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München

Vorkommen verschiedener Verlaufsformen der felinen Leukämievirus-Infektion und deren Nachweis

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INHALTSVERZEICHNIS

ABKÜRZUNGSVERZEICHNIS

AB	antibody (Antikörper)
AG	antigen (Antigen)
bzw.	beziehungsweise
CI	confidence interval (Konfidenzintervall)
DIVA	differentiate infected from vaccinated animals (Differenzieren von infizierten und geimpften Tieren)
DNA	deoxyribonucleic acid (Desoxyribonukleinsäure)
EDTA	ethylenediaminetetraacetic acid (Ethylendiamintetraessigsäure)
ELISA	enzyme-linked immunosorbent assay (enzymgebundener Immunadsorptionstest)
env	envelope (Hüllgen)
et al.	et alii (und andere)
FeLV	feline leukemia virus (felines Leukämievirus)
FIV	feline immunodeficiency virus (felines Immunschwächevirus)
gag	group-specific antigen (gruppenspezifisches Antigen)
IFT	Immunfluoreszenztest
IWV	inactivated whole-virus (inaktiviertes Ganzvirus)
Lab	laboratory (Labor)
min	minutes (Minuten)
Nab	neutralizing antibody (neutralisierender Antikörper)

NPW	negative predictive value (negativer prädiktiver Wert)
OR	odds ratio (Chancenverhältnis)
PoC-Test	Point-of-Care-Test
pol	Polymerasegen
PPW	positive predictive value (positiver prädiktiver Wert)
qPCR	quantitative polymerase chain reaction (quantitative Polymerase-Kettenreaktion)
RNA	ribonucleic acid (Ribonukleinsäure)
RT	reverse transcription (reverse Transkriptase)
RT-PCR	reverse transcription polymerase chain reaction (reverse-Transkriptase-Polymerase-Kettenreaktion)
RT-qPCR	reverse transcription quantitative polymerase chain reaction (quantitative reverse-Transkriptase-Polymerase-Kettenreaktion)
s	seconds (Sekunden)
SPF	specific pathogen free (spezifisch Pathogen-frei)
SU	surface unit (Oberflächeneinheit)
TM	transmembrane (Transmembran)
TNA	total nucleic acids (gesamte Nukleinsäuren)

GENDER-DISCLAIMER

Aus Gründen der besseren Lesbarkeit wurde an einigen Stellen das generische Maskulinum verwendet. Sämtliche Personenbezeichnungen gelten gleichermaßen für alle geschlechtlichen Identitäten

I. EINLEITUNG

Das feline Leukämievirus (FeLV) ist ein weltweit vorkommendes Gammaretrovirus und gehört noch heute zu einem der wichtigsten Infektionserregern bei Katzen. Eine FeLV-Infektion kann progressiv, regressiv, abortiv oder fokal (atypisch) verlaufen. Aufgrund der komplexen Pathogenese und der unterschiedlichen Verlaufsformen ist die Diagnosestellung oftmals schwierig und erfordert häufig die Durchführung mehrerer Testverfahren. Abhängig von der Balance zwischen dem Virus und dem Immunsystem der Katze können verschiedene Infektionsverläufe auftreten und diese auch ineinander übergehen (HARTMANN & HOFMANN-LEHMANN, 2020; HOFMANN-LEHMANN & HARTMANN, 2020).

Die meisten FeLV-Prävalenzstudien basieren ausschließlich auf der Identifikation von progressiv infizierten Katzen. Dabei schwankt die Prävalenz in Europa zwischen 1–9 %. In Deutschland ist nur ein geringer Prozentsatz der Katzen (0,3 %) progressiv infiziert (STUDER et al., 2019). Unter Berücksichtigung aller Verlaufsformen ist die eigentliche Infektionsrate jedoch höher. Untersuchungen aus Süddeutschland zufolge waren 1,8 % der Katzen progressiv, 1,8 % regressiv und 9,2 % der Katzen abortiv infiziert (ENGLERT et al., 2012).

Herkömmliche Point-of-Care-Tests (PoC-Tests) stützen sich auf den Nachweis von p27-Antigen und sind darauf ausgerichtet, progressiv infizierte Katzen zu erkennen. Andere Verlaufsformen sind durch den Nachweis von p27-Antigen nicht detektierbar. Seit 2018 ist in Europa ein kommerziell erhältlicher PoC-Test (v-RetroFel®, scil animal care company GmbH, Viernheim, Deutschland) auf dem Markt, der neben Antikörpern gegen das feline Immunschwächevirus (FIV) und FeLV-p27-Antigen auch FeLV-anti-p15E-Antikörper nachweist, um progressiv, regressiv und abortiv FeLV-infizierte Katzen zu identifizieren.

Die Arbeit besteht aus einer Übersichtspublikation und drei Originalarbeiten (zweimal Erstautor, einmal geteilte Erstautorschaft).

Ziel des Übersichtsartikels dieser Arbeit war es, einen Überblick über die unterschiedlichen FeLV-Verlaufsformen zu geben und die verschiedenen Möglichkeiten zum Nachweis einer FeLV-Infektion darzulegen. Ziel der ersten Originalpublikation war es, die FeLV-Prävalenz in vier Ländern Europas (Italien,

Portugal, Deutschland und Frankreich) unter Berücksichtigung aller FeLV-Verlaufsformen (progressiv, regressiv, abortiv, fokal (atypisch)) zu bestimmen und Risikofaktoren zu ermitteln, die eine FeLV-Infektion begünstigen. Ziel der zweiten Originalpublikation war es, den neuen PoC-Test (v-RetroFel®; Version 2020) in seiner Sensitivität und Spezifität bezüglich des Nachweises von FIV-Antikörpern, FeLV-p27-Antigen und anti-p15E-Antikörpern zu evaluieren. In der dritten Originalpublikation wurde eine laut Herstellerangaben verbesserte Version des PoC-Tests (v-RetroFel®; Version 2021) in Bezug auf dessen Praktikabilität und Performance hinsichtlich anti-p15E-Antikörper bei progressiv, regressiv, abortiv und fokal (atypisch) infizierten Katzen untersucht.

II. PUBLIKATION 1 (ÜBERSICHTSARTIKEL)

Infektion mit dem felinen Leukämievirus – der Weg zur Diagnose

Feline leukemia virus infection – a guide to diagnosis

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Infektion mit dem felinen Leukämievirus – der Weg zur Diagnose

Feline leukemia virus infection – a guide to diagnosis

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Schlüsselwörter: FeLV, p27-Antigen, virale RNA, provirale DNA, anti-FeLV-Antikörper

Key words: FeLV, p27 antigen, viral RNA, proviral DNA, anti-FeLV antibodies

Zusammenfassung

Die Infektion mit dem felinen Leukämievirus (FeLV) kommt bei Katzen weltweit vor. Der Verlauf einer Infektion kann unterschiedlich sein und sich über die Zeit verändern. Die komplexe Pathogenese, die Verfügbarkeit vieler verschiedener Testverfahren und die Interpretation der Testergebnisse stellen Tierärzte oftmals vor eine Herausforderung. Katzen mit einer progressiven Infektion (persistierend p27-Antigen-positiv) scheiden FeLV vorwiegend über den Speichel aus und gelten daher als Ansteckungsquelle für andere nicht infizierte Katzen. Schwieriger zu erkennen sind Katzen mit einer regressiven Infektion, da sie mit herkömmlichen Schnelltests (p27-Antigentest) i. d. R. nicht erfasst werden und unerkannt bleiben. Dennoch sind diese Katzen FeLV-Träger (Provirus-positiv) und bei Schwächung des Immunsystems kann es zu einer Reaktivierung der Infektion und FeLV-assoziierten klinischen Symptomen kommen. Abortiv infizierte Katzen sind zu keinem Zeitpunkt virämisch, scheiden kein Virus aus und entwickeln keine klinischen Symptome. Eine abortive Infektion kann nur durch den Nachweis von

Antikörpern im Blut diagnostiziert werden. Ein neuer Schnelltest zum Nachweis von Antikörpern gegen FeLV-p15E-Antigen wurde kürzlich auf dem europäischen Markt eingeführt und wird gerade evaluiert.

Summary

Feline leukaemia virus (FeLV) infection affects cats worldwide. The course of FeLV infection can change and vary over time. The complex pathogenesis, the availability of many different test methods, and the interpretation of test results are often challenging for veterinarians. Cats with progressive infection (persistently p27 antigen-positive) shed FeLV mainly through saliva and are therefore considered a source of infection for uninfected cats. Diagnosing regressive infection is often challenging, since it usually cannot be detected by commonly used point of care-tests (p27 antigen test) and thus, often remains undetected. Nevertheless, cats with regressive infection are FeLV carriers (provirus-positive) and if the immune system is suppressed, reactivation of the infection and FeLV-associated clinical signs can occur. Abortively infected cats are never viraemic, do not shed virus and do not develop clinical signs. Abortive infection can only be diagnosed by detection of antibodies in blood. A new point-of-care test for the detection of antibodies against FeLV p15E antigen has recently been introduced on the European market and is currently being evaluated.

Einleitung

Das feline Leukämievirus (FeLV) ist ein bei Katzen weltweit verbreitetes Gammaretrovirus und zählt zu den bedeutendsten Infektionserregern der Katze. Die Diagnosestellung ist aufgrund der komplexen Pathogenese und der verschiedenen Infektionsverläufe anspruchsvoll [1-3]. Die Infektion mit FeLV kann bei progressivem Verlauf zu Immunschwäche, Knochenmarkssuppression (Panzytopenien), Neoplasien und im schlimmsten Fall zum Tod führen [4, 5].

Es werden mehrere Subgruppen des FeLV unterschieden. FeLV-A, als relevanteste Subgruppe, ist das infektiöse Hauptvirus und wird von Katze zu Katze übertragen. FeLV-B und FeLV-C entstehen in mit FeLV-A infizierten Katzen durch

Rekombinationen mit endogenen FeLV bzw. durch Mutationen der Subgruppe FeLV-A [6, 7]. FeLV-T, eine weitere experimentell beschriebene Subgruppe, die aus FeLV-A entsteht, besitzt zytolytische Eigenschaften für T-Lymphozyten und verursacht eine schwere Immunsuppression [8], sie scheint jedoch bei Feldinfektionen keine große Bedeutung zu haben.

Durch die Instabilität des FeLV in der Umwelt erfolgt die Virusübertragung vor allem durch engen sozialen Kontakt zu Katzen, die das Virus mit dem Speichel ausscheiden und an nicht infizierte Katzen übertragen. Progressiv infizierte Katzen scheiden Viruspartikel in großen Mengen über den Speichel aus [9, 10]. Auch eine Übertagung über Urin, Kot oder Muttermilch ist möglich [11-13]. Eine vertikale Übertragung von einer infizierten Katze auf ihre Welpen tritt häufig auf. Die Welpen können transplazentar oder durch das Belecken und Säugen infiziert werden [14].

Der Verlauf einer FeLV-Infektion kann unterschiedlich sein (Tab. 1) [1, 2]. Katzen mit einem progressiven Infektionsverlauf benötigen eine besondere medizinische Betreuung und Haltung. Daher sollte der FeLV-Status jeder Katze bekannt sein. Außerdem sollten Impfungen gegen FeLV bei bereits infizierten Katzen vermieden werden [3]. Im Laufe der letzten Jahre wurden die diagnostischen Methoden zum Nachweis der unterschiedlichen Verlaufsformen einer FeLV-Infektion verfeinert und ausgeweitet. Dieser Artikel soll einen Überblick über die derzeit verfügbaren Nachweismethoden zur Diagnose einer FeLV-Infektion geben und als Hilfestellung im Praxisalltag dienen.

Eigenschaften des Virus

FeLV ist ein behülltes Einzelstrang-RNA-Virus, das zur Gattung der Gammaretroviren aus der Familie der Retroviren zählt. Durch die Virushülle ist das Virus in der Umwelt instabil und kann mit gebräuchlichen Desinfektionsmitteln leicht inaktiviert werden. Damit es zu einer Infektion der Wirtszelle kommt, muss die virale RNA durch das Enzym Reverse Transkriptase (RT) zu DNA umgeschrieben werden. Im nächsten Schritt wird die DNA als Provirus, mit Hilfe eines weiteren Enzyms, Integrase, in das Zellgenom der Katze eingebaut (Abb. 1). Das FeLV-Genom wird in 3 Hauptgene unterteilt [15, 16]: (1) Das

gruppenspezifische Antigen Gen (*gag*) kodiert unter anderem das Viruskapsidprotein p27 (Abb. 1), welches in praxistauglichen Antigentests (Point-of-Care- [POC-] Test) nachgewiesen werden kann. (2) Das Polymerasegen (*pol*) kodiert für das Enzym RT, das die virale RNA in DNA (Provirus) umschreibt. (3) Das Hüllgen (*env*) ist für die Kodierung der beiden Proteine gp70 und p15E verantwortlich. Das Oberflächenglykoprotein gp70 spielt eine wesentliche Rolle bei der Induktion virusneutralisierender Antikörper. Das Transmembranprotein p15E wird auf der Oberfläche von FeLV infizierten Zellen exprimiert, ermöglicht dem Virus den Eintritt in die Zelle und hemmt unter anderem die Lymphozytenproliferation [17]. Gegen p15E gerichtete Antikörper enthalten kaum virusneutralisierende Eigenschaften [18].

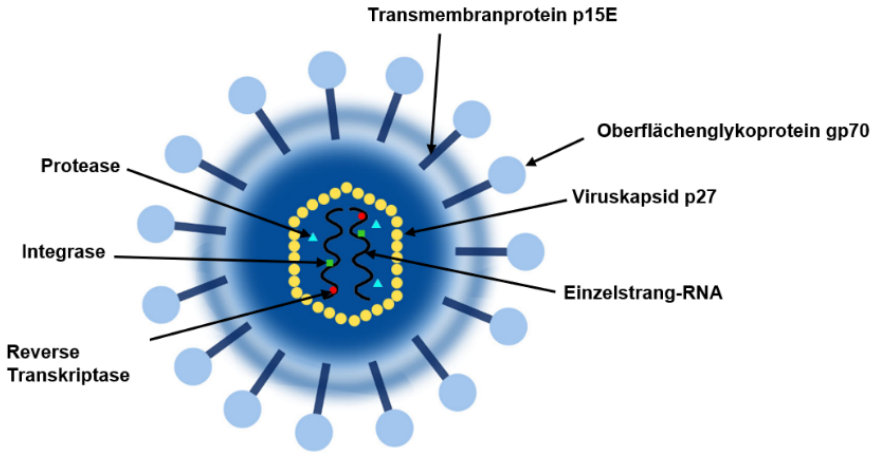


Abb. 1 Aufbau eines felinen Leukämievirus- (FeLV-) Partikels. FeLV ist ein behülltes Retrovirus, aus 2 identischen RNA-Einzelsträngen. Die Reverse Transkriptase transkribiert RNA zu DNA, die im Anschluss mit Hilfe der Integrase in das Genom der Katze eingebaut wird. Das Viruskapsidprotein p27 gehört zu den gruppenspezifischen Antigenen und wir durch *gag* kodiert. Das Hüllgen (*env*) kodiert für das Oberflächenglykoprotein (gp70) und das Transmembranprotein (p15E). Quelle: © J.Giselbrecht

Figure 1 Structure of a feline leukaemia virus (FeLV) particle. FeLV is an enveloped retrovirus consisting of 2 identical RNA single strands. Reverse transcriptase transcribes RNA to DNA, which is then integrated into the cat's genome with the help of integrase. The viral capsid protein p27 belongs to the group-specific antigens and is encoded by *gag*. The envelope gene (*env*) encodes the surface glycoprotein (gp70) and the transmembrane protein (p15E). Source: © J.Giselbrecht

Verlaufsformen

Eine FeLV-Infektion kann progressiv, regressiv, abortiv und selten fokal (atypisch) verlaufen. Die Verlaufsformen können ineinander übergehen und sind daher, gerade bei natürlich infizierten Katzen, oft schwer voneinander zu unterscheiden [1, 19]. Der Verlauf der FeLV-Infektion wird bestimmt durch den Zustand des Immunsystems der betroffenen Katzen (z. B. vorbestehende Immunität oder Immunschwäche) als auch von viralen Charakteristika, wie der Virulenz des Virus oder dem Infektionsdruck. Durch verschiedene Faktoren, wie z. B. Immunsuppression, Koinfektionen und Stress, können die Immunabwehr und somit der Verlauf der Infektion beeinflusst werden [2].

Progressive Infektion

Der Anteil an Katzen, der nach natürlicher Infektion eine progressive Infektion entwickelt, ist abhängig von der getesteten Katzenpopulation (z. B. Infektionsdruck, Alter der Katzen). Vor allem junge und Katzen mit Vorerkrankungen sind anfälliger für einen progressiven Verlauf [20]. Nach experimenteller FeLV-Infektion zeigen etwa ein Drittel aller infizierten Katzen einen progressiven Verlauf [21]. Bei der progressiven Infektion schafft das Immunsystem der betroffenen Katzen es nicht, das Virus und deren systemische Verbreitung zu stoppen. [1, 2]. Durch eine ungenügende Immunantwort haben progressiv infizierte Katzen nur wenige bis keine virusneutralisierende Antikörper (Abb. 2). Außerdem ist ihre zelluläre Immunantwort nur schwach ausgeprägt. Durch das Fehlen einer adäquaten/ausreichenden humoralen und zellulären Immunantwort gegen FeLV, kann die Katze die Virämie nicht überwinden [22]. Die progressive FeLV-Infektion kann in 4 Phasen unterteilt werden: (1) Nach zunächst oronasaler Aufnahme vermehrt sich FeLV in der Schleimhaut des Oropharynx und im lokalen lymphatischen Gewebe. (2) Vom lymphatischen Gewebe ausgehend gelangt das Virus über infizierte Lymphozyten und Monozyten in die Blutbahn (primäre Virämie) und (3) schließlich ins Knochenmark. (4) Im Knochenmark werden die sich rasch replizierenden Vorläuferzellen neutrophiler Granulozyten und Thrombozyten infiziert und ins periphere Blut ausgeschwemmt. Dies wird als sekundäre Virämie bezeichnet [23]. Als Folge der Infektion des Epithels von Speicheldrüsen, Pharynx, Harnblase, Darm und Gesäuge kann das

Virus vor allem mit dem Speichel, aber auch Kot, Urin oder über die Muttermilch ausgeschieden werden [11-13, 23, 24]. Während der virämischen Phasen können freies p27-Antigen im Blut und provirale DNA in Blutzellen sowie virale RNA im Blut und Speichel nachgewiesen werden. Studien haben gezeigt, dass sobald sich der Infektionsverlauf etabliert hat, progressiv infizierte Katzen, im Gegensatz zu regressiv infizierten Katzen, eine höhere Proviruslast und höhere p27-Antigenspiegeln aufweisen und die Proviruslast bei progressiv infizierten Katzen nach einer initialen Phase bleibt konstant hoch [25]. In einer kürzlich veröffentlichten Studie wurden 254 Katzen in einem Tierheim (Texas, USA) auf p27-Antigen (ELISA) und Provirus-DNA (PCR) getestet. Anhand quantitativer Testergebnisse der p27-Antigen- und der Proviruslast-Bestimmung wurden Cut-Off-Werte festgelegt, um zwischen „hoch-positiven“ und „niedrig-positiven“ Katzen zu unterscheiden. Katzen, die als hoch-positiv eingestuft wurden, hatten im Vergleich zu niedrig-positiven Katzen eine signifikant kürzere mittlere Überlebenszeit (1,37 Jahre). 90 % der Katzen, die als niedrig-positiv eingestuft wurden, waren bei der letzten Nachuntersuchung nach 4 Jahren noch am Leben [26]. Progressiv infizierte Katzen gelten als Ansteckungsquelle für andere, nicht infizierte Katzen [10]. Zudem entwickeln progressiv infizierte Katzen häufiger FeLV-assoziierte Symptome [4, 5, 10, 27]. Nichtsdestotrotz können progressiv infizierte Katzen unter optimalen Haltungsbedingungen und bei guter Pflege viele Jahre gesund bleiben [3].

