the specific detection of anti-FCoV antibodies was possible in all plasma, serum, whole blood, and ascitic fluid samples using the ICA kit with protein A blotted as the test line ($_{CJ}$NP/$_{T}$PA). In addition, the specificity and sensitivity of ICA ($_{CJ}$NP/$_{T}$PA) were equivalent to those of the reference ELISA. The development of simple antibody test methods using the principle of ICA ($_{CJ}$NP/$_{T}$PA) for other coronavirus and feline viral infections is expected in the future.

1. Introduction

Feline infectious peritonitis virus (FIPV), a feline coronavirus (FCoV) of the family *Coronaviridae*, causes a fatal disease called FIP in wild and domestic cat species. FCoV is mainly composed of nucleocapsid (N) proteins, membrane proteins (M), and peplomer spike (S) proteins, and has been classified into serotypes I and II according to the amino acid sequence of its S protein (Hohdatsu et al., 1991a; Motokawa et al., 1995). Both serotypes consist of two biotypes: FIPV and feline enteric coronavirus (FECV). FECV infection is asymptomatic in cats, whereas FIPV infection has been shown to cause FIP (Pedersen, 2009). FIPV has been proposed to arise from FECV due to a mutation (Brown, 2011; Chang et al., 2010, 2012); however, the exact mutation and inducing factors have not yet been clarified. Therefore, no reliable method has been established to distinguish FIPV and FECV.

The antemortem diagnosis of FIP is extremely difficult (Addie et al., 2009). Although exudate can be used to diagnose FIP in cats, accompanied by ascites and pleural effusion, not all cats with FIP retain exudate. Therefore, a biopsy is necessary for the antemortem diagnosis of FIP without exudate retention. Tru-cut and fine needle biopsies are minimally invasive, highly specific diagnostic methods; however, their sensitivity is low (Giordano et al., 2005). FIP has typically been diagnosed comprehensively based on the clinical condition, hematological profile, and results of FCoV genomic RNA and FCoV-antibody measurements in cats suspected of FIP (Addie et al., 2009). An indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic assay (ICA) are currently used to measure FCoV-antibodies. IFA and ELISA are highly sensitive and specific, but are also cumbersome, expensive, and time-consuming. ICA is a simple antibody detection method, the results of which can be rapidly obtained at a low cost. However, the sensitivity of commercially available ICA test kits to detect the FCoV antibody was shown to be low (Meli et al., 2013).

The human coronavirus (HCoV) N protein is attracting attention as an important target of antibody test methods because of its high antibody reactivity (Severance et al., 2008). Several antibody-binding liner epitopes were shown to be present in the FCoV N protein, and were common to type I and II FCoV (Satoh et al., 2011). Based on these findings, it is assumed that FCoV antibodies can be detected with high sensitivity using the FCoV N protein regardless of serotypes.

Three types of ICA test kits were prepared using the recombinant N protein of FCoV, and differences in their sensitivity and specificity for FCoV antibodies were investigated.

2. Materials and methods

2.1. Recombinant N protein (rNP)

A cDNA segment representing the N protein of the type I FIPV KU-2 strain was ligated into pGEX4T-1 (GE Healthcare, NJ,

* Corresponding author. Tel.: +81 176 23 4371; fax: +81 176 23 8703.
E-mail address: hohdatsu@vmas.kitasato-u.ac.jp (T. Hohdatsu).

0166-0934/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jviromet.2013.10.014

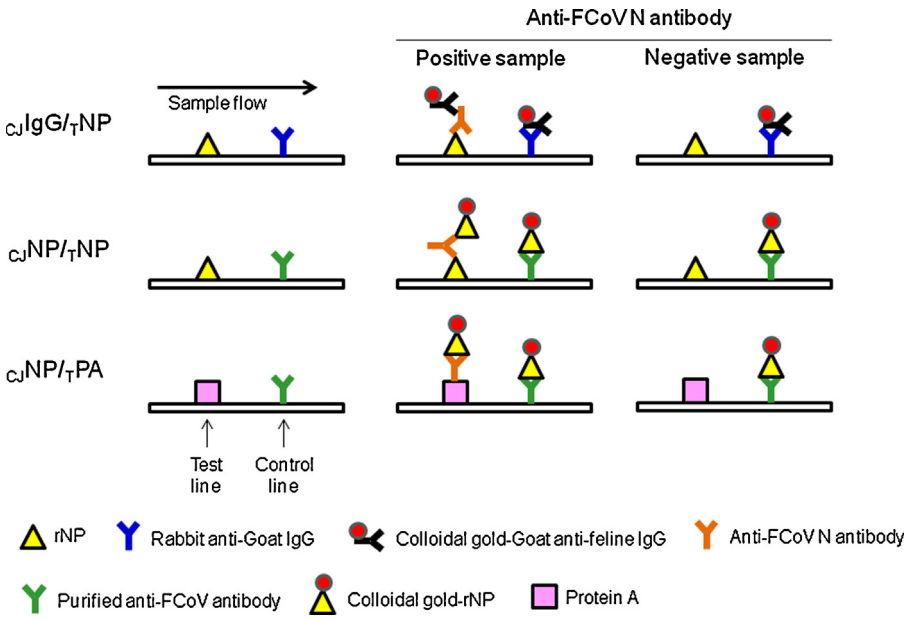

Fig. 1. 3 schematic diagrams of the 3 types of immunochromatographic tests.

