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Diagnosing feline immunodeficiency virus (FIV) infection in FIV-vaccinated and FIV-unvaccinated cats using saliva

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ABSTRACT

We recently showed that two immunochromatography point-of-care FIV antibody test kits (Witness FeLV/FIV and Anigen Rapid FIV/FeLV) were able to correctly assign FIV infection status, irrespective of FIV vaccination history, using whole blood as the diagnostic specimen. A third FIV antibody test kit, SNAP FIV/FeLV Combo (an enzyme-linked immunosorbent assay [ELISA]), was unable to differentiate antibodies produced in response to FIV vaccination from those incited by FIV infection. The aim of this study was to determine if saliva is a suitable diagnostic specimen using the same well characterized feline cohort. FIV infection status of these cats had been determined previously using a combination of serology, polymerase chain reaction (PCR) testing and virus isolation. This final assignment was then compared to results obtained using saliva as the diagnostic specimen utilizing the same three point-of-care FIV antibody test kits and commercially available PCR assay (FIV RealPCR). In a population of cats where one third (117/356; 33%) were FIV-vaccinated, both immunochromatography test kits accurately diagnosed FIV infection using saliva via a centrifugation method, irrespective of FIV vaccination history. For FIV diagnosis using saliva, the specificity of Anigen Rapid FIV/FeLV and Witness FeLV/FIV was 100%, while the sensitivity of these kits was 96% and 92% respectively. SNAP FIV/FeLV Combo had a specificity of 98% and sensitivity of 44%, while FIV RealPCR testing had a specificity of 100% and sensitivity of 72% using saliva. A revised direct method of saliva testing was trialed on a subset of FIV-infected cats ($n = 14$), resulting in 14, 7 and 0 FIV positive results using Anigen Rapid FIV/FeLV, Witness FeLV/FIV and SNAP FIV/FeLV Combo, respectively. These results demonstrate that saliva can be used to diagnose FIV infection, irrespective of FIV vaccination history, using either a centrifugation method (Anigen Rapid FIV/FeLV and Witness FeLV/FIV) or a direct method (Anigen Rapid FIV/FeLV). Collection of a saliva specimen therefore provides an acceptable alternative to venipuncture (i) in fractious cats where saliva may be easier to obtain than whole blood, (ii) in settings when a veterinarian or trained technician is unavailable to collect blood and (iii) in shelters where FIV testing is undertaken prior to adoption but additional blood testing is not required.

1. Introduction

Feline immunodeficiency virus (FIV) and human immunodeficiency virus (HIV) are retroviruses of the genus *Lentivirus*. Both cause life-long infections, resulting in persistently high antibody titres which are useful diagnostically for identifying infected patients [1–4].

Serologic testing for FIV infection is commonly undertaken by veterinarians for patients with severe stomatitis, sequential or persistent opportunistic infections, lymphoma and other malignancies, or signs of non-specific illness when a cause is not apparent after preliminary investigations. Veterinarians in shelters typically perform FIV screening of cats prior to admission into a shelter, or prior to re-homing [5,6]. The introduction of a FIV vaccine[1] in 2002 complicated the serologic diagnosis of FIV infection because the most widely used point-of-care antibody test kit available at the time and western blot analyses were unable to differentiate antibodies produced by FIV-vaccinated and FIV-infected cats [7]. Consequently, in FIV-vaccinated cats and cats of unknown FIV

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[1] Fel-O-Vax® FIV, Boehringer Ingelheim, Fort Dodge, IA, USA.

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vaccination status, diagnosis of FIV required the use of more expensive molecular methods to demonstrate the presence of the virus, such as nucleic acid amplification, with variable results in terms of accuracy and reliability [8–13]. Recently, we reported that two point-of-care FIV antibody test kits (Anigen Rapid FIV/FeLV[2] and Witness FeLV/FIV[3]) were able to accurately diagnose FIV infection in cats, irrespective of FIV vaccination history, using whole blood as the diagnostic specimen [14]. A third point-of-care FIV antibody test kit (SNAP FIV/FeLV Combo[4]) could not distinguish FIV-vaccinated from FIV-infected cats. Since each antibody kit uses a different panel of viral epitopes, we hypothesized that the humoral response to different viral antigens (proteins and glycoproteins) within the formalin-inactivated killed-virus vaccine was more complex than had been appreciated [14].

