Comparative Immunology, Microbiology and Infectious Diseases xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Determining the feline immunodeficiency virus (FIV) status of FIV-vaccinated cats using point-of-care antibody kits

Mark E. Westman[a,*], Richard Malik[b], Evelyn Hall[a], Paul A. Sheehy[a], Jacqueline M. Norris[a,*]

[a] Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia
[b] Centre for Veterinary Education, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 28 July 2015
Accepted 29 July 2015

Keywords:
Feline immunodeficiency virus
FIV diagnosis
FIV vaccination
Serology
Immunochromatography
ELISA
PCR
Cats

ABSTRACT

This study challenges the commonly held view that the feline immunodeficiency virus (FIV) infection status of FIV-vaccinated cats cannot be determined using point-of-care antibody test kits due to indistinguishable antibody production in FIV-vaccinated and naturally FIV-infected cats. The performance of three commercially available point-of-care antibody test kits was compared in a mixed population of FIV-vaccinated ($n = 119$) and FIV-unvaccinated ($n = 239$) cats in Australia. FIV infection status was assigned by considering the results of all antibody kits in concert with results from a commercially available PCR assay (FIV RealPCR™). Two lateral flow immunochromatography test kits (Witness FeLV/FIV; Anigen Rapid FIV/FeLV) had excellent overall sensitivity (100%; 100%) and specificity (98%; 100%) and could discern the true FIV infection status of cats, irrespective of FIV vaccination history. The lateral flow ELISA test kit (SNAP FIV/FeLV Combo) could not determine if antibodies detected were due to previous FIV vaccination, natural FIV infection, or both. The sensitivity and specificity of FIV RealPCR™ for detection of viral and proviral nucleic acid was 92% and 99%, respectively. These results will potentially change the way veterinary practitioners screen for FIV in jurisdictions where FIV vaccination is practiced, especially in shelter scenarios where the feasibility of mass screening is impacted by the cost of testing.

1. Introduction

Feline immunodeficiency virus (FIV) is a retrovirus of the genus *Lentivirus*. It was discovered in 1986 following investigation of an immunodeficiency syndrome in a household of cats in California, USA [1] and shown subsequently to have worldwide distribution. Healthy client-owned cat populations have been reported with infection rates of approximately 3% in Germany [2] and the USA [3], 6% in Canada [4] and the United Kingdom [5], 8% in Australia [6], 10% in New Zealand [7] and 23% in Japan [8]. The prevalence of FIV infection is higher in entire male cats, castrated male cats and feral cats compared to the general client-owned domestic cat population [3,6].

The FIV genome is comprised of three main structural genes, *gag*, *pol* and *env*, which encode internal structural proteins, viral enzymes and envelope glycoproteins, respectively. Six distinct FIV subtypes (A to F) have been identified based on genetic diversity in the variable V3–5 region of the *env* gene [9,10], while an additional subtype has been detected in New Zealand cats [11]. Subtypes A, B and C are most commonly encountered worldwide [12], with subtype A predominant in Australia [9,13]. Nucleotide sequence may vary up to 15% within a subtype and up to 38% between subtypes [14,15]. Subtyping of FIV infections in each geographic area is important as the sole commercially available FIV vaccine contains only subtypes A and D[1] and heterologous challenge may lower vaccine effectiveness [16,17], although subtyping alone appears insufficient to predict vaccine performance [18].

Regardless of the FIV subtype, point-of-care testing to identify antibodies directed against FIV has been the mainstay of diagnostic testing for over 20 years, supplemented by western blot analysis and virus isolation in research settings. Point-of-care test kits are inexpensive, easy to use and reliably diagnose FIV infection in FIV-unvaccinated cats [19]. There is variation between commercially available antibody test kits in the methodology and target viral antigen for antibody detection. SNAP FIV/FeLV Combo[2] is a lateral

* Corresponding authors.
E-mail addresses: mark.westman@sydney.edu.au (M.E. Westman), jacqui.norris@sydney.edu.au (J.M. Norris).

[1] Fel-O-Vax® FIV, Boehringer Ingelheim, Fort Dodge, IA, USA.
[2] IDEXX Laboratories, Westbrook, ME, USA.

http://dx.doi.org/10.1016/j.cimid.2015.07.004

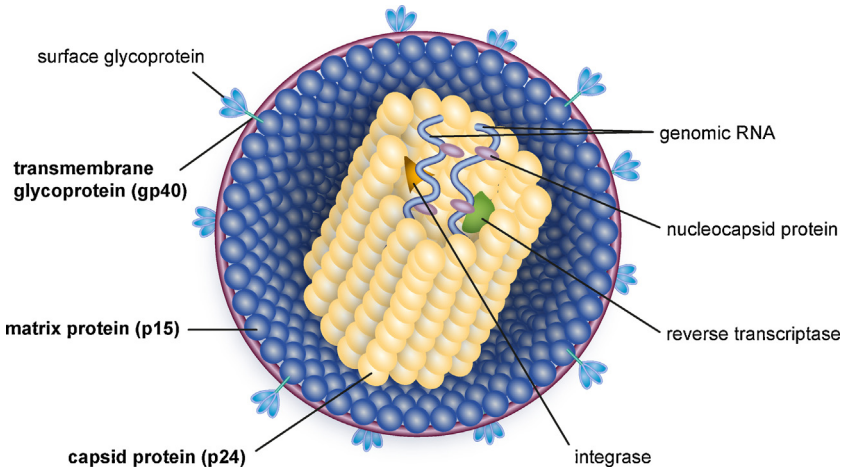

Fig. 1. Schematic of FIV emphasising the different target antigens for antibody testing.