Regressive Infektion

Unter experimentellen Bedingungen entwickeln bis 30 % der Katzen eine regressive Infektion [21]. Regressiv infizierte Katzen schaffen es, mit Hilfe einer effektiven Immunantwort das Virus aufzuhalten und die Virusreplikation zu stoppen oder möglichst gering zu halten. Aufgrund ihrer starken Immunantwort haben regressiv infizierte Katzen in der Regel hohe Konzentrationen von virusneutralisierenden Antikörpern (Abb. 2, Tab. 1) [14, 21, 28, 29]. Im Gegensatz zu progressiv infizierten Katzen kommt es bei regressiv infizierten Katzen meist nur am Anfang einer Infektion oder nach Reaktivierung zur Virämie. Regressiv infizierte Katzen scheiden das Virus nicht aus und ein Nachweis von Antigenen oder viraler RNA im peripheren Blut oder Speichel ist negativ (Tab. 1). Eine

Ausnahme besteht während der Anfangsphase der Infektion, der transienten (vorübergehenden) Virämie oder wenn es durch Schwächung des Immunsystems zur Reaktivierung des Virus kommt. In diesen Fällen ist freies p27-Antigen im peripheren Blut nachweisbar, die Katze ist im Antigentest positiv und scheidet das Virus auch aus [5]. Bei regressiv infizierten Katzen endet die Virämie meist 1–12 Wochen nach der Infektion [30]. Einige regressiv infizierte Katzen schaffen es erst nach mehreren Monaten, im Ausnahmefall erst nach bis zu einem Jahr, die Virämie zu beenden. Faktoren, wie Stress, Koinfektionen oder immunsuppressive Therapie, können zur Unterdrückung des Immunsystems führen und ein verzögertes Ende der Virämie zur Folge haben [2]. Nach Beendigung der transienten Virämie sind regressiv infizierte Katzen wieder p27-Antigen negativ und stellen keine direkte Infektionsquelle für andere Katzen dar. Regressiv infizierte Katzen beherbergen aber provirale DNA in infizierten Lymphozyten und gelegentlich in Monozyten [31]. Folglich ist es möglich, dass auch eine regressiv infizierte Katze Provirus-DNA während einer Bluttransfusion durch Provirus-positive Lympho- und Monozyten übertragen kann [5, 31]. Provirale DNA kann mittels PCR im Blut nachgewiesen werden [32]. Katzen mit einer regressiven Infektion bleiben lebenslang Träger des Virus (in Form von Provirus). In einer Studie wurden regressiv infizierte Katzen über einen Zeitraum von 3 Jahren beobachtet, und in regelmäßigen Abständen wurde die FeLV-Proviruslast bestimmt. Nach 3 Jahren wurden bei den Katzen nur noch geringe Mengen an Provirus nachgewiesen. Zu einer vollständigen Viruseliminierung kam es jedoch bei keiner der Katzen. In Einzelfällen ist es aber möglich, dass eine regressiv infizierte Katze mit sehr geringer Viruslast zeitweilig in der Provirus-PCR negativ getestet wird, wenn die Viruslast unter die Nachweisgrenze der Provirus-PCR fällt [19].

Abortive Infektion

Bei einer abortiven Infektion hat die Katze die Kontrolle über das Virus gewonnen, und die Balance zwischen Virus und Immunsystem neigt sich zugunsten der Katze (Abb. 2). Das Immunsystem abortiv infizierter Katzen ist in der Lage, die Virusreplikation nach oronasaler Aufnahme (Phase 1) und anfänglicher Replikation im lokalen lymphatischen Gewebe des Oropharynx (Phase 2) zu stoppen und neutralisierende Antikörper zu bilden [11, 32, 33]. Eine abortive Infektion kann bei

Katzen durch Exposition mit nur sehr geringen Virusmengen experimentell ausgelöst werden. Katzen die keinen Kontakt zu anderen Katzen und nur Kontakt mit dem Kot progressiv infizierter Katzen hatten, können eine abortive Infektion entwickeln [11, 12]. Abortiv infizierte Katzen sind zu keinem Zeitpunkt virämisch und bleiben daher meist unentdeckt. Weder FeLV-p27-Antigen, noch provirale DNA oder virale RNA können bei diesen Katzen nachgewiesen werden. Das Vorhandensein einer abortiven Infektion kann nur durch den Nachweis von Antikörpern bestätigt werden (Abb. 2, Tab. 1) [12, 19, 32, 34, 35].

Noch ist nicht genau geklärt, wie häufig eine abortive Infektion unter natürlichen Bedingungen vorkommt. Vor kurzem wurde der erste anti-p15E-Antikörpertest zur möglichen Erkennung einer abortiven Infektion entwickelt. Allerdings gibt es noch keine ausreichenden Daten darüber, ob dieser Test Katzen mit einer abortiven FeLV-Infektion unter Feldbedingungen zuverlässig erkennt [2].

	Nachweismethode/ frühestes positives Ergebnis p. i.	progressive Infektion	regressive Infektion	abortive Infektion	fokale (atypische) Infektion	keine Infektion
freies p27-Antigen Serum/Plasma/Vollblut	ELISA, POC-Test 3–6 Wochen	positiv	negativ*	negativ	intermittierend/ schwach positiv	negativ
intrazelluäres p27-Antigen neutrophile Granulozyten, Thrombozyten	IFT 6–9 Wochen (3 Wochen nach freiem p27-Antigen positiv	positiv	negativ*	negativ	negativ/ intermittierend positiv	negativ
virale RNA Blut	RT-PCR/ 1 Woche	positiv	negativ/positiv	negativ	negativ	negativ
virale RNA Speichel	RT-PCR 1–2 Wochen	positiv	negativ/positiv	negativ	negativ	negativ
Provirus-DNA Vollblut	PCR 1–2 Wochen	positiv	positiv	negativ	negativ/ schwach positiv	negativ
replizierendes Virus Plasma/Serum	Virusisolierung 1–2 Wochen	positiv	negativ*	negativ	negativ	negativ
VNA Plasma/Serum	Virus-Neutralisationstest 3 Wochen	negativ (oder geringer Titer)	positiv (hoher Titer)	positiv (Titerhöhe variabel)	positiv (hoher Titer)	negativ
Anti-p15E-AK Plasma/Serum	ELISA, POC-Test 2 Wochen	negativ/positiv	positiv (Titerhöhe variabel)	positiv (Titerhöhe variabel)	(meist) positiv (Titerhöhe variabel)	negativ (eventuell positiv nach Impfung)
Folgen		häufig FeLV-assoziierte Erkrankungen, vorsichtige Prognose	selten Erkrankungen (KM-Suppression, Lymphom) Erkrankung nach Reaktivierung (Immunsuppression)	keine	Erkrankungen unwahrscheinlich	keine

p. i. = nach einer Infektion, ELISA = Enzyme-Linked Immunsorbent Assay, POC = Point-of-Care, IFT = Immunfluoreszenztest, RT-PCR = Reverse-

Transkiptase-Polymerase-Kettenreaktion, PCR = Polymerase-Kettenreaktion, VNA = Virusneutralisierende Antikörper, AK = Antikörper, KM-Suppression = Knochenmarkssuppression, *positiv während transienter Virämie oder nach Reaktivierung

Tab. 1 Verlaufsformen und Folgen einer felinen Leukämievirus-Infektion und die zu erwartenden Testergebnisse basierend auf *Hartmann und Hofmann-Lehmann 2020* [1], *Hofmann-Lehmann und Hartmann 2020* [2].

Table 1 Courses and consequences of feline leukaemia virus infection and expected test results, based on *Hartmann and Hofmann-Lehmann* 2020 [1], *Hofmann-Lehmann and Hartmann* 2020 [2].

Fokale (atypische) Infektion

Fokale FeLV-Infektionen kommen bei natürlich infizierten Katzen wahrscheinlich selten vor. Auf der Grundlage alter experimenteller Studien wurde die Anzahl fokal infizierter Katzen auf 10–25 % geschätzt. Jedoch sollte berücksichtigt werden, dass keine neueren Daten auf Grundlage einer molekularen PCR-Analyse verfügbar sind. [36]. Bei der fokalen (atypischen) Infektion wird das Virus durch das Immunsystem der Katze zurückgedrängt und kann nur in bestimmten Organen persistieren, wie z. B. Milz, Lymphknoten, Milchdrüsen, Organe des Harntrakts oder Dünndarm; es kommen daher meist nur wenig Virus und infizierte Zellen im peripheren Blut vor [12, 13, 21]. Sollten infizierte Zellen ins Blut gelangen, werden diese i. d. R. durch das Immunsystem der Katze bekämpft. Durch die Abwesenheit infizierter Zellen im peripheren Blut ist bei fokal infizierten Katzen keine oder nur eine sehr geringe Menge Provirus-DNA oder virale RNA mittels PCR im Blut nachweisbar. Allerdings ist zu beachten, dass die lokal infizierten Zellen lösliches p27-Antigen produzieren können und p27-Antigen so in das periphere Blut gelangen kann, was manchmal zu einem positiven p27-Antigennachweis (ELISA, POC-Test) führen kann (Tab. 1) [37]. Es gibt einen Fallbericht zu einer Katze, bei der sich die FeLV-Infektion auf die Milchdrüsen beschränkte und das Virus über die Milch auf die Welpen übertragen wurde. Die Katze selbst wurde während dieser Phase negativ auf FeLV-p27-Antigen getestet [13].

	Progressive Infektion	Fokale (atypische) Infektion	Regressive Infektion	Abortive Infektion
				
Immunantwort	**sehr gering**	**stark**	**stark**	**sehr stark**
Provirus	im Genom der Katze integriert	im Genom der Katze in betroffenen Organen sequestriert	im Genom der Katze integriert	nicht vorhanden
Virusreplikation	systemische, persistierende Virusreplikation	lokale Virusreplikation	transiente Virusreplikation	nicht vorhanden
FeLV-assoziierte Erkrankungen	häufig	selten	selten	keine

Abb. 2 Die Abbildung soll das Ausmaß der Anti-FeLV-Immunität (grüne Kontur), der Virusreplikation (Viruspartikel) und der Antikörperbildung (grün) bei Katzen mit unterschiedlicher Verlaufsform darstellen. Progressiv infizierte Katzen zeigen eine sehr schwache oder gar nicht vorhandene Immunantwort. Bei einer fokalen Infektion ist die Virusreplikation auf ein bestimmtes Gewebe begrenzt. Regressiv

infizierte Katzen schaffen es, durch ihre ausgeprägte Immunantwort das Virus in Schach zu halten, jedoch nicht vollständig zu eliminieren. Abortiv infizierte Katzen sind im Stande, durch ihre starke Immunantwort und in der Regel hohe Antikörperkonzentrationen, das Virus erfolgreich abzuwehren. Quelle: © J.Giselbrecht

Figure 2 The figure is intended to show the extent of anti-FeLV immunity (green outline), viral replication (viral particles) and antibody formation (green) in cats with different FeLV courses. Progressively infected cats show a very weak or non-existent immune response. During focal infection, virus replication is localised to certain tissues. Regressively infected cats are able to keep the virus under control because of their strong immune response. Abortively infected cats manage to defend themselves against FeLV through their strong immune response and high antibody concentrations. Source: © J.Giselbrecht

Prävalenz

Die FeLV-Prävalenz variiert weltweit und liegt in Europa zwischen 1–9 % [38]. Durch Testung und das anschließende Separieren von Katzen sowie durch die Einführung wirksamer Impfstoffe in vielen Ländern konnte die Prävalenz deutlich gesenkt werden [3]. Seit einiger Zeit jedoch stagniert die Abnahme der FeLV-Prävalenz und hat vielerorts ein Plateau erreicht [20, 38-40]. Es sind daher weiterhin Maßnahmen (Test- und Eradikationsprogramme sowie Impfung) erforderlich, um die Prävalenz weiter zu senken und einen Wiederanstieg zu verhindern. Die Infektionshäufigkeit variiert geographisch und hängt von der untersuchten Population ab [41, 42]. In einer 2019 durchgeführten europaweiten Studie, an der 32 Länder beteiligt waren, lag die europaweite Prävalenz virämischer Katzen bei 2,3 % (141/6005 Katzen). Die Mehrheit der beprobten Katzen (85,9 %; 5151/6005 Katzen) kam aus Privathaushalten. Die restlichen Katzen stammten von Züchtern (2,9 %; 177/6005 Katzen), waren Straßenkatzen (7,4 %; 446/6005 Katzen) und Katzen aus Tierheimen (3,0 %; 179/6005 Katzen). Die höchsten Prävalenzen wiesen Portugal (8,8 %), Ungarn (5,9 %), Italien (5,7 %) und Malta (5,7 %) auf. Deutschland zählte mit 0,3 % zu den Ländern mit niedriger Prävalenz (Tab. 2) [38]. Die meisten Prävalenz-Studien basieren auf dem Nachweis von FeLV-Antigen. Werden alle Verlaufsformen einer FeLV-Infektion berücksichtigt,

liegt die Prävalenz höher [34]. In einer Studie von Englert und Mitarbeiter (2012) in Süddeutschland waren 1,8 % (9/495) der Katzen progressiv infiziert, 1,2 % (6/495) der Katzen regressiv und 9,2 % (22/246) der Katzen abortiv mit FeLV infiziert [34]. In der Türkei wurden 1008 Blutproben gesunder Straßenkatzen auf das Vorhandensein von FeLV-p27-Antigen, proviraler DNA und Antikörper untersucht. Die Antikörper wurden mittels im Labor durchgeführten anti-gp70-Antikörper ELISA (Biopronix FeLV gp70 Ab, Agrolabo, Italy; Sensitivität 96 %, Spezifität 100 %) bestimmt. 3,3 % (32/1008) der Katzen waren progressiv infiziert (p27-Antigen positiv, Provirus positiv, Antikörper negativ). Ein hoher Prozentsatz an Katzen (69,7 %; 703/1008) waren in der Provirus-PCR positiv. Es ist zu berücksichtigen, dass die von den Autoren verwendete PCR-Methode nicht nur exogenes FeLV, sondern auch Sequenzen von endogenem FeLV amplifizieren kann. Es ist bekannt, dass endogene FeLV-Sequenzen im Genom der Katze integriert sind und daher auch bei nicht infizierten Katzen vorkommen [43]. Schlussendlich wurden 43,6 % der beprobten Katzen einer regressiven Verlaufsform zugeordnet. Diese Katzen waren sowohl für provirale DNA als auch für FeLV-Antikörper positiv. Die beachtliche Anzahl an regressiv infizierten Katzen muss jedoch aufgrund der verwendeten Labormethoden kritisch betrachtet werden. 1,6 % (16/1008) besaßen ausschließlich FeLV-anti-gp70-Antikörper und wurden als abortiv infiziert bezeichnet [44].

Land	**FeLV-Prävalenz % (95% Konfidenzintervall)**	**Impfrate %**
Deutschland	0,3 (1,7 x 10^{-4}–1,8)	22,9
Vereinigtes Königreich	0,7 (3,8 x 10^{-4}–4,0)	81,5
Frankreich	1,0 (3,8 x 10^{-4}–4,0)	52,1
Österreich	1,3 (0,3–2,9)	46,0
Schweiz/Lichtenstein	2,7 (1,4–5,2)	67,6
Italien/Malta	5,7 (3,7–8,7)	17,8
Ungarn	5,9 (3,5–9,9)	26,9
Portugal	8,8 (6,2–12,3)	14,2

In verschiedenen Studien wurden anhand multivariater Analysen Risikofaktoren für eine FeLV-Infektion identifiziert. Europaweit hatten Freigänger (FeLV-Prävalenz

5,9 %), Katzen aus Mehrkatzenhaushalten (bestehend aus ≥ 5 Katzen) (FeLV-Prävalenz 5,1 %), männlich nicht kastrierte Katzen (FeLV-Prävalenz 4,0 %), kranke Katzen (FeLV-Prävalenz 3,9 %) und Katzen zwischen 1 und ≤ 6 Jahre (FeLV-Prävalenz 3,5 %) ein signifikant höheres Infektionsrisiko [38]. In Nordamerika wurden Untersuchungen an insgesamt 18.038 Katzen durchgeführt, um Risikofaktoren festzulegen. Kranke Katzen (FeLV-Prävalenz 6,3 %), Freigänger (FeLV-Prävalenz 3,6 %) und adulte Katzen (FeLV-Prävalenz 3,3 %) hatten ein signifikant höheres Risiko für eine progressive FeLV-Infektion [45].

Tab. 2 FeLV-Prävalenz und FeLV-Impfraten in verschiedenen Ländern Europas. Bei den FeLV-Impfraten wurden Katzen mit unbekanntem Impfstatus nicht berücksichtigt (~10 % der Katzen). Quelle: Studer N, Lutz H, Saegerman C et al. Pan-European study on the prevalence of the feline leukaemia virus infection - reported by the European Advisory Board on Cat Diseases (ABCD Europe). Viruses 2019; 11: 993. doi:10.3390/v11110993

Table 2 FeLV prevalence and FeLV vaccination rates in different countries in Europe. Cats with unknown vaccination status were not included (~10% of the cats) in the FeLV vaccination rates. Source: Studer N, Lutz H, Saegerman C et al. Pan-European study on the prevalence of the feline leukaemia virus infection - reported by the European Advisory Board on Cat Diseases (ABCD Europe). Viruses 2019; 11: 993. doi:10.3390/v11110993

Zur Verfügung stehende Tests

Für den Nachweis einer FeLV-Infektion werden in der Regel direkte Nachweisverfahren verwendet. Dazu gehören der Nachweis von freiem FeLV-p27-Antigen, viraler RNA und proviraler DNA. Aber auch ein indirekter Nachweis der Infektion, mittels Antikörpernachweis, gewinnt immer mehr an Bedeutung.

FeLV-p27-Antigen

FeLV-p27-Antigen kann sowohl extrazellulär (freilöslich) als auch intrazellulär (zellgebunden) nachgewiesen werden. Die am häufigsten verwendeten Testverfahren zur Diagnose einer FeLV-Infektion beruhen auf dem Nachweis von

freilöslichem, zirkulierendem FeLV-p27-Antigen im Blut, basierend auf dem ELISA- oder Lateral-Flow-Prinzip. Der Nachweis von freilöslichem p27-Antigen ist frühestens 3 Wochen nach einer FeLV-Infektion im Blut möglich [46, 47] und korreliert bei progressiv infizierten Katzen mit einer Virämie und der damit verbundenen Virusausscheidung [37]. Für den Praxisalltag wurden zahlreiche in-house-Tests zum Nachweis von freilöslichem p27-Antigen entwickelt. Seit deren Markteinführung wurden mehrere vergleichende Studien durchgeführt, um die Genauigkeit dieser Tests anhand verschiedener Parameter (Sensitivität, Spezifität, positiver und negativer prädiktiver Wert, Durchführbarkeit) zu überprüfen (Tab. 3) [48-53]. Eine wesentliche Rolle spielt die Spezifität (falsch-positiv-Rate) der Tests, da vermieden werden muss, dass Katzen aufgrund von falsch-positiven Testergebnissen von anderen Katzen separiert oder gar euthanasiert werden. Die Zuverlässigkeit eines Tests (Vorhersagewert, prädiktiver Wert) hängt stark von der Infektionsrate (Prävalenz) innerhalb einer Katzenpopulation ab. Der positive prädiktive Wert (PPV) sagt voraus, ob eine positiv getestete Katze tatsächlich mit FeLV infiziert ist. Mangelt es den Tests an Sensitivität (richtig-positiv-Rate), können infizierte Katzen unentdeckt bleiben und so weitere Katzen infizieren. Unterschiede in der Testleistung können zur Fehldiagnose führen, vor allem, wenn die Prävalenz der Infektion niedrig und das erwartete FeLV-Expositionsrisiko der getesteten Katzen gering ist. Ein positives Testergebnis sollte daher unmittelbar bestätigt werden [2]. Zur Bestätigung einer FeLV-Ausscheidung kann eine RT-PCR auf virale RNA im Speichel durchgeführt werden, da dieser Nachweis eine gute Korrelation mit einer Virämie und Virusausscheidung zeigt. Alternativ kann eine PCR auf provirale DNA zur Bestätigung durchgeführt werden. Sind keine der angeführten Labormethoden zugänglich, gibt es die Möglichkeit, die Katze erneut mit einem zweiten, vorzugsweise anderen POC-Test zu testen um so das Ergebnis zu überprüfen [2, 49, 54]. Der negative prädiktive Wert (NPV) gibt die Wahrscheinlichkeit an, dass eine negativ getestete Katze auch tatsächlich negativ ist. Liegt eine niedrige Prävalenz vor, ist der negativ prädiktive Wert meist hoch.