USA), and transfected into *Escherichia coli* strain BL-21. Expression of the glutathione-*S*-transferase (GST) fusion protein was examined using the GST gene fusion system following the manufacturer's instructions. rNP was purified by affinity chromatography on glutathione-Sepharose 4B (GE Healthcare, NJ, USA).

2.2. Monoclonal antibodies (MAbs)

MAbs E22-2 (IgG1) and F19-1 (IgG1) were used as hybridoma cell culture supernatants. These hybridoma cells were previously established (Hohdatsu et al., 1991b). MAb E22-2 was shown to react with the N protein of FCoV, while MAb F19-1 reacts with the M protein of FCoV (Hohdatsu et al., 1991b).

2.3. Western blotting

rNP was separated employing 12% SDS–PAGE and transferred to a nitrocellulose membrane. A standard protein marker (Precision Plus Protein Standards) was purchased from Bio-Rad (Hercules, CA, USA). The membrane was blocked with 5% non-fat dry milk powder in TBST (20 mM Tris–HCl, 150 mM sodium chloride, and 0.05%Tween-20, pH 8.0) for 1 h at 37 °C, incubated for 1 h at 37 °C with MAb F19-1 or E22-2, and then incubated with peroxidase-conjugated goat anti-mouse IgG (MP Biomedicals, LLC-Cappel products, CA, USA) for 1 h at 37 °C. It was then visualized in the substrate for 10 min.

2.4. Sample specimens

Plasma, serum, and whole blood samples were collected from control uninfected specific pathogen-free (SPF) cats inoculated orally with FIPV. Twenty-three cats were inoculated with the type I FIPV KU-2 strain. Twenty-four cats were inoculated with the type II FIPV 79-1146 strain. Samples from 37 control uninfected SPF cats were used as negative controls.

2.5. Preparation of the ICA test strip

The ICA test strip consisted of three main components: a sample pad (C083 Cellulose Fiber Sample Pad Strips, Millipore Corporation, MA, USA), nitrocellulose membrane (Hi-Flow Plus 240 Membrane Cards, Millipore Corporation, MA, USA), and absorbent pad (the same one as the sample pad). Three types of ICA test strips were prepared in this study (Fig. 1). Preparation methods for the ICA strips were as follows:

ICA (conjugate: IgG/test line: NP) [ICA ($_{CJ}$IgG/$_{T}$NP)]: purified rNP was dispensed on the test line of the nitrocellulose membrane using a brush. Rabbit anti-goat IgG (Bethyl Laboratories, Inc., TX, USA) was dispensed on the control line of the nitrocellulose membrane using a brush.

ICA (conjugate: NP/test line: NP) [ICA ($_{CJ}$NP/$_{T}$NP)]: Purified rNP was dispensed on the test line of the nitrocellulose membrane using a brush. Affinity-purified cat anti-FCoV IgG (prepared by the laboratory of the present study) was dispensed on the control line of the nitrocellulose membrane using a brush.

ICA (conjugate: NP/test line: PA) [ICA ($_{CJ}$NP/$_{T}$PA)]: Protein A (Sigma–Aldrich, MO, USA) was dispensed on the test line of the nitrocellulose membrane using a brush. Affinity-purified cat anti-FCoV IgG was dispensed on the control line of the nitrocellulose membrane using a brush.

2.6. Preparation of colloidal gold-labeled goat anti-cat-IgG

Goat anti-cat-IgG (MP Biomedicals, Tokyo, Japan) was diluted with 20 mM Borax (20 mM sodium tetraborate containing 1% bovine serum albumin (BSA) and 0.1% sodium azide) to 0.5 mg/ml, and 40 μl of this dilution was added to 1 ml of colloidal gold solution (40 nm; BBI Solutions, Cardiff, UK). After stirring well, the mixture was left standing for 30 min at room temperature, followed by the addition of 100 μl of 20 mM Borax containing 10% BSA, following which the mixture was once again left standing for 30 min at room temperature. After centrifugation at 22,000 × *g* for 10 min, the precipitate was suspended in 0.5 ml of preserving solution (1% (w/v) BSA, 0.1% (w/v) sodium azide, and 150 mM sodium chloride in 20 mM Tris–HCl buffer, pH 8.0). This suspension (colloidal gold-labeled goat anti-cat-IgG) was used as a conjugate in ICA ($_{CJ}$IgG/$_{T}$NP).

2.7. Preparation of colloidal gold-labeled rNP

rNP (0.5 mg/ml) was diluted with PBS to 0.5 mg/ml, and 30 μl of this dilution was added to 1 ml of colloidal gold solution. After