In general, it is easier and less invasive to collect a saliva specimen than a blood specimen from feline patients. Indeed, venipuncture is impossible in some cats without sedation or skilled manual restraint. Antibody testing using saliva accurately detects HIV infection in people; a *meta*-analysis of the OraQuick Advance Rapid HIV-1/2 In-Home HIV Test[5] identified similar specificity, and only a 2% reduction in sensitivity, when saliva was used instead of whole blood [15]. As a result, this test kit has been approved by the USA Food and Drug Administration for self-testing using saliva[6] [16]. Surprisingly, despite IgG being reliably detectable in cat saliva [17], only three studies have investigated using saliva to diagnose FIV infection in cats. Poli and colleagues reported that detection of FIV antibodies in saliva using ELISA was extremely unreliable, with a high frequency of false-positive and false-negative results, although the exact numbers and details of the commercial ELISA kits used were not provided [18]. In contrast, an indirect immunofluorescence assay and Western blot testing (WB-IgG) detected FIV antibodies in the saliva of 15/16 (94%) FIV-seropositive cats and no false-positive results were recorded amongst the 16 FIV-seronegative cats [18]. Matteucci et al. [19] attempted to isolate FIV from the saliva, plasma and peripheral blood mononuclear cells (PBMC) of naturally FIV-infected cats; the isolation rate of FIV from saliva was considerably lower than from PBMC (18% versus 81% of cats). The third study investigating saliva testing to diagnose FIV infection was a prevalence survey of client-owned cats using a later generation of a commercially available ELISA kit (SNAP FIV/FeLV Combo) to detect FIV antibodies in addition to utilizing nucleic acid amplification (polymerase chain reaction [PCR] testing) to detect proviral DNA [20]. Although blood was not obtained in the main study, preliminary evaluation using three FIV-infected and two FIV-uninfected cats found results for FIV antibody testing using the ELISA kit to be identical when blood and saliva from the same cat were tested concurrently. There was also good correlation between the ELISA antibody test kit and combined results from the three PCR assays using saliva (Kappa value 0.76; 95% confidence interval [CI] 0.64–0.87) [20].

The aim of the present study was to systematically investigate the use of saliva to diagnose FIV infection in FIV-vaccinated and FIV-unvaccinated cats, using three point-of-care FIV antibody test kits and a commercially available real-time PCR (qPCR) assay, in a well characterized cohort.

2. Material and methods

2.1. Sample population

Client-owned cats were recruited as part of another study into FIV diagnostic testing using whole blood [14]. Briefly, cats of known FIV vaccination history were recruited through veterinary clinics and classified as 'FIV-vaccinated' (had received at least one FIV vaccine at any time in their life) or 'FIV-unvaccinated' (had never received a FIV vaccine). Clinical records were interrogated to enforce this criterion. Practices where the prevalence of FIV infection was perceived to be high were targeted. Animal ethics approval was granted by The University of Sydney (Approval number N00/1-2013/3/5920).

2.2. Blood collection, blood testing and defining FIV infection status

The procedures for blood collection, FIV antibody testing of whole blood using three point-of-care test kits, nucleic acid amplification of blood (FIV RealPCR), use of virus isolation (VI) in rare discrepant cases and final assignment of FIV status have been described previously [14]. In summary, consideration of all four FIV test results (three antibody tests and PCR testing) led to FIV status being assigned when there was a majority, either of negative or positive FIV results (i.e. 3–1 or 4–0). In seven cases, where test results were equally split (i.e. 2–2), VI was undertaken as the 'tie-breaker'. VI was also undertaken to confirm FIV-vaccinated/FIV-infected cats, even though in all cases there was a clear FIV-positive test majority.

Four FIV-vaccinated cats (4/117; 3%) were determined to be FIV-infected. All four cats tested FIV-positive using whole blood with SNAP FIV/FeLV Combo, Witness FeLV/FIV and Anigen Rapid FIV/FeLV. Two of the four cats tested FIV-negative with FIV RealPCR initially, although with repeat testing (three times over 18 months) these two cats eventually tested positive with FIV RealPCR. Serial re-testing was undertaken following positive VI results to investigate whether FIV RealPCR would be sensitive enough to detect FIV infection in these two cats.

Of the 113 FIV-vaccinated/FIV-uninfected cats, SNAP FIV/FeLV Combo recorded zero FIV-negative results (i.e. all 113 cats tested FIV-positive using this kit), Witness FeLV/FIV recorded 107 FIV-negative results and Anigen Rapid FIV/FeLV recorded 113 FIV-negative results. A total of 112/113 cats tested FIV-negative with FIV RealPCR.

Twenty-one of the 239 FIV-unvaccinated cats (9%) were determined to be FIV-infected. All 21 cats tested FIV-positive using whole blood with SNAP FIV/FeLV Combo, Witness FeLV/FIV, Anigen Rapid FIV/FeLV and FIV RealPCR. Of the 218 FIV-unvaccinated/FIV-uninfected cats, SNAP FIV/FeLV Combo recorded 212 FIV-negative results, Witness FeLV/FIV recorded 217 FIV-negative results and Anigen Rapid recorded 218 FIV-negative results. A total of 215/218 cats tested FIV-negative with FIV RealPCR.

2.3. Saliva collection and saliva testing

Saliva collection was performed immediately following blood collection. Two sterile, individually cased cotton swabs mounted on plastic rods[7] were used to obtain saliva. Each swab was rubbed one after the other against the buccal mucosa on each side of the mouth, with the cheek pressed gently against the upper dental arcade while slowly twisting the swab, for approximately 10 s per side. Swabs

[2] BioNote, Gyeonggi-do, Korea.
[3] Zoetis Animal Health, Lyon, France.
[4] IDEXX Laboratories, Westbrook, ME, USA.
[5] OraSure Technologies Inc., PA, USA.
[6] www.fda.gov/ForConsumers/ConsumerUpdates/ucm310545.htm

[7] Sarstadt, Mawson Lakes, South Australia, Australia (Plastic Stem Cotton Tip Catalogue No. 80.625; 1.5 mL Micro Tube Catalogue No. 72.706.400).