Neben kommerziell erhältlichen POC-Tests gibt es die Möglichkeit, anhand industriegefertigter ELISA-Platten mehrere Katzen gleichzeitig zu untersuchen. Dieses Verfahren wird ausschließlich im Großlabor angewendet und kommt nicht in der Praxis zum Einsatz.

Der Nachweis von intrazellulärem Antigen beruht auf dem Verfahren der

Immunfluoreszenz. Der Immunfluoreszenztest (IFT) weist spezifische *gag*-Proteine (inklusive p27) des FeLV in neutrophilen Granulozyten und Thrombozyten nach [55]. Der Nachweis erfolgt anhand eines Blut- oder auch Knochenmarksausstrich. Ein positives Testergebnis ist frühestens 6–9 Wochen nach einer FeLV-Infektion, also 3 Wochen nach einem positiven Nachweis von freiem p27-Antigen im Blut, nach Befall des Knochenmarks, zu erwarten [3]. Ist eine Katze sowohl im ELISA, als auch im IFT positiv, gilt sie mit hoher Wahrscheinlichkeit als progressiv infiziert (Tab. 1). Falsch negative Ergebnisse im IFT sind möglich z. B. durch eine vorhandene Leukopenie, ein zu geringer Prozentsatz an infizierten peripheren neutrophilen Granulozyten, durch technische Fehler oder eine fehlerhafte Interpretation. Zu dicke Blutausstriche können wiederum zu falsch positiven Ergebnissen führen. Bei leukopenischen Katzen ist ein IFT aus einer Knochenmarksprobe anstatt aus peripherem Blut zu bevorzugen, da diese eine höhere Konzentration an Leukozyten aufweisen [37].Der IFT findet heute wegen der Fehleranfälligkeiten und der aufwendigen Durchführung kaum noch Verwendung.

				SNAP® FIV/FeLV Kombi Plus		WITNESS® FeLV/FIV		ONE-Step®		DUO Speed® FeLV/FIV		FASTest®		Anigen® Rapid FIV Ab/FeLV Ag	
	FeLV-Prävalenz	**Anzahl beprobter Katzen**	**Referenz-standard**	**Sens. (NPV) %**	**Spez. (PPV) %**	**Sens. (NPV) %**	**Spez. (PPV) %**	**Sens. (NPV) %**	**Spez. (PPV) %**	**Sens. (NPV) %**	**Spez. (PPV) %**	**Sens. (NPV) %**	**Spez. (PPV) %**	**Sens. (NPV) %**	**Spez. (PPV) %**
Hartmann et al. 2001	**8,6 %**	800	Virus-isolierung	91,3 (99,2)	98,2 (82,9)	66,6 (96,9)	98,7 (83,3)	88,4 (98,8)	91,8 (50,8)	89,6 (99,0)	98,1 (81,1)	85,5 (98,6)	98,2 (81,9)	n. u.	n. u.
Hartmann et al. 2007	**7,4 %**	536	Virus-isolierung	92,3 (99,4)	97,3 (73,5)	92,1 (99,4)	97,5 (74,5)	96,8 (99,7)	95,4 (62,0)	94,7 (99,6)	99,2 (90,0)	94,7 (99,6)	98,8 (85,7)	n. u.	n. u.
Sand et al. 2010	**5,0 %**	300	Provirus-PCR	53,3 (97,6)	100,0 (100,0)	n. u.	n. u.	n. u.	n. u.	n. u.	n. u.	n. u.	n. u.	40,0 (96,9)	100,0 (100,0)
Westman et al. 2017	**8,0 %**	563	Provirus-PCR	63,0 (94,0)	94,0 (62,0)	57,0 (94.0)	98,0 (80,0)	n. u.	n. u.	n. u.	n. u.	n. u.	n. u.	57,0 (94,0)	98,0 (79,0)
Levy et al. 2017	**5,0 %**	300	2 Platten-ELISA*	100,0 (100,0)	100,0 (100,0)	89,0 (99,0)	95,5 (51,0)	n. u.	n. u.	n. u.	n. u.	n. u.	n. u.	91,8 (100,0)	95,5 (52,0)

*Einschluss in die Studie nur dann, wenn Ergebnisse der 2 verschiedenen Mikrotiterplatten-ELISA-Tests übereinstimmten.

Sens. = Sensitivität, NPV = negativ prädiktiver Wert, Spez. = Spezifität, PPV = positiv prädiktiver Wert, n. u. = nicht untersucht

Tab. 3 Vergleich verschiedener Point-Of-Care-Tests zum Nachweis von p27-Antigen zu unterschieidlichen Referenzmethoden in verschiedenen Studien unter Berücksichtigung von Sensitivität, Spezifität, positiv und negativ prädiktivem Wert.

Table 3 Comparison of different point-of-care-tests based on various studies for the detection of p27 antigen considering sensitivity, specificity, positive and negative predictive value.

FeLV-Provirus-DNA

Bei progressiv und regressiv infizierten Katzen wird provirale DNA in das Genom der Katze integriert und kann 1–2 Wochen nach einer FeLV-Infektion mittels PCR aus peripheren Blut nachgewiesen werden (Tab. 1) [16]. Wird die Katze positiv auf das Vorhandensein von Provirus-DNA getestet, ist sie daher entweder progressiv oder regressiv infiziert. Die Bestimmung der Proviruslast (= die Quantifizierung der vorhandenen DNA) kann bei der Zuordnung des Infektionsverlaufs helfen [25]. Bei natürlich infizierten Katzen ist der Zeitpunkt der Infektion meist unbekannt und die Zuordnung einer Verlaufsform nicht immer einfach, da sich die Mengen an Provirus-DNA zwischen progressiv und regressiv infizierten Katzen während einer frühen Infektion nicht unterscheiden [19]. Im weiteren Verlauf sind Katzen mit einer hohen Proviruslast und einer vorliegenden Virämie (p27-Antigen-positiv) progressiv infiziert, während niedrige Mengen an proviraler DNA, ohne vorliegende Virämie mit einer regressiven Infektion verbunden sind [25, 26]. Es gilt zu berücksichtigen, dass für die Durchführung einer PCR ein qualifiziertes Labor notwendig ist, welches entsprechende Kontrollen (Negativ- und Positivkontrollen) mitführt und eine Aussage über die analytische Sensitivität und Spezifität des verwendeten Verfahren treffen kann [25].

Ein Provirus-negativer Befund kann bei einer Katze auftreten, wenn die Katze entweder keinen Kontakt zu FeLV hatte, sich erst kürzlich (im Zeitraum von 1–2 Wochen) mit FeLV infiziert hat oder die Viruslast so gering ist, dass sie unter der Detektionsgrenze der verwendeten Provirus-PCR liegt. Weitere Möglichkeiten sind, dass die Katze abortiv oder fokal infiziert ist [2, 19].

FeLV-RNA

FeLV-RNA ist innerhalb 1 Woche nach Infektion im Speichel oder 1–2 Wochen nach Infektion im Blut (Plasma) nachweisbar [47]. Der Nachweis viraler RNA im Speichel ist durch die im Speichel vorhandene hohe Viruslast ein zuverlässiger Parameter für eine Virämie und eine damit verbundene Virusausscheidung [10, 46]. So wurde die Reverse-Transkriptase-PCR (RT-PCR) zum Nachweis viraler RNA im Speichel natürlich infizierter Katzen evaluiert [9]. Dazu wurden Blutproben von 445 Katzen auf das Vorhandensein von Provirus-DNA (PCR), viraler RNA im Plasma und Speichel (RT-PCR) und p27-Antigen (Mikrotiterplatten-ELISA)

getestet. Im Vergleich zum Mikrotiterplatten-ELISA lag die Sensitivität und Spezifität für den Nachweis von FeLV-RNA im Speichel mittels RT-PCR bei 98,1 % und 99,2 %. Der FeLV-RNA-Nachweis im Speichel kann daher als alternative Testmethode zu p27-Antigen-Tests herangezogen werden oder als Bestätigungstest, um ein positives oder fragliches p27-Antigen-Testergebnis zu bestätigen [9, 10, 46]. Angesichts der hohen Kosten und der relativ langen Bearbeitungszeit für die Durchführung einer RT-PCR (1–3 Tage), wird diese Testmethode noch selten eingesetzt. Bei Straßenkatzen oder Katzen in Tierheimen ohne tierärztliche Betreuung vor Ort könnten mit dieser Methode jedoch eine Blutentnahme umgangen und der Infektionsstatus einfach bestimmt werden. Es besteht die Möglichkeit, bis zu 10 Speichelproben im Labor zu poolen und gleichzeitig zu analysieren. Sollte eine gepoolte Speichelprobe positiv ausfallen, ist es notwendig, die einzelnen Katzen mittels RT-PCR aus einzelnen Speichelproben oder mit einem p27-Antigentest aus Blut zu testen, um die FeLV-ausscheidende(n) Katze(n) zu identifizieren. Wird eine Katze positiv auf virale RNA getestet, ist sie zum Zeitpunkt der Testung virämisch und somit FeLV-Ausscheider. Das Ergebnis ist daher vergleichbar mit einem positiven Test auf p27-Antigen aus Blut. Auch hier ist wichtig, dass für die Durchführung der RT-PCR ein qualifiziertes Labor gewählt wird und entsprechende Kontrollen mitgetestet werden [25].

Wird bei einer Katze keine virale RNA im Speichel nachgewiesen, ist die Katze zum Zeitpunkt der Testung nicht virämisch und scheidet kein Virus aus. Das kann bedeuten, dass die Katze nicht infiziert ist oder sich in einem sehr frühen Stadium der FeLV-Infektion befindet (innerhalb der ersten Woche); sie könnte aber auch eine regressive oder abortive Infektion haben [47, 56].

Anti-FeLV-Antikörper

Sowohl die humorale als auch die zelluläre Immunantwort sind wichtig für den Schutz vor einer FeLV-Infektion. Nach der Exposition mit FeLV können bei Katzen mit einer abortiven oder regressiven Infektion virusneutralisierende Antikörper nachgewiesen werden, ebenso nach manchen Impfungen, abhängig vom verwendeten Impfstoff. Katzen mit einer progressiven Infektion und hoher Viruslast entwickeln in der Regel weder Antikörper, noch hohe Konzentrationen FeLV-spezifischer zytotoxischer Lymphozyten. Dies weist auf eine unzureichende

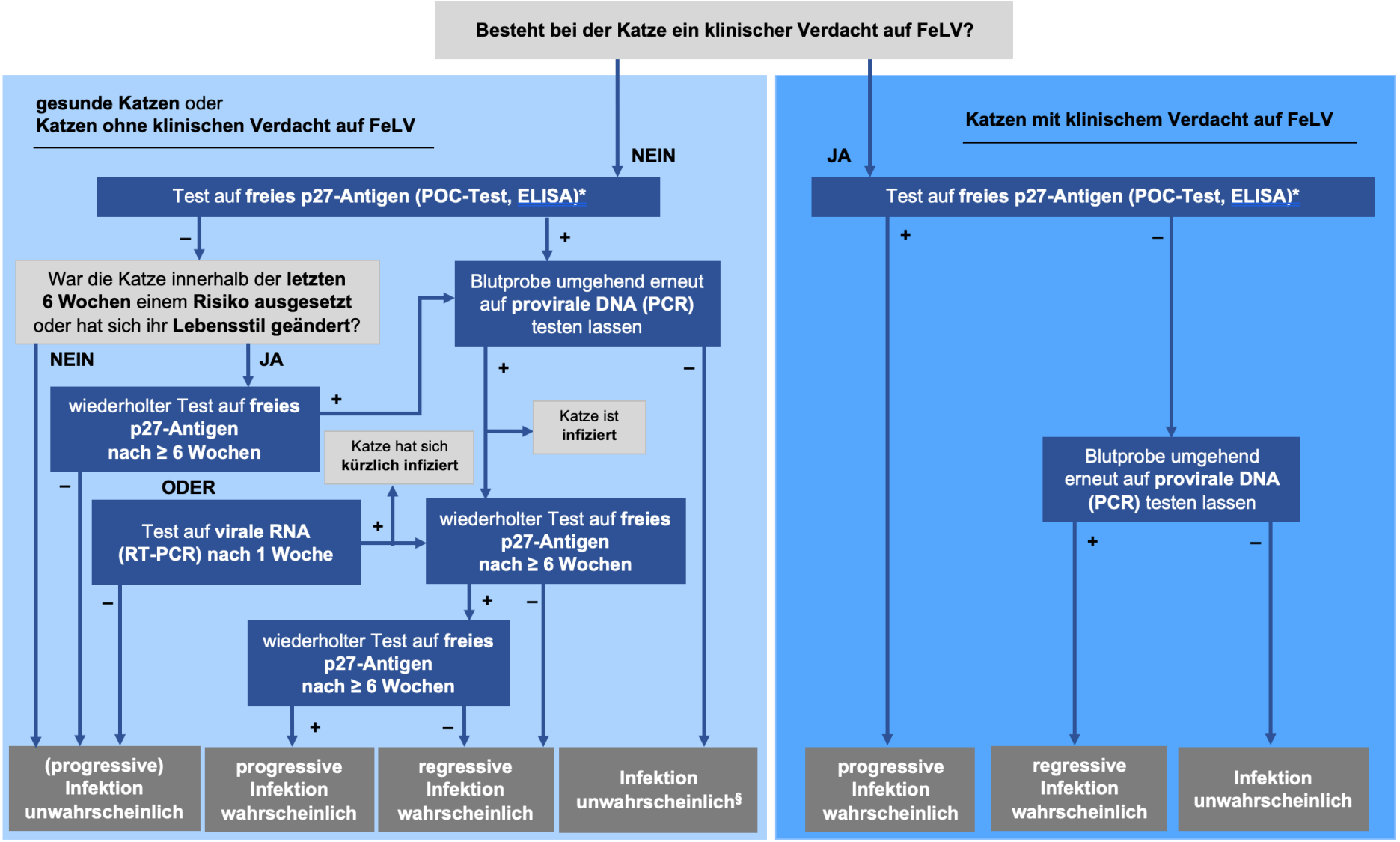
humorale und zellvermittelte Immunantwort hin [22]. Die virusneutralisierenden Antikörper sind vorwiegend gegen das Oberflächenglykoprotein gp70 (Abb. 1) gerichtet und stellen wahrscheinlich den wichtigsten Teil des Abwehrmechanismus dar. In einer Studie zur Untersuchung der humoralen Immunantwort bei Katzen wurden 123 mit FeLV infizierte Katzen mittels eines neuem ELISA auf Antikörper gegen das FeLV-Oberflächenglykoprotein gp70 (Abb. 1) untersucht. Als Referenzstandard wurde ein Virusneutralisationstest verwendet. Katzen, die im Verlauf eine regressive Infektion entwickelten (8,1 %; 10/123) zeigten höhere neutralisierende anti-gp70 Antikörper und eine niedrigere Proviruslast im Vergleich zu Katzen mit progressiver Infektion (68,3 %; 84/123). Angesichts der niedrigen Katzenanzahl mit bekanntem Impfstatus war es jedoch nicht möglich, die Sensitivität und Spezifität des neuen ELISA festzulegen [29].

Das Transmembranprotein p15E (Abb. 1) hemmt T-Zell-Funktionen und hat immunsuppressive Eigenschaften [57]. Durch Verwendung von rekombinantem FeLV-p15E wurde ein Antikörper-Test entwickelt. Es wurde nachgewiesen, dass FeLV-infizierte Katzen Antikörper gegen p15E entwickelten [58]. Katzen, die nach einer FeLV-Infektion immun oder virämisch wurden, hatten hohe Antikörperspiegel gegen p15E [28]. In einer Folgestudie wurde untersucht, inwiefern sich der Nachweis von anti-p15E-Antikörpern für die Diagnose einer FeLV-Infektion eignet. Insgesamt wurden Serumproben von 294 Katzen aus der Schweiz unter anderem auf das Vorhandensein von p15E-Antikörpern untersucht. Als Referenzstandard wurde die Untersuchung auf Provirus-DNA mittels PCR herangezogen. Die Sensitivität und Spezifität von p15E-Antikörpern bei experimentell infizierten Katzen betrugen 95,7 % bzw. 100,0 %. Bei natürlich infizierten Katzen zeigte der Nachweis von anti-p15E-Antikörper eine Sensitivität von 77,1 % und eine Spezifität von 85,6 %. Gegen FeLV geimpfte Katzen wiesen niedrige Antikörpertiter gegen p15E auf, was darauf hindeutet, dass anti-p15E-Antikörper eher eine vorangegangene Infektion als eine Impfung anzeigen. Jedoch hing das Ergebnis von dem verwendeten Impfstoff ab. Ein POC-Test zum Nachweis von Antikörpern gegen FeLV p15E-Antigen wurde kürzlich auf dem europäischen Markt eingeführt. Allerdings liegen derzeit nicht genügend Daten vor, um den Nutzen dieses Tests für die Diagnose einer FeLV-Infektion im Feld beurteilen zu können [2]. Zudem ist unklar, wie gut das Vorhandensein von p15E-Antikörpern mit dem Schutz vor FeLV übereinstimmt und wie lange Antikörper

(nach Impfung und Infektion) nachweisbar sind.

Diagnostisches Vorgehen bei Verdacht auf FeLV-Infektion

Um die diagnostische Vorgehensweise und die Testinterpretation zu erleichtern, hat das Expertengremium „European Advisory Board on Cat Diseases“ (ABCD) einen Diagnosealgorithmus (abcdcatsvets.org, „ABCD FeLV diagnostic tool“) erstellt (Abb. 3). Dieser Diagnosealgorithmus basiert auf der Risikobewertung sowie der klinischen Präsentation der Katze. Er berücksichtigt verschiedene Testmethoden, deren Testcharakteristika und gibt Empfehlungen für das weitere Vorgehen bei einem positiven p27-Antigen-Testergebnis. Wird eine Katze positiv auf freies p27-Antigen (POC-Test, ELISA) getestet, sollte das Ergebnis unmittelbar, z. B. anhand einer Provirus-PCR, oder virale RNA mittels RT-PCR in Blut oder Speichel oder anhand eines vorzugsweise anderen POC-Tests (oder ELISA) überprüft werden [3] [1, 2]. Die Überprüfung eines positiven Ergebnisses ist insbesondere bei niedriger FeLV-Prävalenz und geringem Expositionsrisiko der getesteten Katze (z. B. reine Wohnungskatze ohne Kontakt zu anderen Katzen) wichtig. Bestätigt sich das Ergebnis als positiv, wird die Katze nach 6 Wochen erneut auf freies p27-Antigen getestet. Ist die Katze wiederholt positiv, wird sie nach weiteren 6 Wochen noch einmal getestet, um festzustellen, ob es sich um eine progressive oder regressive Infektion handelt. Ist die Katze weiterhin p27-Antigen-positiv, ist sie mit hoher Wahrscheinlichkeit progressiv infiziert. Ein negatives Ergebnis auf p27-Antigen bei wiederholter Untersuchung macht dagegen eine regressive Infektion sehr wahrscheinlich.



FeLV = Felines Leukämievirus, POC = Point-Of-Care, ELISA = Enzyme-linked Immunosorbent Assay, DNA = Desoxyribonukleinsäure, PCR = Polymerase-Kettenreaktion, RNA = Ribonukleinsäure, RT-PCR = Reverse-Transkriptase-Polymerase-Kettenreaktion.