Table 1
Summary of the antibodies detected using four different point-of-care FIV antibody test kits.

FIV antibody detection kit	FIV target antigen		
	p15	p24	gp40
SNAP FIV/FeLV Combo (Australia, NZ, North America)	●	●	
SNAP FIV/FeLV Combo Plus (Europe)[a]	●	●	●
Witness FeLV/FIV			●
Anigen Rapid FIV/FeLV		●	●

[a] Not used in this study, but used by Hartmann et al. [19].

flow enzyme-linked immunosorbent assay (ELISA) kit that detects antibodies to p15 (matrix protein) and p24 (capsid protein), Witness FeLV/FIV[3] is a lateral flow immunochromatography kit that detects antibodies to gp40 (transmembrane glycoprotein), while Anigen Rapid FIV/FeLV[4] is a lateral flow immunochromatography kit that detects antibodies to p24 and gp40 (Fig. 1 and Table 1). SNAP FIV/FeLV Combo Plus, available only in Europe, is a lateral flow ELISA kit that detects antibodies to p15, p24 and gp40. Published sensitivity and specificity of each test kit in FIV-unvaccinated cats are 94% and 100% for SNAP FIV/FeLV Combo (https://www.idexx.com/files/small-animal-health/products-and-services/snap-products/snap-fiv-felv-combo/snap-combo-test-accuracy.pdf),[35] 100% and 100% for SNAP FIV/FeLV Combo Plus, 95% and 99% for Witness FeLV/FIV [19], and 89% and 100% for Anigen Rapid FIV/FeLV [20].

The introduction of the FIV vaccine in 2002 complicated FIV diagnosis because vaccination was reported to result in the production of antibodies to FIV indistinguishable from those produced in response to natural infection [21]. Consequently, for FIV-vaccinated cats and cats of unknown vaccination status, FIV diagnostics shifted towards molecular methods such as nucleic acid amplification [22,23]. Some studies have also explored alternative methods for FIV diagnosis with excellent results, such as a discriminatory ELISA based on antibody response to two different FIV antigens [24,25], and by calculating the CD4:CD8^{low} T-lymphocyte ratio [26].

In this study, we reappraised the assertion that point-of-care kits are unable to distinguish antibodies produced following FIV vaccination from antibodies produced in response to natural FIV infection, and therefore are unable to determine the true FIV infection status of FIV-vaccinated cats, using three commercially available test kits.

2. Material and methods

2.1. Sample population

Cats with known FIV vaccination history were recruited through veterinary clinics in Australia during 2013–2014, most commonly at the same time as an annual health check or some routine procedure (e.g. dental scaling and polishing). Very occasionally, cats were sampled during hospitalisation for further work up of systemic illness; however no FIV-infected cats would have been classified as being in the feline-AIDS (FAIDS) phase of infection. Cats or kittens were excluded from the study if they were less than six months of age (due to the possibility of maternal antibodies being present), had an unclear FIV vaccination history or had a known FIV infection status (due to prior testing). Cats were included in the 'FIV-vaccinated' group if they had received one or more FIV vaccines at any time in their life, regardless of whether or not the administration of vaccine had been in accordance with the manufacturer's guidelines[1]. Cats were included in the 'FIV-unvaccinated' group if they had never been vaccinated against FIV. Clinical records of all patients from both groups were carefully interrogated to enforce this inclusion criterion. Cases were recruited from veterinary practices servicing areas where the prevalence of FIV infection was perceived to be high [27].

Animal ethics approval was granted by the University of Sydney (Approval number 5920).

2.2. Serological and molecular detection of FIV infection

Blood was collected by the primary author using jugular venipuncture and immediately aliquoted into three EDTA tubes and stored at 4 °C. Testing for FIV antibodies was performed within 24 hours of blood collection[5] with three commercially available point-of-care kits tested concurrently, using whole blood from the same EDTA tube, according to the manufacturer's instructions. The antibody kits tested were SNAP FIV/FeLV Combo[2], Witness FeLV/FIV[3], and Anigen Rapid FIV/FeLV[4] (Table 1). The antibody results panel for each cat was digitally photographed at the time of testing. Blood from this tube was also used for routine haematologic

[3] Zoetis Animal Health, Lyon, France.
[4] BioNote, Gyeonggi-do, Korea.
[5] Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia.

Please cite this article in press as: M.E. Westman, et al., Determining the feline immunodeficiency virus (FIV) status of FIV-vaccinated cats using point-of-care antibody kits, Comp Immunol Microbiol Infect Dis (2015), http://dx.doi.org/10.1016/j.cimid.2015.07.004