Abb. 3 Diagnoseschlüssel des Expertengremiums „European Advisory Board on Cat Diseases“ (ABCD) zum Nachweis einer Infektion mit dem felinen Leukämievirus. *Als Alternative zur Bestimmung von freiem p27-Antigen im Blut kann auch eine RT-PCR auf virale RNA im Speichel durchgeführt werden. §In seltenen Fällen kann eine fokale FeLV-Infektion der Grund für einen positiven Test auf freies p27-Antigen und ein negatives Provirus-PCR-Ergebnis in Blutproben sein. Quelle: www.abcdcatsvets.org

Figure 3 Diagnostic tool of the expert organisation "European Advisory Board on Cat Diseases" (ABCD) for the detection of feline leukaemia virus infection. *As an alternative to the determination of free p27 antigen in the blood, a test for viral RNA in saliva can also be evaluated by means of RT-PCR. §In rare cases, a focal FeLV infection can be the reason for positive test for free p27 antigen and negative provirus PCR result in blood samples. Source: www.abcdcatsvets.org

Testung auf FeLV-Infektion in verschiedenen Situationen

Einzelkatze

Der FeLV-Status jeder Katze sollte bekannt sein. Bei jeder Katze sollte individuell abgeschätzt werden, wie hoch das Risiko einer FeLV-Infektion ist. Daher sollte das Testverfahren dementsprechend angepasst werden. Um Hinweise auf eine vorliegende Virämie und damit verbundene Virusausscheidung zu erlangen, sollte bei jeder Katze, unabhängig davon, wie hoch das Infektionsrisiko ist, eine Testung auf p27-Antigen mittels POC-Test erfolgen. Alternativ kann eine RT-PCR auf virale RNA im Speichel durchgeführt werden. Insbesondere bei Katzen mit geringem Infektionsrisiko sollte ein positives Testergebnis überprüft werden. Negative Testergebnisse sind meist zuverlässig, wenn POC-Tests mit einer hohen Sensitivität (Tab. 3) verwendet werden und die getestete Katze aus einer Population mit niedriger FeLV-Prävalenz stammt. Eine Infektion kann nicht ausgeschlossen werden, wenn sich die Katze in der frühen Phase der Infektion (< 3 Wochen), vor der Entwicklung einer Virämie, befindet, wenn ein Antigennachweis noch nicht möglich ist [2, 3].

Des Weiteren sollte vor jeder Impfung gegen FeLV der FeLV-Status der Katze bestimmt werden. Eine Impfung von bereits infizierten Katzen hat keinen Nutzen [59] und sollte vermieden werden. Jede Impfung kann mit unerwünschten Reaktionen und schwerwiegenden Nebenwirkungen, wie z. B. das „feline injection site sarcoma“ (FISS), verbunden sein [60]. Gemäß den aktuellen Impfrichtlinien der „Ständigen Impfkommission (StIKo Vet)“ und der „American Association of Feline Practitioners (AAFP)“ wird empfohlen, nur so oft wie nötig und so selten wie möglich zu impfen (z. B. keine Impfung bei FeLV-Antigen-positiven Katzen und FeLV-PCR-positiven Katzen [61, 62]. Darüber hinaus ist es sinnvoll, vor allem bei Katzen mit Freigang, den FeLV-Status in jährlichen Abständen (z. B. im Rahmen der Gesundheitsvorsorge) oder nach einer potenziellen FeLV-Exposition zu bestimmen. Auch sollte bei reinen Wohnungskatzen die Bestimmung des FeLV-Status zumindest einmalig erfolgen. Sofern die Katze als reine Wohnungskatze gehalten wird, keinen Kontakt zu anderen Katzen hat, klinisch gesund ist und eine FeLV-Infektion bereits ausgeschlossen wurde, ist eine erneute Bestimmung des FeLV-Status nicht notwendig.

Private Mehrkatzenhaushalte

Das Ziel sollte sein, nur Katzen desselben Infektionsstatus gemeinsam zu halten. Im Idealfall sind in einem Mehrkatzenhaushalt keine Virusträger, d. h. weder progressiv, noch regressiv infizierte Katzen, vorhanden. Um dies sicherzustellen, ist es notwendig, alle Katzen mittels PCR auf Provirus-DNA zu testen, um auch regressiv infizierte Katzen zu erkennen. Soll eine Katze mit unbekanntem FeLV-Status aus einer fremden Herkunft (z. B. Fundkatze) oder mit unbekannter Exposition (z. B. Katze mit Zugang ins Freie, Katze aus Tierheim) in eine Gruppe von nicht FeLV-infizierten Katzen integriert werden, ist es wichtig, die Katze vor der Zusammenführung auf Virusausscheidung (p27-Antigen) oder Provirus zu testen. Kann eine kürzlich stattgefundene Virusexposition nicht ausgeschlossen werden, sollte zusätzliche eine Quarantänezeit von möglichst 6 Wochen (mindestens 3 Wochen) eingehalten werden und eine wiederholte Testung auf Virusausscheidung erfolgen [63].

Tierheime

Grundsätzlich sollte jede Katze, die in ein Tierheim kommt, auf FeLV-p27-Antigen getestet werden und für mindestens 3 Wochen, besser 6 Wochen, zunächst in Quarantäne gehalten werden. In Gebieten mit einer hohen FeLV-Prävalenz sollten Katzen, die beim ersten Mal p27-Antigen-negativ getestet wurden, trotzdem unbedingt eine Quarantäne von 6 Wochen einhalten. Bevor die isolierten Katzen in Kontakt mit anderen Katzen kommen, sollten sie erneut getestet werden, da es nach einer FeLV-Exposition in der Regel mindestens 3–6 Wochen dauert, bevor p27-Antigen im peripheren Blut einer infizierten Katze nachgewiesen werden kann. Dies gilt insbesondere für Tierheime, bei denen die Katzen nicht getrennt gehalten werden. Nur so können auch erst vor Kurzem infizierte Katzen erkannt werden. Wird eine Katze positiv auf p27-Antigen getestet, muss diese als infiziert und Virusausscheider betrachtet und getrennt von anderen Katzen untergebracht werden [2, 63]. Eine erneute Testung zu einem späteren Zeitpunkt ist zudem wichtig, um die Verlaufsform der Infektion zu bestimmen (Abb. 3).

Progressiv infizierte, gesunde Katzen sollten so schnell wie möglich in ein geeignetes Zuhause vermittelt werden. Es muss sichergestellt sein, dass solche Katzen kein Infektionsrisiko für andere Katze darstellen und daher getrennt von

FeLV-negativen Katzen in reiner Wohnungshaltung gehalten werden. Es besteht jedoch die Möglichkeit, sie gemeinsam mit anderen progressiv infizierten Katzen zu halten. Idealerweise sollten auch regressiv infizierte Katzen (p27-Antigen-negativ, Provirus-positiv) getrennt von nicht FeLV-infizierten Katzen gehalten werden, da eine regressive Infektion unter Stresssituationen reaktiviert werden kann und die Katze dann wieder Virus ausscheidet [63, 64].

Katzenzuchten

Die FeLV-Prävalenz in Katzenzuchten ist in vielen, vor allem nordeuropäischen Ländern, mittlerweile sehr niedrig [38]. Ein Katzenkontakt zwischen verschiedenen Zuchten sollte nur dann erfolgen, wenn die jeweiligen Züchter ähnliche FeLV-Untersuchungsprogramme durchführen. Wenn junge und adulte Katzen Zugang ins Freie und/oder Kontakt zu Katzen mit unbekanntem FeLV-Status haben, sollten diese geimpft werden [64].

Blutspenderkatzen

Bei einer Bluttransfusion kann eine FeLV-Infektion auf die Empfängerkatze übertragen werden. Sowohl progressiv als auch regressiv infizierte Katzen können provirale DNA über Bluttransfusionen auf ungeschützte Empfängerkatzen übertragen und bei diesen zur Virämie und FeLV-assoziierten Erkrankungen führen [65]. Methoden zum Nachweis von freiem p27-Antigen sind daher nicht ausreichend, um die Übertragung einer FeLV-Infektion bei einer Bluttransfusion zu verhindern. Jede Katze, die für eine Blutspende in Frage kommt, sollte daher auf provirale DNA mittels PCR getestet werden [2].

Fazit für die Praxis

Der FeLV-Status jeder Katze sollte bekannt sein, da eine FeLV-Infektion die Prognose und das klinische Management einer kranken Katze beeinflusst. Das Verständnis der Pathogenese einer FeLV-Infektion sowie die Kenntnis über die verschiedenen Nachweisverfahren ist eine wichtige Voraussetzung für die korrekte Interpretation von Testergebnissen und die Bestimmung des FeLV-Status einer

Katze. Katzen, die eine progressive Infektion durchlaufen, können eine tödlich verlaufende FeLV-assoziierte Erkrankung (z. B. Anämie, Lymphom) entwickeln. Diese Katzen stellen epidemiologisch gesehen das größte Risiko dar, da sie FeLV in großen Mengen (vor allem über den Speichel) ausscheiden. Regressiv infizierte Katzen sind oft schwer detektierbar, da sie durch diagnostische Routineuntersuchungen (p27-Antigentest) nicht erkannt werden. Diese Katzen sind jedoch auch in der Lage, die Infektion (z. B. über eine Bluttransfusion) weiterzugeben und oder sie zu reaktivieren.

Interessenkonflikt

Die Autoren bestätigen, dass kein Interessenkonflikt besteht.

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III. PUBLIKATION 2 (ORIGINALPUBLIKATION)

Prevalence of different courses of feline leukaemia virus infection in four European countries

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Article

Prevalence of Different Courses of Feline Leukaemia Virus Infection in Four European Countries

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Abstract: Prevalence of progressive feline leukaemia virus (FeLV) infection is known to still be high in cats in Europe, especially in Southern Europe, but the prevalence of other outcomes of FeLV infection has not been determined in most countries. The present study aimed to investigate the prevalence of progressive, regressive, abortive, and focal infection in four European countries, two with a high (Italy, Portugal) and two with a low expected prevalence (Germany, France). Blood samples of 934 cats (Italy: 269; Portugal: 240; France: 107; Germany: 318) were evaluated for the p27 antigen, as well as anti-whole virus, anti-SU, and anti-p15E antibodies by enzyme-linked immunosorbent assay (ELISA) in serum and for proviral DNA by quantitative polymerase chain reaction (qPCR) in whole blood. Positive p27 antigen ELISA results were confirmed by reverse transcriptase-qPCR (RT-qPCR) detecting viral RNA in saliva swabs and/or blood. The outcome of FeLV infection was categorised as progressive (antigen-positive, provirus-positive), regressive (antigen-negative, provirus-positive), abortive (antigen- and provirus-negative, antibody-positive), and focal (antigen-positive, provirus-negative) infection. Overall FeLV prevalence was 21.2% in Italy, 20.4% in Portugal, 9.5% in Germany, and 9.3% in France. Prevalence of progressive, regressive, abortive, and focal infection in Italy was 7.8%, 4.5%, 6.3%, and 2.6%; in Portugal 3.8%, 8.3%, 6.7%, and 1.7%; in Germany 1.9%, 1.3%, 3.5%, and 2.8%; in France 1.9%, 3.7%, 2.8%, and 0.9%, respectively. In conclusion, overall FeLV prevalence is still very high, especially in Southern European countries. Therefore, testing, separation of infected cats, and vaccination are still important measures to reduce the risk of FeLV infection.

Keywords: FeLV; retrovirus; prevalence; p27 antigen; proviral DNA; viral RNA; antibody levels; Europe

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1. Introduction

Feline leukaemia virus (FeLV) is a gammaretrovirus that is widespread worldwide and one of the most important infectious agents in cats [1–3]. Due to the complex pathogenesis and the different courses of FeLV infection, diagnosis is challenging and often not possible using a single test. FeLV infection can take progressive, regressive, abortive, or focal (atypical) courses [1,2]. However, even when established, courses can change into each other. For example, cats that are initially progressively infected can develop a regressive course of infection. Conversely, regressively infected cats can become progressively infected. Differentiation between the FeLV outcomes is difficult, especially in

naturally infected cats [1–4]. The individual outcome in a FeLV-infected cat is determined by the immune status of the infected cat, influenced by pre-existing immunity or age, and by viral characteristics, such as the virulence of the virus or infection pressure. Several factors, such as immunosuppression, coinfections, and stress can influence the immune response, and thus the course of infection [2]. In progressive infection, the immune system of the affected cats is unable to sufficiently control virus replication and its systemic spread, and viraemia persists. During the viraemic phases, free p27 antigen can be detected in serum/plasma, proviral DNA (deoxyribonucleic acid) in blood, and viral RNA (ribonucleic acid) in blood and saliva [5]. Progressive infection can lead to immunodeficiency, bone marrow suppression, and neoplasia, and is commonly fatal [4,6,7]. On the contrary, with the help of an effective immune response, cats that are regressively infected are able to stop or significantly inhibit viral replication. Due to the pronounced immune response, regressively infected cats generally have high levels of virus-neutralising antibodies. In contrast to progressively infected cats, in regressively infected cats, viraemia never occurs or only lasts briefly at the beginning of the infection and potentially (rarely) reoccurs later, after reactivation [6]. Abortively infected cats produce virus-neutralising antibodies and are able to effectively control virus replication [8–10]. Neither FeLV p27 antigen, proviral DNA, nor viral RNA can be detected in these cats. Abortive infection can only be diagnosed by the detection of antibodies [4,9,11–13].

FeLV prevalence of progressive FeLV infection, which is easily detected, varies worldwide, ranging from 1 to 9% in Europe [14]. According to a recent Europe-wide study of the Advisory Board on Cat Diseases [15] including 6005 cats in 30 European countries, the highest prevalence was found in Portugal (8.8%), Hungary (5.9%), Italy (5.7%), and Malta (5.7%). France and Germany were considered to be low-prevalence countries, with a prevalence of 1.0% and 0.3%, respectively [14]. In this and many other prevalence studies, however, only progressive infections were assessed. Nevertheless, when considering all courses of FeLV infection, the overall FeLV prevalence is considered to be much higher. This was demonstrated in a German study in 2012, in which 1.8% (9/495) of cats were progressively, 1.2% (6/495) regressively, and 9.2% (22/246) abortively infected with FeLV [12]. However, the prevalence of regressive and abortive infection is largely unknown in most European countries.

Therefore, the aim of the present multicentre, prospective, and cross-sectional study was to determine the prevalence of all courses of FeLV infection in cats from four different countries in Europe with different FeLV prevalence, including two countries with high suspected prevalence (Italy and Portugal) and two countries with low suspected prevalence (Germany and France).

2. Materials and Methods

2.1. Samples and Data Collection

A total of 934 cats from four different European countries were prospectively included. Two countries with a suspected high overall FeLV prevalence (Italy and Portugal) and two countries with a suspected low overall FeLV prevalence (Germany and France) according to a previous study [14] were included. Of all cats, 318 originated from Germany, 269 from Italy, 240 from Portugal, and 107 from France. A body weight of >1 kg was a prerequisite for inclusion. Overall, eleven veterinary clinics in Italy, four veterinary clinics in Portugal, two veterinary clinics in Germany, and one veterinary clinic in France participated. Cats from which blood was collected for different reasons were included and originated from private homes, shelters, or were stray. The study was accepted by the ethical committee of the Centre for Clinical Veterinary Medicine of the LMU Munich, Germany (reference number 142–25–08–2018).

Serum and ethylenediaminetetraacetic acid (EDTA) whole blood samples were collected from all cats. In addition, saliva swabs (cotton swabs with plastic shafts) were collected from 815/934 cats and transferred to a reagent tube (1.5 mL, Sarstedt, Nümbrecht, Germany). Both blood and saliva samples from Italy, Portugal, and France were stored at

−20 °C, while samples from Germany were stored at −80 °C, in all cases for a maximum of 24 months before the samples were sent on dry ice to the Clinical Laboratory from the Department of Clinical Diagnostics and Services, and the Center for Clinical Studies at the Vetsuisse Faculty, University of Zurich, for examination. In Zurich, all samples were stored at −80 °C until analysis for a maximum of four months.

Data were provided from 777/934 owners using a questionnaire that included questions on cat identification (name of the veterinary practice, cat and cat owner, date of collection) and demographic data of the cat (age, sex, neutering status, cat breed), on housing conditions (indoor only, outdoor only, or indoor and outdoor access, number of cats in the household), history of illness, previous FeLV infection status if available (FeLV antigen-positive, FeLV antigen-negative, unknown), and FeLV vaccination status.

2.2. Sample Analysis

2.2.1. Detection of FeLV p27 Antigen

All 934 cats were tested for the presence of free p27 antigen in serum by the sandwich enzyme-linked immunosorbent assay, as described previously [16]. All samples were tested in duplicates and absorbances were read using a microplate reader (Synergy H1, Biotek, Winooski, VT, USA). Values > 4% of the positive control were considered positive [17].

2.2.2. FeLV Viral RNA in Saliva and Blood

To confirm positive p27 antigen results, blood and saliva samples from all p27 antigen-positive cats were tested for FeLV viral RNA using a published reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay [14,18]. Positive and negative controls were run in parallel with each RT-qPCR. All negative samples were retested, and diluted at 1:5 and 1:10 in a neutral buffer with a pH of 7.4 (0.15 M sodium chloride, 1 mM EDTA, 0.05 M Tris-base, 0.1% BSA, 0.1% Tween 20) to make possible inhibition unlikely. The viral RNA copy number was determined using a standard curve (serial tenfold dilutions of purified RNA from FEA/FeLV-A infected cells [18]) run with the same conditions (reaction composition, instrument and thermal profile) as described [14].

2.2.3. FeLV Proviral Load in Blood

FeLV proviral DNA was evaluated in all 934 cats. Total nucleic acids were extracted from 100 µL EDTA anticoagulated whole blood using the MagNA Pure 96 instrument (Roche Diagnostics AG, Rotkreuz, Switzerland) and Viral NA SV Kit (Roche Diagnostics AG, Rotkreuz, Switzerland) according to the manufacturer's instructions with 100 µL elution buffer [10]. For all samples, the viral nucleic acid (NA) plasma external lysis SV 4.0 protocol was applied and negative controls of phosphate-buffered saline (PBS) run in parallel with each batch of samples to monitor for cross-contamination. Proviral DNA was amplified and quantified using 5 µL of TNA and 20 µL of DNA quantitative PCR (qPCR) Mastermix (Eurogentec, Seraing, Belgium) containing 480 nM primers (exoFeLV-U3F2, exoFeLV-U3R3) and a 160 nM probe (exoFeLV-U3p). All oligonucleotides were synthesised by a Microsynth AG (Balgach, Switzerland). The temperature profile consisted of 2 min (min) at 50 °C, denaturation of 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s (s) and 60 °C for 1 min. The FeLV proviral copy numbers in the single samples were determined by coamplifying 10-fold serial dilutions of a DNA standard template, as described [18]. All samples that tested positive in the p27 antigen ELISA were diluted 1:5 and 1:10 and retested to avoid a false-negative result in the provirus qPCR due to possible inhibition. To verify the quantity and quality of the TNA, a qPCR for feline albumin was performed on all 934 TNA samples [19].

2.2.4. Detection of Anti-FeLV Antibodies

Serum samples of all 934 cats were analysed for the presence of antibodies against FeLV whole virus (FL-74), FeLV SU (p45), and FeLV p15E using indirect ELISA, as previously described [4,10,20].

Detection of Anti-Whole Virus (FL-74) and Anti-SU (p45) Antibodies

Serum samples were analysed for the presence of antibodies to FeLV p45 and to FeLV whole virus by ELISA, using 100 ng of the p45 antigen and 100 ng of gradient-purified FL-74 FeLV per well and serum dilutions of 1:200, respectively, as described previously [21]. Positive and negative controls were run with each assay. For negative controls, serum samples from specific pathogen-free (SPF) cats were used. For positive controls, serum samples collected from cats known to have antibodies against FeLV (=100% positive control) were used. Values ≥25% of the positive control were considered positive [12].

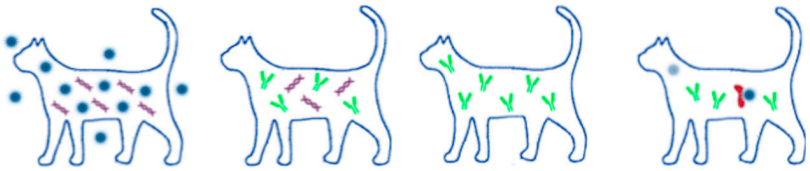
Detection of Anti-p15E Antibodies

An ELISA for the detection of anti-p15E antibodies was used, as previously described [10]. Positive and negative controls were included in each assay. For negative controls, serum from SPF cats was used. For positive controls, pooled serum samples from cats experimentally infected with FeLV-A/Glasgow-1 (=100% positive control) were included. Relative optical density (ROD) values were determined using the formula ROD = [(sample OD − negative control OD)/(positive control OD − negative control OD)]. Samples with an ROD that tested >16.3% (ROD value 0.163) compared to the positive control were considered anti-p15E antibody-positive [10].

2.3. FeLV Infection Status

According to the European Advisory Board on Cat Diseases [15], the different courses of FeLV infection were defined as follows [3]: (1) progressive infection (p27 antigen-positive, provirus-positive), (2) regressive infection (p27 antigen-negative, provirus-positive), (3) abortive infection (p27 antigen-negative, provirus-negative, anti-p15E- and anti-SU antibody-positive), (4) focal infection (p27 antigen weakly positive, provirus-negative), and (5) FeLV-uninfected (p27 antigen-negative, provirus-negative, anti-p15E and anti-SU antibody-negative) (Table 1). Samples that were p27 antigen-negative, provirus-negative, anti-p15E antibody-positive, but anti-SU antibody-negative were considered "unclassified".

Table 1. Courses of feline leukaemia virus infection (FeLV) and the expected test results. Adapted from the European Advisory Board on Cat Diseases FeLV diagnostic tool [15].

	Progressive	**Regressive**	**Abortive**	**Focal (Atypical)**
				
p27 AG	positive	negative	negative	(weakly) positive
viral RNA	positive	negative	negative	negative or positive
proviral DNA	positive	positive	negative	negative
anti-SU/whole virus AB	negative	(usually) positive (variable titre)	positive	(usually) positive (variable titre)
anti-p15E AB	negative or positive	(usually) positive (variable titre)	positive	(usually) positive (variable titre)

AG, antigen; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; AB, antibody.

2.4. Statistical Analysis

Laboratory data of all cats were analysed using Excel (Microsoft Germany GmbH, Munich, Germany) and the R statistical language (version 4.1.2; R Core Team, 2020). The normality of data distribution was determined by the Shapiro–Wilk test. Due to non-normally distributed data, a nonparametric test was used for statistical analysis. The Mann–Whitney U test was used to compare p27 antigen concentration in progressively and focally infected cats. In addition, Mann–Whitney U test was used to compare the proviral load of progressively and regressively infected cats. The Kruskal–Wallis and Dunn–Bonferroni tests were used to compare anti-FeLV antibody levels in progressively, regressively, abortively, and focally infected cats. The Spearman correlation was conducted for the correlation between p27 antigen concentration in blood, viral RNA loads in blood and saliva, and anti-p15E antibody levels. Results with a p-value <0.05 were considered statistically significant.

3. Results

3.1. Study Population

At the time of sampling, 56% (377/670) of cats were healthy and had no history of illness, whereas 44% (293/670) of cats were either sick at the time of testing or had a history of illness. According to the questionnaires, in 70% (654/934) of all cats, FeLV vaccination status was known; 12% (81/654) of these cats were vaccinated against FeLV. The vaccine manufacturers and vaccination dates were available in 67% (54/81) of the vaccinated cats. Age was known in 82% (765/934) of the cats and ranged from 8 weeks to 20 years (median: 3 years). Table 2 summarises the signalment, history of illness, vaccination, and housing condition of the cats. Table 3 gives an overview of the vaccines used in the individual countries.

Table 2. Signalment and history of the cats included in the study.

Variables	Modalities	Italy n = 269 (%)	Portugal n = 240 (%)	Germany n = 318 (%)	France n = 107 (%)	Total n = 934 (%)
age	kitten (<1 year)	80 (30)	47 (20)	80 (25)	6 (6)	213 (23)
	young adult (1–6 years)	98 (36)	55 (23)	122 (38)	22 (20)	297 (32)
	mature adult (7–10 years)	51 (19)	20 (8)	56 (18)	13 (12)	140 (15)
	senior (>10 years)	33 (12)	8 (3)	54 (17)	20 (19)	115 (12)
	unknown	7 (3)	110 (46)	6 (2)	46 (43)	169 (18)
sex	female	126 (47)	88 (37)	154 (48)	35 (33)	403 (43)
	male	139 (52)	68 (28)	161 (51)	24 (22)	392 (42)
	unknown	4 (1)	84 (35)	3 (1)	48 (45)	139 (15)
neutering status	intact	77 (29)	13 (5)	60 (19)	5 (4)	155 (16)
	neutered	176 (5)	131 (55)	218 (69)	51 (48)	576 (62)
	unknown	16 (6)	96 (40)	40 (12)	51 (48)	203 (22)
breed	mixed breed cat *	236 (88)	133 (55)	195 (61)	48 (45)	612 (66)
	purebred cat	28 (10)	17 (7)	121 (38)	3 (3)	169 (18)
	unknown	5 (2)	90 (38)	2 (1)	56 (52)	153 (16)
housing status	outdoor only	65 (24)	50 (21)	61 (19)	2 (2)	178 (19)
	in- and outdoor	55 (21)	27 (11)	26 (8)	1 (1)	109 (12)
	indoor only	138 (51)	70 (29)	141 (44)	2 (2)	351 (37)
	shelter	0 (0)	0 (0)	74 (23)	0 (0)	74 (8)
	unknown	11 (4)	93 (39)	16 (6)	102 (95)	222 (24)
cats in household	1 cat	79 (29)	11 (5)	131 (41)	1 (1)	222 (24)
	2–4 cats	90 (33)	26 (11)	68 (22)	0 (0)	184 (20)
	≥5 cats	42 (16)	41 (17)	33 (10)	0 (0)	116 (12)
	shelter, colony, stray	26 (10)	8 (3)	76 (24)	0 (0)	110 (12)
	unknown	32 (12)	154 (64)	10 (3)	106 (99)	302 (32)

Table 2. *Cont.*

Variables	Modalities	Italy n = 269 (%)	Portugal n = 240 (%)	Germany n = 318 (%)	France n = 107 (%)	Total n = 934 (%)
history of illness	healthy	170 (63)	112 (47)	95 (30)	0 (0)	375 (40)
	pre-existing illness/sick	88 (33)	27 (11)	177 (56)	1 (1)	293 (32)
	unknown	11 (4)	101 (42)	46 (14)	106 (99)	264 (28)
FeLV vaccination status	vaccinated	34 (13)	11 (5)	24 (7)	12 (11)	81 (9)
	not vaccinated	224 (83)	115 (48)	231 (73)	3 (3)	573 (61)
	unknown	11 (4)	114 (47)	63 (20)	92 (86)	280 (30)

* European Shorthair, European Longhair, Domestic Shorthair, Domestic Longhair; n, number of cats.

Table 3. Number of cats in which the feline leukaemia virus (FeLV) vaccination status was known that had received different FeLV vaccines in the individual countries.

Vaccine *	Italy n = 269 (%)	Portugal n = 240 (%)	Germany n = 318 (%)	France n = 107 (%)	Total n = 934 (%)
Purevax® FeLV	24 (9)	4 (2)	15 (5)	-	43 (5)
Leucogen®	2 (1)	1 (0)	4 (1)	-	7 (0)
Versifel® FeLV	2 (1)	1 (0)	-	-	3 (0)
Fevaxyn® Pentofel	-	-	-	1 (1)	1 (0)
unknown vaccine	6 (2)	5 (2)	5 (1)	11 (10)	27 (3)
total	34 (13)	11 (4)	24 (7)	12 (11)	81 (9)

* In total, four different FeLV vaccines were used. The vaccines used against FeLV included recombinant canarypox virus (vCP97) vaccine (Purevax® FeLV, Boehringer Ingelheim Vetmedica GmbH, Rohrdorf, Germany), a monovalent FeLV subunit vaccine (Leucogen®, Virbac Animal Health, Carros, France), an inactivated whole virus vaccine containing FeLV-A, FeLV-B, and FeLV-C (Versifel® FeLV, Zoetis Animal Health, Parsippany-Troy Hills, NJ, USA), and an inactivated feline leukaemia virus vaccine (Fevaxyn® Pentofel, Zoetis, Ottignies-Louvain-la-Neuve, Belgium).

3.2. FeLV Test Results

A summary of testing results in the different countries is shown in Table 4.

Table 4. Number and percentage of positive test results of all cats included in the study from Italy, Portugal, Germany, and France.

Test	Italy n = 269 (%)	Portugal n = 240 (%)	Germany n = 318 (%)	France n = 107 (%)	Total n = 934 (%)
p27 AG	27 (10.0)	13 (5.4)	15 (4.7)	3 (2.8)	58 (6.2)
viral RNA	21 (7.8)	9 (3.7)	6 (1.9)	2 (1.9)	38 (4.1)
proviral DNA	33 (12.3)	29 (12.1)	10 (3.1)	6 (5.6)	78 (8.3)
anti-SU AB	53 (19.7)	74 (30.8)	66 (20.7)	15 (14.0)	208 (22.2)
anti-whole virus AB	33 (12.3)	35 (14.6)	42 (13.2)	23 (21.5)	133 (14.2)
anti-p15E AB	57 (21.2)	54 (22.5)	45 (14.1)	39 (36.4)	195 (19.8)

AG, antigen; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; AB, antibodies; SU, surface unit; n, number of cats.

3.3. Prevalence of Different FeLV Courses in the Four European Countries

Overall, considering all courses, FeLV infection prevalence was 15.6% (146/934), with 20.8% (56/269) in Italy, 20.4% (49/240) in Portugal, 9.3% (10/107) in France, and 9.1% (29/318) in Germany (Table 5). Not all cats could be assigned to a definitive course of infection; cats with an unknown vaccination status that were p27 antigen-negative, provirus-negative, anti-p15E antibody-positive but anti-SU antibody-negative were considered "unclassifiable" (total 2.1% (20/934); Italy, 2.2% (6/269), Portugal 0.8% (2/240),

Germany 1.3% (4/318), and France 7.5% (8/107)) and were not included in the total infection prevalence.

Table 5. Prevalence of the different courses of feline leukaemia virus (FeLV) infection in Italy, Portugal, France, and Germany.

Course of Infection	Italy n = 269 (%)	Portugal n = 240 (%)	Germany n = 318 (%)	France n = 107 (%)	Total n = 934 (%)
progressive	21 (7.8)	9 (3.8)	6 (1.9)	2 (1.9)	38 (4.1)
regressive	12 (4.5)	20 (8.3)	4 (1.3)	4 (3.7)	40 (4.3)
abortive	17 (6.3)	16 (6.7)	11 (3.5)	3 (2.8)	47 (5.0)
focal (atypical)	7 (2.6)	4 (1.7)	9 (2.8)	1 (0.9)	21 (2.3)
total infected	57 (21.2)	49 (20.4)	30 (9.4)	10 (9.4)	146 (15.6)
unclassified [1]	6 (2.2)	2 (0.8)	4 (1.3)	8 (7.5)	20 (2.1)

[1] Samples that were p27 antigen-negative, provirus-negative, anti-p15E antibody-positive, but anti-SU antibody-negative were considered "unclassified".

3.4. Viral Loads and p27 Antigen Levels in the Different Courses of Infection

All cats that tested positive for p27 antigen by ELISA were considered to have either a progressive or a focal infection. Cats with progressive infection had significantly higher antigen levels (median: 91%, range: 5–197%) compared to cats with focal infection (median: 6%, range: 4–20%, $p < 0.001$) (Figure 1).

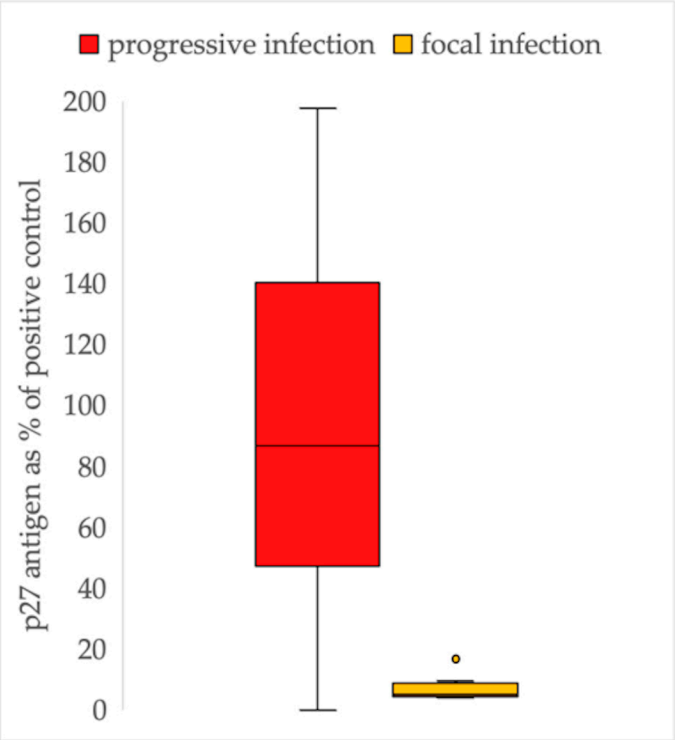


Figure 1. Results of p27 antigen enzyme-linked immunosorbent assays (ELISA) in cats with progressive (p27 antigen-positive, provirus-positive) and focal feline leukaemia virus infections (p27 antigen weakly positive, provirus-negative). Progressively infected cats had significantly higher antigen concentrations (median: 91%, range: 5–197%, $p < 0.001$) than cats with focal infection (median: 6%, range: 4–20%).

The blood proviral loads (Figure 2) were significantly higher in progressively infected cats (n = 37) (range: 43–9,501,447 copies) than in regressively infected cats (n = 40) (range: 1–428 copies). Two cats with a progressive course had a low proviral load (copy numbers < 60 (43, 59)). All other progressively infected cats (n = 35) had high proviral loads (copy numbers > 60 (237–9,501,447 copies). Among the regressively infected cats, one cat had a high proviral load (428 copies). All other regressively infected cats (n = 39) had low proviral loads (copy numbers < 60 (1–59 copies)).

In cats with progressive infection, a strong significant correlation was observed between the p27 antigen concentration and viral RNA load in saliva (Spearman correlation = 0.65; $p < 0.001$) (Figure 3A). There was also a correlation between the p27 antigen concentration and viral RNA load in blood (Spearman correlation = 0.38; $p = 0.027$) (Figure 3B).

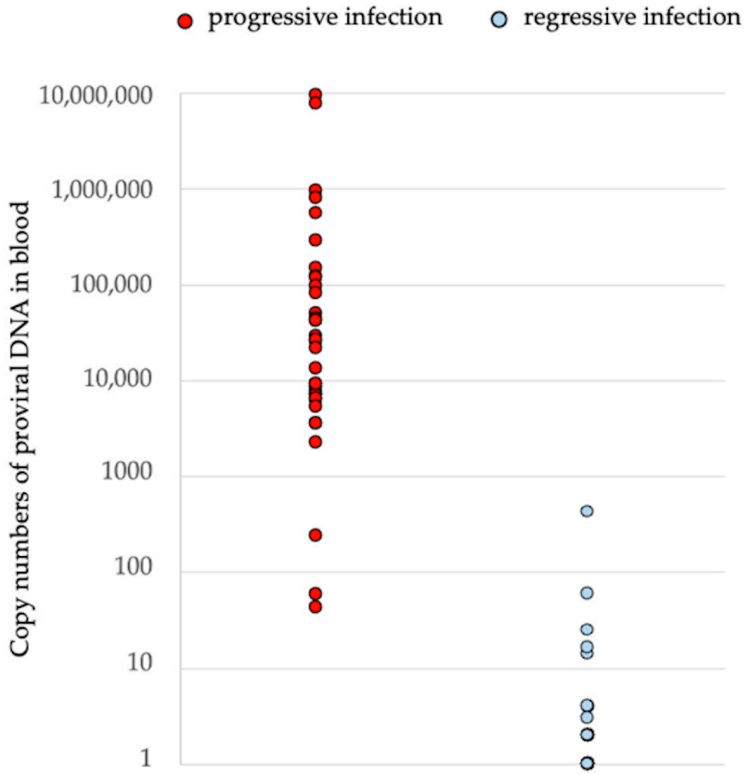
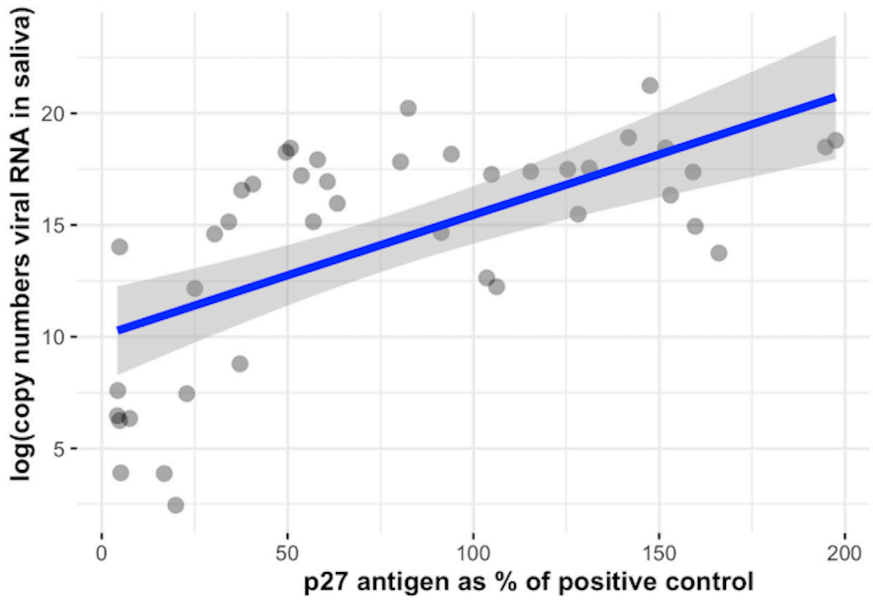


Figure 2. Proviral load in progressively (p27 antigen-positive, viral RNA in saliva and/or blood detectable, provirus-positive) and regressively infected cats (p27 antigen-negative, provirus-positive). Cats with progressive infection had significantly higher copy numbers (median: 21,543, range: 43–9,501,447) compared to cats with regressive infection (median: 1, range: 1–428) ($p < 0.001$).

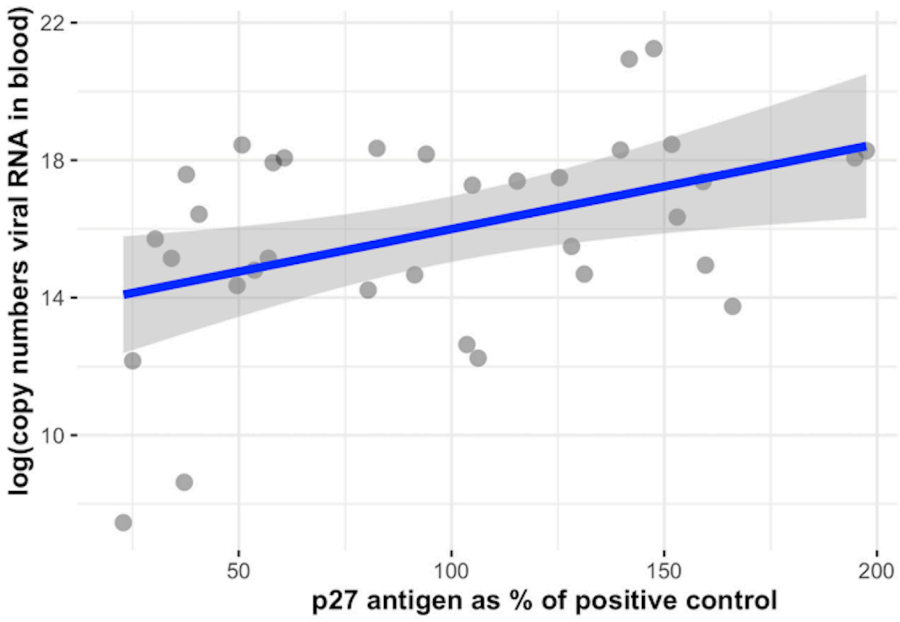
3.5. Antibody Levels in Different Courses of Infection

Cats with an abortive infection had significantly higher anti-SU (p45) antibody levels (median: 37%, range: 25–116%) compared to cats with progressive (median: 15%, range: 0–62%) ($p < 0.001$), regressive (median: 20%, range: 1–113%) ($p < 0.001$), and focal (median: 23%, range: 6–53%) ($p < 0.001$) infection. Regressively infected cats had significantly higher anti-whole virus antibody levels (median: 49%, range: 0–190%) compared to progressively (median: 4%, range: 0–63%) ($p < 0.001$) and abortively (median: 18%, range: 0–82%) ($p = 0.011$) infected cats. Progressively infected cats had significantly lower antibody levels compared to abortively infected ($p = 0.005$) and focally infected cats (median: 23%, range: 5–123%) ($p \leq 0.001$). In contrast, progressively infected cats had significantly higher anti-p15E antibody levels (median: 64%, range: 0–121%) than abortively (median: 25%, range: 17–114%) ($p < 0.001$) infected cats. Abortively infected cats had significantly higher antibody levels compared to regressively (median: 19%, range: 0–152%) ($p < 0.001$) and

focally infected cats (median: 11%, range: 0–40%) ($p = 0.002$) (Figure 4). In addition, there was a significant correlation between anti-p15E antibody levels in blood and p27 antigen in the blood (Spearman correlation = 0.4709; $p \leq 0.001$) (Figure 5).



(**A**)



(**B**)

Figure 3. Correlation between p27 antigen concentration in blood and viral RNA loads in blood and saliva. The grey shading indicates 95% confidence intervals. (**A**) A strong significant correlation was observed between the p27 antigen concentration and viral RNA load in saliva (Spearman correlation = 0.65; $p < 0.001$). (**B**) A significant correlation was also observed between the p27 antigen and viral RNA load in the blood (Spearman correlation = 0.38; $p = 0.027$). RNA, ribonucleic acid.

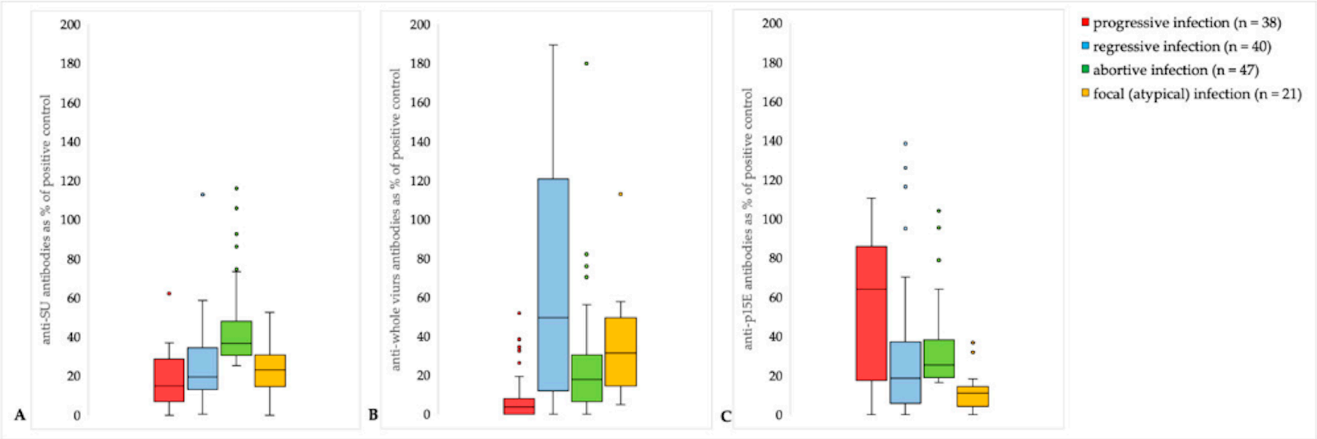


Figure 4. Box plots of anti-whole virus (FL-74), anti-SU (p45), and anti-p15E antibodies in progressively (red), regressively (blue), abortively (green), and focally (yellow) infected cats. (**A**) Cats with abortive infection had significantly higher anti-SU (p45) antibody levels (median: 37%, range: 25–116%) compared to cats with progressive (median: 15%, range: 0–62%) ($p < 0.001$), regressive (median: 20%, range: 1–113%) ($p < 0.001$), and focal (median: 23%, range: 6–53%) ($p < 0.001$) infection. (**B**) Regressively infected cats had significantly higher anti-whole virus antibody levels (median: 49%, range: 0–190%) compared to progressively (median: 4%, range: 0–63%) ($p < 0.001$) and abortively (median: 18%, range: 0–82%) ($p = 0.011$) infected cats. Progressively infected cats had significantly lower antibody tires compared to abortively infected ($p = 0.005$) and focally infected cats (median: 23%, range: 5–123%) ($p \leq 0.001$). (**C**) Progressively infected cats had significantly higher anti-p15E antibody levels (median: 64%, range: 0–121%) compared to abortively (median: 25%, range: 17–114%) ($p < 0.001$) infected cats. Abortively infected cats had significantly higher antibody levels compared to regressively (median: 19%, range: 0–152%) ($p < 0.001$) and focally infected cats (median: 11%, range: 0–40%) ($p = 0.002$).

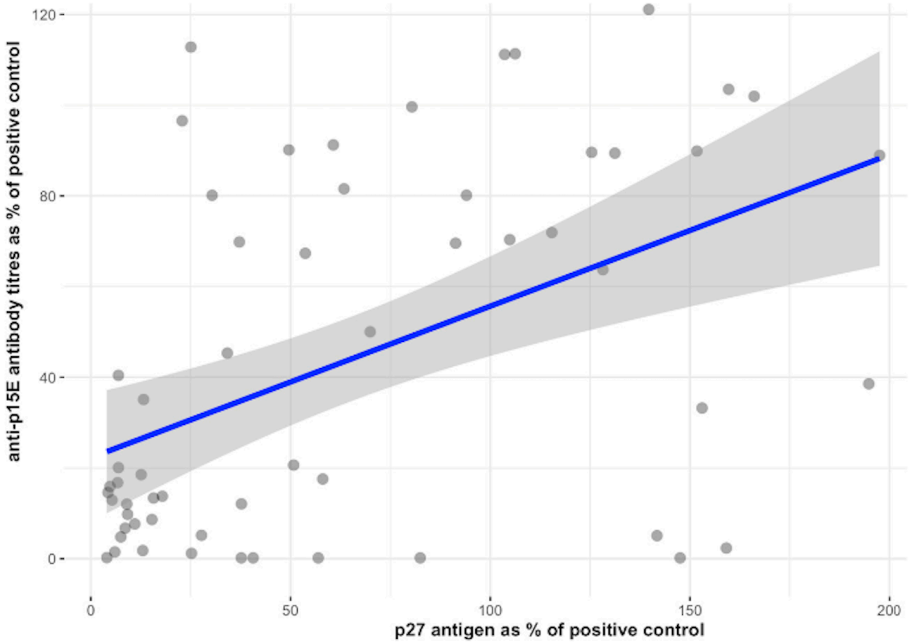


Figure 5. Correlation between anti-p15E antibody levels in blood and p27 antigen levels in blood. A significant positive correlation was observed between the anti-p15E antibody concentration and p27 antigen concentration in blood (Spearman correlation = 0.431; $p \leq 0.001$.

4. Discussion

This is the first study to determine the FeLV prevalence in four European countries considering all courses of FeLV infection (progressive, regressive, abortive, focal (atypical)). There was still a larger number of infected cats than expected when considering all infection stages, with more cats being infected in Southern Europe (Italy 21.2%, Portugal 20.4%) than in Western Europe (Germany 9.5%, France 9.3%).

In the present study, more cats in Southern Europe (Italy 7.8%, Portugal 3.8%) had a progressive course of infection in contrast to cats in Western Europe (Germany 1.9%, France 1.9%). Similar results on FeLV prevalence have been obtained in a pan-European study conducted by the Advisory Board of Cat Diseases [15] in 2019, in which 6005 cats were tested for FeLV shedding by RT-qPCR from saliva swabs [14]. The prevalence in Portugal, Italy, Germany, and France was 8.8%, 5.7%, 0.3%, and 1.0%, respectively. One possible explanation why the prevalence of progressively infected cats in Germany and France was higher in the present study compared to the study in 2019 could be that, in Germany in particular, several cats from animal shelters were sampled, and FeLV prevalence could be higher in shelter cats compared to those presented at veterinary clinics [22]. In France, only one veterinary clinic, and therefore only a small proportion of the country, was included in the study. The prevalence might be lower in other parts of France. It is unlikely that the higher prevalence in the present study is caused by false-positive results as in the present study, all progressively infected cats also tested positive for viral RNA in blood; and all progressively infected cats in which a saliva swab was available were positive for viral RNA in RT-qPCR. In a study in Switzerland including blood samples from 445 cats, the sensitivity and specificity for the detection of FeLV RNA in saliva by RT-qPCR were 98.1% and 99.2%, respectively, and the detection of viral RNA in saliva and viral RNA in blood, each tested by RT-qPCR, showed almost perfect agreement (kappa = 0.96) [5].

In focally (atypically) infected cats, there are usually little replicating virus and infected cells in the peripheral blood. However, focally infected cells can produce soluble p27 antigen and release it into the peripheral blood, which can lead to a positive p27 antigen test [11,23–25]. In the present study, viral RNA was also detected in saliva in these cats. Therefore, detection of RNA in the saliva of focally infected cats is possible when the replication of the virus after oronasal ingestion is limited to the mucosa of the oropharynx and local lymphoid tissue and affects the epithelium of the salivary glands and pharynx [5,26,27].

The determination of the course of infection often necessitates several samplings, especially at the beginning of the infection, when a battle between the cat's immune system and the virus can prevail; but what the clinician usually sees is only a snapshot (one-time consultation) [2]. Determining the course of infection is therefore based on an assumption. For example, regressively infected animals can become progressively infected because of reactivation of the virus [1]. In the present study, there was one cat that was negative in the p27 antigen ELISA but tested positive for viral RNA in the blood. The reason why this cat was assigned to a progressive course was that all other laboratory results indicated a progressive course of FeLV infection (viral-RNA-positive in blood, proviral-DNA-positive, anti-p15E antibody-positive). It is possible that the p27 antigen ELISA in this cat was false-negative, although this is unlikely due to several repetitions of the ELISA. Another explanation could be that the cat was at the beginning of the FeLV infection and the p27 antigen could not yet be detected. The cat had a high proviral load in blood (8503 copies/PCR reaction). However, it should be noted that, during the early stages of infection, both progressively and regressively infected cats can exhibit a high proviral load [4]. In the present study, as well as in a recently published study, it was shown that progressively infected cats had higher anti-p15E antibody levels compared to regressively infected cats [28]. The cat classified as progressively infected had a high antibody level of 92%, which is also indicative of a progressive course.

Studies have shown that, once the course of infection is established, progressively infected cats, in contrast to regressively infected cats, have a higher proviral load, and the proviral load in progressively infected cats remains consistently high after an initial

phase [29]. In the present study, progressively infected cats had a significantly higher proviral load compared to regressively infected cats. This suggests that the determination of proviral DNA in blood by PCR alone can provide an indication of the course of the infection. A study from Switzerland showed that cats that tested negative for p27 antigen but positive for proviral DNA in blood had significantly lower proviral load (by a factor of 300) [29]. However, it was impossible to draw a cut-off that distinguished all regressively infected cats from progressively infected cats; there was some overlap the proviral DNA loads between the two groups of cats. Thus, it is important to repeat the test at a later timepoint and to initiate further diagnostic measures (p27 antigen, viral RNA), especially in case of unclear results, to allow a clear assignment to a course of infection. Especially in naturally infected cats, the assignment of a course is not always possible, as the time of infection is usually unknown and the amounts of proviral DNA do not differ between progressively and regressively infected cats during early infection [4].

The reason for the different distribution of FeLV course in the individual countries in the present study is still unclear. In general, several subgroups of exogenous FeLV (exoFeLV) (FeLV-A, -B, -C, -D, -E, and -T) are distinguished in addition to endogenous FeLV (enFeLV). Multiple studies provided evidence of an association between enFeLV loads and the replication of FeLV-A. In one study, it was observed that cats with high enFeLV loads were more likely to develop progressive FeLV infection, while cats with low enFeLV loads had a lower risk [8,23,24,30]. There might be differences between the FeLV subtypes in the respective countries. A study from the USA in 2018 showed that cats with a progressive course and a higher proviral load were significantly more likely to be FeLV-B-positive. In contrast, regressive, abortive, and uninfected cats had higher levels of enFeLV [31]. The properties of the cat, such as age or genetic background, including the endogenous viral load, might also play a role in the development of the respective courses in the present study. In order to strengthen this assumption, further investigations, such as genetic analyses, would be necessary.

There are only a few studies that determined FeLV prevalence considering all courses of FeLV infection. In a study performed in Southern Germany in 2012, 7.5% (37/495) of cats were infected with FeLV. Out of these, 1.8% (9/495) of cats were progressively infected (p27 antigen-positive), 1.2% (6/495) were regressively infected (p27 antigen-negative, provirus-positive), and 9.2% (22/246) of unvaccinated cats were abortively infected with FeLV (anti-p45 antibody-positive; >25%). The presence of focally infected cats was not investigated [12]. The prevalence of progressively and regressively infected cats was comparable to the results from Germany in the present study; however, the proportion of abortively infected cats in the present study was 3.5%, in contrast to 9.2% previously. One reason for this difference could be that the criteria for the classification of an abortive course were chosen differently. In the German study, all SU antibody-positive cats were assigned to an abortive course of infection. In the present study, cats had to be anti-SU antibody-positive and anti-p15E antibody-positive to be considered abortively infected. Cats with anti-SU antibody concentrations >25% of the positive control were defined as positive [12]. This cut-off was chosen because SPF cats can have antibody concentrations of up to 25% because of unspecific reactions or antibodies against enFeLV (H Lutz, E Boenzli, data not published, personal communication). Anti-p15E antibody levels >16.3% (relative optical density (ROD) value 0.163) of the positive control were therefore considered positive, as defined previously for field cats when establishing the p15E ELISA [10]. A recently published study showed that, in Australia, 57.4%, and in Germany, 8.2% of FeLV-unexposed cats had anti-p15E antibody tires >16.3%. These findings highlight that the cut-off value of the p15E laboratory ELISA should be considered critically and should be reconsidered [28]. Anti-SU antibodies can be strongly increased after vaccination against FeLV, depending on the vaccine used, as well as after FeLV infection. Cats vaccinated against FeLV using canarypox vectored vaccines might not develop detectable antibody levels before coming into contact with FeLV [18,32]. In a study from Germany, anti-SU antibodies could not be detected in 64.3% of cats vaccinated against FeLV [12]. In contrast, cats vaccinated

with a recombinant FeLV subunit vaccine containing p45 (unglycosylated SU) showed a high anti-SU antibody response [4,33]. Curiously, there were more vaccines used in Italy than in Portugal, Germany, and France, yet the FeLV prevalence was higher in Italy. It should, however, be noted that the FeLV vaccination status of many cats was unknown and therefore the number of cats vaccinated against FeLV was likely much higher in all countries. Since the vaccination status of many cats was unknown in the present study, anti-p15E antibodies were additionally selected to assign the cats to an abortive course of infection. In a study carried out in Switzerland, serum samples from 294 cats were used to test the suitability of the detection of anti-p15E antibodies for the diagnosis of FeLV infection [10]. Anti-p15E antibodies were found to be useful to differentiate infected from uninfected animals, suggesting that anti-p15E antibodies indicate previous infection rather than vaccination [10,20]. In the present study, cats with a progressive course had higher anti-p15E antibody levels compared to cats with regressive, abortive, or focal infection. Furthermore, a positive correlation between the concentration of p27 antigen in blood and the level of anti-p15E antibody levels in the blood was found. Regressively infected cats had high anti-whole virus antibody levels, while progressively infected cats had low concentrations of anti-whole virus and anti-SU antibodies, except for a few outliers. This suggests that the presence of anti-whole virus and anti-SU antibodies is more likely to be a sign of protection against FeLV infection when compared to anti-p15E antibodies. It is still unclear whether the presence of anti-p15E antibodies also corresponds to protection against FeLV and how long these antibodies are detectable (after vaccination and infection). In the present study, the presence of anti-p15E antibodies was more likely a marker for viral replication. It is also possible that cats that were classified as progressively infected and that had high anti-whole virus antibodies could be in a phase of switching to regressive infection if the antibodies were constantly high [34]. Since all cats were only sampled on a single date, no statement can be made about the long-term course.

Therefore, the most important limitation of the present study was that the FeLV status was determined only based on one blood sample. The second limitation was the presence of selection bias, as only cats in which blood was taken any way for diagnostic reasons were included. This unavoidable preselection might have influenced FeLV prevalence. However, it is important to note that these data provide insights into the FeLV situation among cats admitted to veterinary practices in the respective countries.

5. Conclusions

In conclusion, this is the first study that determined the prevalence of FeLV in four European countries considering all courses of FeLV infection (progressive, regressive, abortive, focal (atypical)). When all courses of infection were taken into account, the overall FeLV prevalence was still very high, especially in Italy and Portugal (Southern Europe). The number of infected cats in Germany and France (Western Europe) was also still remarkably high. Therefore, the FeLV status of each cat should be known. If a cat cannot be assigned to a course of infection, follow-up testing and the use of further laboratory diagnostic methods can be helpful. Even though FeLV prevalence in the tested countries decreased in previous years, further steps (testing, separation of infected cats, and vaccination) are needed to control infection risk and reduce prevalence further.

Author Contributions: Conceptualisation, K.H., M.B. and R.H.-L.; methodology, J.G., S.J. and M.B.; validation J.G.; formal analysis, J.G., Y.Z., M.B., K.H. and R.H.-L.; investigation, J.G. and M.B.; resources, K.H. and R.H.-L.; data curation, J.G., S.J., S.T.-K., M.-G.P., N.L., R.S. and S.B.; writing—original draft preparation, J.G.; writing—review and editing, M.B., S.J., M.L.M., B.P., E.B., S.T.-K., Y.Z., M.-G.P., N.L., R.S., S.B., R.H.-L. and K.H.; visualisation, J.G. and Y.Z.; supervision, M.B., R.H.-L. and K.H.; project administration, M.B., R.H.-L. and K.H.; funding acquisition, K.H. and R.H.-L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The present study was approved by the ethical committee (reference number 142-25-08-2018) of the Centre for Clinical Veterinary Medicine of the LMU Munich. All cat owners gave their written consent to participate.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors confirm that the datasets analysed during the study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The salary for Juliana Giselbrecht's employment as a research assistant at the Small Animal Clinic, Centre for Clinical Veterinary Medicine LMU Munich, was paid by scil animal care company GmbH (Viernheim, Germany). However, there are no conflict of interests since the company had no role in the design of the study, the collection, analyses, or interpretation of data, writing of the manuscript, or in the decision to publish the results. The University of Zurich holds a patent on feline leukaemia virus (FeLV) transmembrane protein p15E for the diagnosis of FeLV infection, and R.H.-L. is one of the coinventors of this test.

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IV. PUBLIKATION 3 (ORIGINALPUBLIKATION)

Field performance of a rapid test to detect progressive, regressive, and abortive feline leukemia virus infections in domestic cats in Australia and Germany

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Article

Field Performance of a Rapid Test to Detect Progressive, Regressive, and Abortive Feline Leukemia Virus Infections in Domestic Cats in Australia and Germany

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Abstract: Different feline leukemia virus (FeLV) infection outcomes are possible in cats following natural exposure, such as progressive infections (persistent viremia), regressive infections (transient or no viremia followed by proviral persistence) and abortive infections (presence of only antibodies). Laboratory-based testing is currently required for categorization of infection outcomes in cats. The aim of this study was to evaluate the field performance of a novel, rapid, combination point-of-care (PoC) test kit commercially available in Europe (v-RetroFel®Ag/Ab; 2020–2021 version) to determine different FeLV infection outcomes by concurrent detection of FeLV antigen (p27) and antibodies against FeLV transmembrane envelope protein (p15E). A secondary aim was to evaluate the performance of the same test kit (v-RetroFel®FIV) to determine positive/negative feline immunodeficiency virus (FIV) infection status by the detection of antibodies to FIV capsid protein (p24) and transmembrane glycoprotein (gp40). Two cohorts of domestic cats were recruited and tested with v-RetroFel® using plasma or serum, including cats in Australia (n = 200) and cats in Germany (n = 170). Results from p27 antigen PoC testing, proviral DNA PCR, and neutralizing antibody testing or testing for antibodies against non-glycosylated surface unit envelope protein (p45) were used to assign cats to groups according to different FeLV infection outcomes. Testing with a laboratory-based FeLV p15E antibody ELISA was also performed for comparison. In the first cohort, v-RetroFel®Ag/Ab correctly identified 89% (109/122) FeLV-unexposed cats and 91% (21/23) progressive infections, but no regressive (0/23) or abortive (0/32) infections. In the second cohort, v-RetroFel®Ag/Ab correctly identified 94% (148/158) FeLV-unexposed cats and 100% (4/4) progressive infections, but no regressive (0/2) and only 17% (1/6) abortive infections. There was test agreement between v-RetroFel®Ab and the p15E laboratory ELISA in 58.9% of samples. As a secondary outcome of this study, the sensitivity and specificity of v-RetroFel®FIV testing in cohort 1 were 94.7% (18/19) and 98.3% (178/181), and in cohort 2, 30.0% (3/10) and 100.0% (160/160), respectively. Prior history of FIV vaccination did not produce any false-positive FIV results. In conclusion, v-RetroFel®Ag/Ab (2020–2021 version) was unable to accurately determine different FeLV infection outcomes in the field. Improvements of the test prior to application to field samples are required.

Keywords: antibodies; FeLV; FIV; infection; humoral immunity; v-RetroFel®; vaccination; veterinary science

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1. Introduction

Feline leukemia virus (FeLV) is a *Gammaretrovirus* that infects domestic and non-domestic felids worldwide [1–5]. Both exogenous and endogenous forms of FeLV have been identified, with recombination and mutation events giving rise to different FeLV subgroups [1,6–13]. Exogenous FeLV-A is the subgroup almost exclusively transmitted horizontally between cats [14].

Exposure to exogenous FeLV-A produces a spectrum of possible outcomes in cats, depending on the challenge dose, virus virulence, infection pressure (e.g., single exposure vs. extended contact), cat age, and host immunity factors [15–21]. Terminology used to describe different categories of FeLV infection has developed over time with the advent of molecular testing [15,22–24]. Currently, both European and North American guidelines on the prevention, diagnosis, and management of FeLV have adopted the nomenclature of progressive, regressive, and abortive infections [25,26].

Progressively infected cats are persistently viremic, with a primary viremia involving local oropharyngeal lymphoid tissue (duration 1–12 weeks) followed by a secondary viremia caused by infection of the bone marrow (2–16 weeks and beyond) [15,26,27]. Progressively infected cats have a poor prognosis, and can eventually develop disorders of hematopoiesis, immune suppression, and neoplasia, resulting in a prognosis for survival of only three years for up to 80–90% of infected cats [25,27–30].

In regressively infected cats, a primary viremia usually (but not always) occurs before a sufficient host immune response is mounted to clear the viremia [15,26,27], but lifelong infection in the form of proviral DNA integration results [15,26,29]. The prognosis for regressive infections varies, with some studies reporting an association with lymphomagenesis; additionally, regressive infections can be reactivated [31–35].

Cats with abortive infections are never viremic and resist proviral integration due to a timely and robust immune response, and carry the same long-term prognosis as FeLV-uninfected cats [15,21,26].

A gamut of testing is required to classify the type of FeLV infection following exposure. The mainstay of FeLV screening is detection of viral capsid protein (p27) with rapid point-of-care (PoC) test kits (antigen testing) [15,26,36]. Other available FeLV testing options, depending on the country, include PCR testing to detect proviral DNA in blood or bone marrow, immunofluorescent antibody testing to detect cell-associated p27-antigen, virus isolation (VI) to detect viable virus presence in body fluids, and reverse-transcriptase (RT)-PCR testing to detect viral RNA in blood or saliva [15,21,37–42]. Since progressive and regressive infections can be indistinguishable very early in the course of infection (both being p27-positive, PCR-positive and RT-PCR-positive during the viremic phase), repeat p27-antigen testing can be required to differentiate progressive (persistently p27-positive) and regressive infections (transiently p27-positive) [15,29,43–45].

Antibody testing can be useful for identifying FeLV exposure and assigning a category of infection [15,16,42,44,46]. Regressive and abortive infections, but not progressive infections, usually have a detectable neutralizing antibody (NAb) response [15,20,43,47]. All FeLV-infected cats, irrespective of infection outcome (i.e., progressive, regressive, or abortive), are assumed to develop antibodies against the FeLV transmembrane protein (p15E) [48,49]. Progressive infections, however, have been reported to have weaker immunoblot and p15E enzyme-linked immunosorbent assay (ELISA) reactions than regressive and abortive infections [50]. Based on results from a laboratory-based ELISA to detect anti-p15E antibodies [49], the first commercially available FeLV antibody PoC test kit (v-RetroFel®, Scil Animal Care Company, Viernheim, Germany) was launched in Europe in April 2018, with the manufacturer claiming test results can discriminate between different infection types.

Regressively infected cats have been demonstrated to transmit FeLV infection to FeLV-naive cats via blood transfusion [51]. Consequently, FeLV PCR testing of all donor cats prior to blood transfusion is recommended to identify regressive infections and facilitate the removal of these cats from blood donor programs [52]. Since commercial PCR testing usu-

ally involves a 1–3 day turnaround for results, when a rapid blood transfusion is required, this approach is usually not possible. Therefore, having the ability to identify regressively infected cats in an acute emergency setting quickly and easily by PoC testing prior to blood transfusion would be advantageous [52]. Similarly, rapid identification of regressively infected cats in multi-cat household situations by PoC testing would be useful [15,21]. Accurate and rapid identification of regressive and abortive infections would also be beneficial for veterinarians trying to make informed risk–benefit assessments regarding FeLV vaccination for cats in their local area, since it provides a more accurate estimate of the true FeLV prevalence and therefore, in general, of the risk of FeLV exposure [53]. FeLV PoC antigen testing is able to differentiate infected from vaccinated animals (DIVA) [26], and laboratory-based p15E antibody testing has shown promise as a potential DIVA test [48,49].

The primary aim of this study was to assess the field performance of v-RetroFel® Ag/Ab (2020–2021 version) to detect different FeLV infection outcomes using samples collected in Australia and Germany. A secondary aim was to evaluate the performance of v-RetroFel®FIV (a third strip present within the same v-RetroFel® combination test kit) to determine feline immunodeficiency virus (FIV) infection status in the same population of cats.

2. Materials and Methods

2.1. Australian Samples (Cohort 1; n = 200)

Residual plasma samples from previous studies were utilized for FeLV antibody testing [47,54]. Samples included client-owned cats and cats residing in two rescue facilities [47]. Blood was collected by jugular or cephalic venipuncture following application of a local anesthetic cream and was then immediately aliquoted into multiple ethylenediamine tetra-acetic acid (EDTA) tubes. An EDTA tube was centrifuged for 3 min at 12,000× *g*, and harvested plasma was aliquoted into two plain tubes using a sterile pipette and stored at −80 °C until use.

In total, 93/200 (46.5%) cats were FeLV-unvaccinated and 107/200 (53.5%) cats were FeLV-vaccinated: 38 cats (19%) had been vaccinated against FeLV with a monovalent inactivated whole-virus (IWV) vaccine (Fel-O-Vax® Lv-K, Boehringer Ingelheim Animal Health, Fort Dodge, IA, USA); 50 cats (25%) had been vaccinated with a polyvalent vaccine, which included inactivated whole FeLV antigen (Fel-O-Vax® 5, Boehringer Ingelheim Animal Health); and 19 cats (9.5%) were vaccinated with a monovalent FeLV subunit p45 vaccine (Leucogen®, Virbac Animal Health, Bendigo, Victoria, Australia). Additionally, 29 cats (14.5%) had been vaccinated against FIV with a dual-subtype IWV vaccine (Fel-O-Vax® FIV, Boehringer Ingelheim Animal Health). One cat had been vaccinated against both FeLV (Fel-O-Vax® 5) and FIV.

Commercially available FeLV PoC testing (SNAP Combo®, IDEXX Laboratories, Westbrook, ME, USA; Witness®, Zoetis Animal Health, Lyon, France; or Anigen Rapid®, BioNote, Gyeonggi-do, Republic of Korea) was performed with fresh EDTA anticoagulated whole blood to detect p27-antigenemia. SNAP Combo® has published sensitivity and specificity under the Australian conditions of 100% and 94%, while Witness® and Anigen Rapid® both have published sensitivity and specificity values of 91% and 98% under Australian conditions [55]. All p27-positive results were confirmed either by testing with a second FeLV PoC test (from a different manufacturer), or, following transfer of plasma, stored at −80 °C on dry ice and transported to Clinical Laboratory, Vetsuisse Faculty, the University of Zurich, testing with a laboratory-based p27 antigen sandwich ELISA [56]. Some results were confirmed by both methods. Residual plasma from regressive and abortive infections was also used for p27 antigen laboratory ELISA testing when available.

Plasma stored at −80 °C and transferred on dry ice to Clinical Laboratory, Zurich was also tested for anti-p15E antibodies using a laboratory ELISA as described [49]. Cloned and purified whole p15E subunit of FeLV-A (GenBank accession no. AAA93093.1), without the membrane-spanning helix part of the viral envelope protein, was used as the ELISA capture antigen [49]. Relative optical density (ROD) values were determined using the formula

ROD = [(Sample OD − Negative control OD)/(Positive control OD − Negative control OD)]. Samples with ROD that tested > 16.3% (ROD value 0.163) compared to the positive control (pooled serum sample from cats experimentally infected with FeLV-A/Glasgow-1) were considered antibody-positive, as was determined for cats in a previous Swiss field study [49].

FeLV real-time (q)PCR testing to detect proviral DNA was performed on EDTA anticoagulated whole blood samples in duplicate at Veterinary Pathology Diagnostic Services (VPDS), Sydney School of Veterinary Science (SSVS), the University of Sydney, as described [57,58].

Plasma stored at −80 °C was transferred on dry ice to Veterinary Diagnostic Services (VDS), the University of Glasgow, for FeLV NAb testing, as described [47,59]. Twofold serial dilutions of plasma samples (1/4, 1/8, 1/16, and 1/32) were tested, and any dilution that reduced the focus count of FeLV by 75% compared with the virus control was considered a positive result [47].

Table 1 summarizes the testing approach used to classify cats in Australia as progressively infected (n = 23), regressively infected (n = 23), abortively infected (n = 32), or FeLV-unexposed (n = 122).

Table 1. Classification of feline leukemia virus (FeLV) infection status in Australian cats (n = 200). PoC = point-of-care, Ag = antigen, Lab = laboratory, NAb = neutralizing antibodies, NP = not performed.

FeLV Infection Status	Results			
	PoC p27 Ag	Lab-ELISA p27 Ag	Proviral DNA PCR	NAb
Progressive (n = 23)	+	+ [1]	+	− [2]
Regressive (n = 23)	−	− [3]	+	+
Abortive (n = 32)	−	− [4]	−	+
FeLV-unexposed (n = 122)	−	NP	−	−

[1] Residual sample was available for laboratory-based p27 testing in 20/23 cats. [2] One cat was classified as progressively infected based on p27-antigenemia, but tested NAb-positive. [3] Residual samples were available for laboratory-based p27 testing in 20/23 cats. [4] Residual samples were available for laboratory-based p27 testing in 7/32 cats.

The accuracy of v-RetroFel®FIV to determine FIV infection status was also evaluated. In total, samples from 19 FIV-infected cats (including 2 annually FIV-vaccinated cats) and 181 FIV-uninfected cats (including 27 annually FIV-vaccinated cats) were tested. The FIV status of all 200 samples had been previously determined with fresh EDTA anticoagulated whole blood using FIV PoC test kits from the same three manufacturers as the FeLV PoC test kits (i.e., SNAP Combo®, Witness®, or Anigen Rapid®). The sensitivity and specificity of each test kit under Australian conditions were reported (100% and 64% for SNAP Combo®, 100% and 98% for Witness®, and 100% and 100% for Anigen Rapid®) [60]. FIV-positive results were confirmed by testing with the other two FIV PoC tests, and in most FIV-positive cases (15/19), FIV PCR testing to detect viral RNA and proviral DNA was conducted (FIV RealPCR®, IDEXX Laboratories, East Brisbane, QLD, Australia). The sensitivity and specificity of FIV RealPCR® testing under Australian conditions were reported to be 92% and 99% [60]. Virus isolation was used to confirm FIV infection in the two FIV-vaccinated cats at the University of Florida and the University of Glasgow [60,61].

Plasma stored at −80 °C was thawed for testing with v-RetroFel®PoC kits at SSVS. Two observers (Jennifer Green, and E.B-B. or A.C.), blinded to the FeLV and FIV infection status of all samples, performed v-RetroFel® PoC testing in accordance with manufacturer's instructions. Both observers were in agreement for all test results.

2.2. German Samples (Cohort 2; n = 170)

Serum and EDTA anticoagulated whole blood samples from 170 cats in Germany were collected prospectively. Samples originated from cats that presented at the Clinic of Small Animal Medicine of the Centre for Clinical Veterinary Medicine LMU Munich and had blood drawn for various reasons, as well as shelter cats with unknown FeLV and FIV status. Both cats with a history of illness and healthy cats were tested. Of the 170 cats, 11 were vaccinated against FeLV. Five cats were vaccinated with a recombinant canarypox virus (vCP97) vaccine (Purevax® FeLV, Boehringer Ingelheim Vetmedica GmbH, Rohrdorf, Germany), four cats with a monovalent FeLV subunit vaccine (Leucogen®, Virbac Animal Health, Carros, France), and in two cats, the FeLV vaccine administered was unknown. None of the cats were vaccinated against FIV.

Samples were stored at −80 °C for a maximum of 24 months before being sent on dry ice to Clinical Laboratory, Vetsuisse Faculty, the University of Zurich.

Samples were tested for the presence of free FeLV p27 antigen in serum by sandwich ELISA, as described previously [56]. All samples were tested in duplicate and the absorbances were read using a microplate reader (Synergy H1, Biotek, VT, USA).

To confirm positive p27 antigen results, blood and saliva samples from all p27 antigen-positive cats (n = 4) were tested for viral RNA. A published RT-qPCR assay [37,58] was used to detect FeLV viral RNA, with each sample being tested once. Positive and negative controls were run in parallel with each RT-qPCR. All negative samples were diluted 1:5 and 1:10 in a neutral buffer at pH 7.4 (0.15 M sodium chloride, 1 mM EDTA, 0.05 MTris-base, 0.1% BSA, 0.1% Tween 20) to make possible inhibition unlikely.

For FeLV proviral DNA testing [58], total nucleic acids [49] were extracted from 100 µL EDTA anticoagulated whole blood using the MagNa Pure 96 instrument (Roche Diagnostics AG, Rotkreuz, Switzerland) and the Viral NA SV Kit (Roche Diagnostics AG, Rotkreuz, Switzerland) with a 100 µL elution buffer according to the manufacturer's instructions. For all samples, the viral NA plasma external lysis SV 4.0 protocol (Roche Diagnostics AG) was applied, with each sample being tested once, and negative controls of phosphate-buffered saline (PBS) were run in parallel with each batch of samples to monitor for cross-contamination.

The proviral DNA copy number was amplified and quantified using 5 µL of TNA and 20 µL of DNA quantitative PCR Mastermix (Eurogentec, Seraing, Belgium) containing 480 nM primers (exoFeLV-U3F2, exoFeLV-U3R3) and a 160 nM probe (exoFeLV-U3p). All oligonucleotides were synthetized by Microsynth AG (Balgach, Switzerland). The temperature profile consisted of 2 min at 50 °C, denaturation for 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. The FeLV proviral copy numbers in the single samples were determined by co-amplifying 10-fold serial dilutions of a DNA standard template, as described previously [6]. All samples that tested positive in the p27 antigen ELISA were diluted 1:5 and 1:10 in the neutral buffer to avoid a false negative result in the provirus qPCR due to possible inhibition. To verify the quantity and quality of viral load, quantitative PCR for feline albumin was performed on all 170 TNA samples [62].

Serum samples were analyzed for the presence of antibodies against FeLV surface unit (SU) non-glycosylated protein (p45), FeLV whole virus (FL-74), and FeLV p15E using indirect ELISAs, as described previously [16,44,49]. Anti-SU and anti-whole virus antibody concentrations > 25% (ROD value 0.250) [63] and anti-p15E antibody concentrations > 16.3% (ROD value 0.163) [49], compared to the positive control (pooled serum sample from cats experimentally infected with FeLV-A/Glasgow-1), were defined as antibody-positive.

Table 2 summarizes the testing approach used to classify cats in Germany as progressively infected (n = 4), regressively infected (n = 2), or abortively infected (n = 6), or FeLV-unexposed (n = 158).

Table 2. Classification of feline leukemia virus (FeLV) infection status in German cats (n = 170). Lab = laboratory, Ag = antigen, RT = reverse-transcriptase, Ab = antibody, SU = surface unit protein.

FeLV Infection Status	Results				
	Lab-ELISA p27 Ag	Viral RT-PCR	Proviral DNA PCR	Lab-ELISA Anti-SU Ab	Lab-ELISA Anti-Whole Virus Ab
Progressive (n = 4)	+	+	+	−	−
Regressive (n = 2)	−	−	+	−	± [2]
Abortive (n = 6)	−	−	−	+	+
FeLV-unexposed (n = 158)	−	−	−	± [1]	± [3]

[1] Of the 158 FeLV-unexposed cats, 30 cats had anti-SU antibodies. Of these, five cats were vaccinated with a monovalent FeLV subunit p45 vaccine (Leucogen®, Virbac Animal Health) that is known to produce an antibody response. [2] One cat classified as regressively infected tested positive, and one cat classified as regressively infected tested negative, for anti-whole virus antibodies. [3] Of the 158 FeLV-unexposed cats, 11 cats had anti-whole virus antibodies, including two FeLV-vaccinated cats. One cat was vaccinated with a monovalent FeLV subunit vaccine (Leucogen®, Virbac Animal Health), and for one cat, the vaccine manufacturer was unknown.

In addition, all samples were tested for the presence of FIV antibodies by Western blotting (WB) to determine FIV status. The WB was performed as described [64–67], and samples were considered WB-positive (i.e., FIV-infected) if two bands with a molecular weight of 15,000 (p15) and 24,000 (p24) Daltons, respectively, were identifiable on the blotting strip [64]. If both bands were absent, the sample was classified as WB-negative (i.e., FIV-uninfected). Samples that had only one band, either p15 or p24, were classified as FIV-negative at the time of sampling.

Serum centrifuged directly after blood sampling was used to perform v-RetroFel® PoC testing. Two observers (Juliana Giselbrecht and a second person) performed and interpreted the tests at the Small Animal Clinic, LMU Munich. The tests were performed according to the manufacturer's instructions. At the time that v-RetroFel® PoC testing was performed, the results of the FeLV and FIV laboratory results were unknown. Both observers were in agreement for all test results.

2.3. Evaluation of v-RetroFel® Test Results

The v-RetroFel® PoC test consists of three separate test strips designed to detect (i) FeLV p27 antigen, (ii) antibodies to FeLV transmembrane protein (p15E), and (iii) antibodies to FIV capsid protein (p24) and transmembrane glycoprotein (gp40).

For the current study, when presenting results from v-RetroFel® PoC testing, the following abbreviations will be used hereafter: (i) v-RetroFel®Ag for PoC FeLV p27 antigen results; (ii) v-RetroFel®Ab for PoC FeLV p15E antibody results; and (iii) v-RetroFel®FIV for PoC FIV antibody results. For combined p27 antigen/p15E antibody results, v-RetroFel®Ag/Ab will be used.

For v-RetroFel®Ag/Ab testing, the manufacturer claims that:

- p27-positive/p15E antibody-positive results indicate progressive or early regressive FeLV infections;
- p27-negative/p15E antibody-positive results indicate regressive or abortive FeLV infections;
- p27-negative/p15E antibody-negative results indicate no exposure to FeLV;
- p27-positive/p15E antibody-negative results are unlikely to be observed (but would also be considered indicative of progressive infections).

For v-RetroFel®FIV testing, results are reported as antibody-positive (FIV-infected) or antibody-negative (FIV-uninfected).

Test agreement between v-RetroFel®Ab and p15E laboratory ELISA results was calculated using both negative and positive test results. Test outcomes for v-RetroFel® were

compared between groups with each study population by Fisher's exact testing, and results from p15E laboratory ELISA testing were compared between groups by Mann–Whitney *U*-testing since data were not normally distributed. Ages were compared by two-tailed *t*-testing. For all analyses, a p value < 0.05 was considered significant. Sensitivity and specificity for FIV testing with 95% confidence intervals (CI) were calculated using Microsoft Excel®.

3. Results

3.1. Australian Samples (n = 200)

A summary of the results is shown in Table 3.

Table 3. Results of v-RetroFel®Ag/Ab PoC testing to detect FeLV p27 antigen and FeLV p15E antibodies, and p15E laboratory ELISA testing to detect p15E antibodies, in Australian cats (n = 200). Positive results are shown. Refer to Table 1 for the testing approach used to classify cats in Australia as progressively infected, regressively infected, abortively infected, or FeLV-unexposed. p15E antibody test results were not used for classification of FeLV infection status. None of the progressively infected or regressively infected cats had been vaccinated against FeLV, 25/32 abortively infected cats had been vaccinated against FeLV (11 with Fel-O-Vax® Lv-K and 14 with Fel-O-Vax® 5), and 82/122 FeLV-unexposed cats had been vaccinated against FeLV (27 with Fel-O-Vax® Lv-K, 36 with Fel-O-Vax® 5, and 19 with Leucogen®). FeLV = feline leukemia virus, PoC = point-of-care, Ag = antigen, Ab = antibody, Lab = laboratory.

FeLV Infection Status	Positive Results		
	v-RetroFel®Ag PoC p27 Ag	v-RetroFel®Ab PoC p15E Ab	Lab-ELISA p15E Ab
Progressive (n = 23)	21 (91%)	13 (57%)	16 (70%)
Regressive (n = 23)	0 (0%)	0 (0%)	16 (70%)
Abortive (n = 32)	0 (0%)	0 (0%)	25 (78%)
FeLV-unexposed (n = 122)	1 (0.8%)	12 (10%) [1]	70 (57%)

[1] Only 2/12 of these cats also tested p15E antibody-positive with the laboratory ELISA. All 12 cats that were p15E antibody-positive with v-RetroFel®Ab PoC testing had been vaccinated against FeLV (5 cats with Fel-O-Vax® Lv-K, 4 cats with Fel-O-Vax® 5, and 3 cats with Leucogen®).

3.1.1. Results of v-RetroFel®Ag/Ab Testing

Overall, using v-RetroFel®Ag/Ab, 122/200 (61%) of cases werein agreement with this study's definitions of FeLV infection status.

Testing with v-RetroFel®Ag/Ab correctly identified 91% (21/23) progressive infections (13 cats tested antigen-positive/antibody-positive and 8 cats tested antigen-positive/antibody-negative) but did not correctly identify any regressive (0/23) or abortive (0/32) infections (all 55 cats incorrectly tested antibody-negative). Two progressively infected cats tested falsely antigen-negative (and antibody-negative) with v-RetroFel®Ag/Ab. One FeLV-unexposed cat tested falsely antigen-positive (and antibody-negative) with v-RetroFel®Ag/Ab.

Testing with v-RetroFel®Ag/Ab correctly identified 89% (109/122) FeLV-unexposed cats (antigen-negative and antibody-negative). In addition to the one cat that tested falsely antigen-positive (and antibody-negative), 12 cats tested antigen-negative/antibody positive (all 12 had been vaccinated against FeLV). In the 122 FeLV-unexposed cats, antibody-positive results with v-RetroFel®Ab were more likely to occur in younger cats than older cats (1.3 years mean age for antibody-positive results vs. 5.1 years for antibody-negative results; p = 0.0003; two-tailed t-test). Males were less likely to test antibody-positive than females (3/67 males vs. 9/55 females; p = 0.035; Fisher's exact test). FeLV-unexposed cats that

had been vaccinated against FeLV (n = 82) were more likely to test antibody-positive with v-RetroFel®Ab than unvaccinated FeLV-unexposed cats (n = 40) (12/82 vs. 0/40; p = 0.02; Fisher's exact test). None of the three FeLV vaccines was more likely than the others to produce antibody-positive results with v-RetroFel®Ab testing in the 82 FeLV-vaccinated/FeLV-unexposed cats (Fel-O-Vax® Lv-K—5/27, Fel-O-Vax® 5—4/36, and Leucogen®—3/19; p > 0.48; Fisher's exact testing).

The sensitivity and specificity of v-RetroFel®Ag for p27 antigen were 91.3% (21/23) and 99.4% (176/177) respectively. For anti-p15E antibodies, the sensitivity and specificity of v-RetroFel®Ab (based on the assumption that all progressively, regressively, and abortively infected cats produce antibodies against p15E) were 16.7% (13/78) and 90.2% (110/122) respectively.

3.1.2. Results of p15E Laboratory ELISA Testing

Testing with the p15E laboratory ELISA detected antibodies in 70% (16/23) progressive infections, 70% (16/23) regressive infections, and 78% (23/32) abortive infections. There were no differences in p15E laboratory ELISA levels between progressive, regressive, and abortive infections (p < 0.35; Mann–Whitney U-testing). More than half of FeLV-unexposed cats, however, also tested p15E laboratory ELISA-positive (70/122; 57%). In the 122 FeLV-unexposed cats, p15E laboratory ELISA-positive results were more likely to occur in older cats than younger cats (6.9 years mean age for antibody-positive results vs. 1.9 years for antibody-negative results; p < 0.00001; two-tailed t-test). Males were more likely to test antibody-positive than females (46/67 males vs. 24/55 females; p = 0.0062; Fisher's exact test). Overall, FeLV-unexposed cats had lower p15E laboratory ELISA levels than progressively, regressively, and abortively infected cats (p = 0.007, p = 0.03, and p = 0.005, respectively; Mann–Whitney U-testing), although there was a substantial overlap in results (Figure 1).

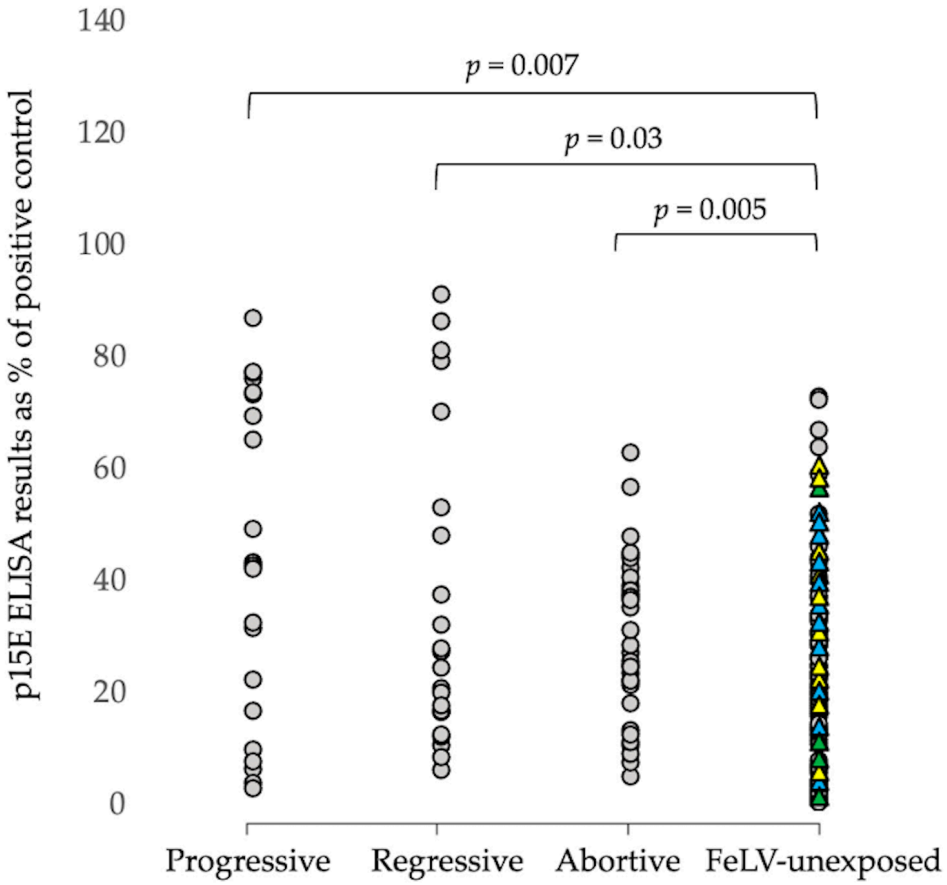

Figure 1. Results from p15E laboratory ELISA testing in cats in Australia (cohort 1, n = 200). Feline

leukemia virus (FeLV)-unexposed cats had significantly lower antibody levels than progressive, regressive, and abortive infections, although there was a substantial overlap in results. The triangles represent the FeLV-vaccinated unexposed cats. The yellow triangles represent cats that had been vaccinated with Fel-O-Vax® Lv-K; the blue triangles, Fel-O-Vax® 5; and the green triangles, Leucogen®. Higher anti-p15E antibody levels were found in cats vaccinated with Fel-O-Vax® Lv-K or Fel-O-Vax® 5 than cats vaccinated with Leucogen®.

Both FeLV-vaccinated/FeLV-unexposed cats (32/82) and unvaccinated FeLV-unexposed cats (38/40) tested p15E laboratory ELISA-positive. Surprisingly, FeLV-unvaccinated unexposed cats had higher anti-p15E antibody levels than FeLV-vaccinated unexposed cats (ROD 0.310 vs. 0.155; $p < 0.00001$; Mann–Whitney *U*-test). Of the FeLV-vaccinated unexposed cats, Fel-O-Vax® Lv-K (13/27) and Fel-O-Vax® 5 (18/36) produced more p15E laboratory ELISA-positive results than Leucogen® (1/19; $p < 0.01$ for both, Fisher's exact testing), and higher p15E antibody levels were observed in cats vaccinated with Fel-O-Vax® Lv-K or Fel-O-Vax® 5 ($p < 0.002$ for both; Mann–Whitney *U*-testing).

There was test agreement between v-RetroFel®Ab and p15E laboratory ELISA testing in only 78/200 (39%) of samples.

3.1.3. Results of v-RetroFel®FIV Testing

Results are summarized in Table 4. The sensitivity and specificity of FIV testing were 94.7% (18/19; 95% CI 84.7 to 100) and 98.3% (178/181; 95% CI 96.5 to 100), respectively. Prior history of FIV vaccination did not impact the results, with all 27 FIV-vaccinated/FIV-uninfected cats testing FIV-negative, while both FIV-vaccinated/FIV-infected cats tested FIV-positive.

Table 4. Results of v-RetroFel®FIV PoC testing to detect antibodies against FIV capsid protein (p24) and glycoprotein (gp40) in 200 Australian cats (cohort 1). FIV infection status was determined by results from three commercially available PoC tests, PCR testing to confirm FIV-positive results, and virus isolation to confirm FIV infection in two FIV-vaccinated cats. FIV = feline immunodeficiency virus, PoC = point-of-care.

FIV Infection Status	v-RetroFel®FIV PoC Result	
	Negative	Positive
Uninfected ($n = 181$)	178	3
Infected ($n = 19$)	1	18

3.2. German Samples (n = 170)

A summary of the results is shown in Table 5.

3.2.1. Results of v-RetroFel®Ag/Ab Testing

Overall, using v-RetroFel®Ag/Ab, 149/170 (88%) of cases were in agreement with this study's definitions of FeLV infection status.

V-RetroFel®Ag/Ab correctly identified 100% (4/4) progressive infections (all 4 cats tested antigen-positive/antibody-negative) but did not correctly identify any regressive infections (0/2) and only correctly identified 17% (1/6) of the abortive infections.

Testing with v-RetroFel®Ag/Ab correctly identified 94% (148/158) FeLV-unexposed cats. No cat tested falsely antigen-positive (and antibody-negative). There was no sex effect on antibody-positive results (4/70 males vs. 6/88 females; $p = 1.0$; Fisher's exact test). Ten FeLV-unexposed cats tested antigen-negative/antibody-positive (two cats had been vaccinated against FeLV, one cat with Purevax® FeLV and one cat with Leucogen®).

The sensitivity and specificity of v-RetroFel®Ag for p27 antigen were 100% (4/4) and 100% (166/166) respectively. For anti-p15E antibodies, the sensitivity and specificity of v-RetroFel®Ab (based on the assumption that all progressively, regressively, and abortively infected cats produce antibodies against p15E) were 8.3% (1/12) and 93.7% (148/158) respectively.

Table 5. Results of v-RetroFel®Ag/Ab PoC testing to detect FeLV p27 antigen and FeLV p15E antibodies, and p15E laboratory ELISA testing to detect p15E antibodies, in German cats (n = 170). Positive results are shown. Refer to Table 2 for the testing approach used to classify cats in Germany as progressively infected, regressively infected, abortively infected, or FeLV-unexposed. p15E antibody test results were not used for classification of FeLV infection status. None of the progressively infected, regressively infected, or abortively infected cats had been vaccinated against FeLV, and 11/158 FeLV-unexposed cats had been vaccinated against FeLV (5 cats with Purevax®, 4 cats with Leucogen®, and 2 cats with an unknown vaccine). FeLV = feline leukemia virus, PoC = point-of-care, Ag = antigen, Ab = antibody, Lab = laboratory.

FeLV Infection Status	Positive Results		
	v-RetroFel®Ag PoC p27 Ag	v-RetroFel®Ab PoC p15E Ab	Lab-ELISA p15E Ab
Progressive (n = 4)	4 (100%)	0 (0%)	3 (75%)
Regressive (n = 2)	0 (0%)	0 (0%)	0 (0%)
Abortive (n = 6)	0 (0%)	1 (17%) [1]	2 (33%)
FeLV-unexposed (n = 158)	0 (0%)	10 (6%) [2]	13 (8%) [3]

[1] The one abortive infection that tested positive with the v-RetroFel®Ab PoC test was negative with the p15E laboratory ELISA. [2] One of these ten cats also tested positive with the p15E laboratory ELISA. Two FeLV-vaccinated cats tested positive with v-RetroFel®Ab and negative with the p15E laboratory ELISA (one cat was vaccinated with Purevax® FeLV shortly before sampling, and one cat had been vaccinated eight times with Leucogen®). [3] Three of these 13 cats had been vaccinated against FeLV (one cat with Purevax® FeLV; one cat with Leucogen®; and for one cat, the vaccine manufacturer was unknown).

3.2.2. Results of p15E Laboratory ELISA Testing

Testing with the p15E laboratory ELISA detected antibodies in 75% (3/4) progressive infections, 0% (0/2) regressive infections, and 33% (2/6) abortive infections. However, of the FeLV-unexposed cats, 8% (13/158) tested antibody-positive with the p15E laboratory ELISA (Figure 2). There was no sex effect in the FeLV-unexposed group on antibody-positive results (7/70 males vs. 6/88 females; p = 0.56; Fisher's exact test). FeLV-unvaccinated unexposed cats did not have significantly different anti-p15E antibody levels compared to FeLV-vaccinated unexposed cats (ROD 0.048 vs. 0.213; p = 0.063; Mann–Whitney U-test). Sample numbers were too low to investigate a possible age effect on p15E laboratory ELISA-positive results. Antibody levels between progressively, regressively, and abortively infected cats, as well as FeLV-unexposed cats, were also not compared statistically, since the numbers of progressive, regressive, and abortive infections were too low for statistical comparison.

There was agreement between v-RetroFel®Ab and p15E laboratory ELISA test results in 141/170 (83%) of samples.

3.2.3. Results of v-RetroFel®FIV Testing

Results are summarized in Table 6. Sensitivity and specificity of FIV testing were 30.0% (3/10) and 100.0% (160/160), respectively. There was no history of FIV vaccination in any cat.

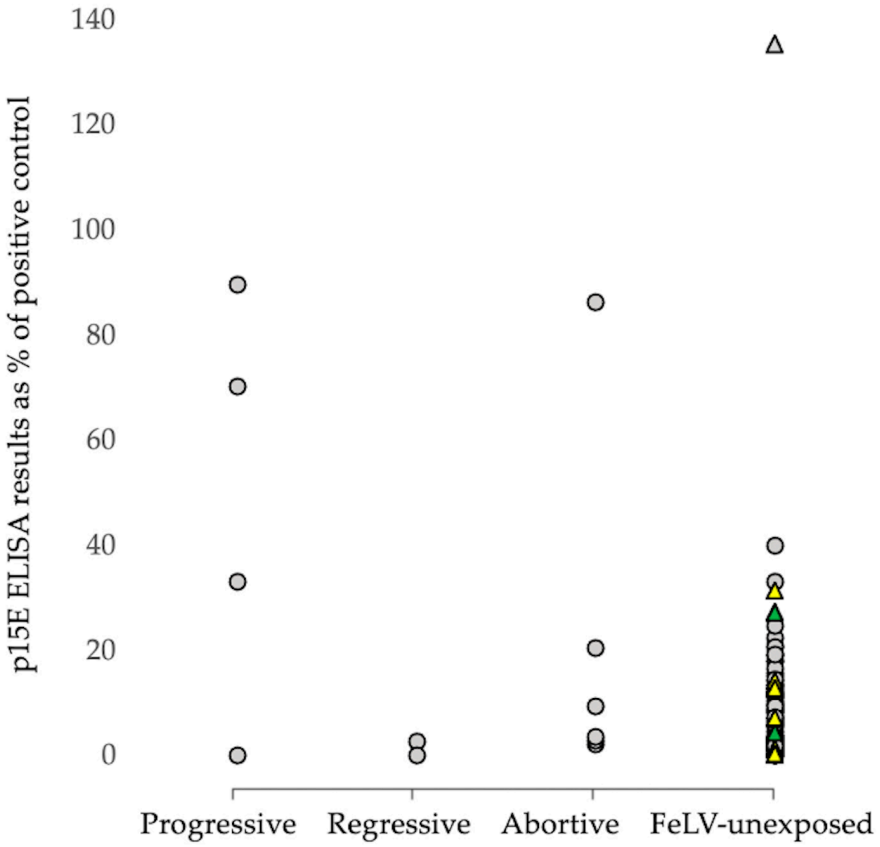

Figure 2. Results from p15E laboratory ELISA testing in cats in Germany (cohort 2, $n = 170$). The small numbers of cats in the progressive, regressive, and abortive categories precluded statistical analysis. The triangles represent the FeLV-vaccinated unexposed cats. The yellow triangles represent cats that had been vaccinated with Purevax® FeLV; the green triangles, Leucogen®; and the grey triangles, an unknown vaccine manufacturer. The small numbers of vaccinated cats in each group precluded statistical analysis.

Table 6. Results of v-RetroFel®FIV PoC testing to detect antibodies against FIV capsid protein (p24) and glycoprotein (gp40) in 170 German cats (cohort 2). FIV infection status was determined by results from Western blotting (WB). FIV = feline immunodeficiency virus, PoC = point-of-care.

FIV Infection Status	v-RetroFel®FIV PoC Result	
	Negative	Positive
Uninfected [1] ($n = 160$)	160	0
Infected ($n = 10$)	7	3

[1] Seventy-eight cats had one band with WB and were categorized as FIV-negative at the time of testing. The other 82 cats had no bands with WB.

3.3. Comparing Results from Cohorts 1 and 2

Overall, correct FeLV infection status was determined with v-RetroFel®Ag/Ab testing in 271/370 (73%) cases. There was no difference between countries (i.e., Australia vs. Germany) in terms of v-RetroFel®Ag/Ab test performance for each infection category ($p > 0.15$; Fisher's exact tests). There was test agreement between v-RetroFel®Ab and the p15E laboratory ELISA in overall 58.9% (218/370) of samples.

Overall, FeLV-unexposed cats had significantly lower p15E laboratory ELISA antibody titers than progressively, regressively, and abortively infected cats ($p = 0.00002$, $p = 0.000005$,

and $p < 0.00001$, Mann–Whitney *U*-tests). There was, however, a substantial overlap in antibody results between different categories (Figure 3).

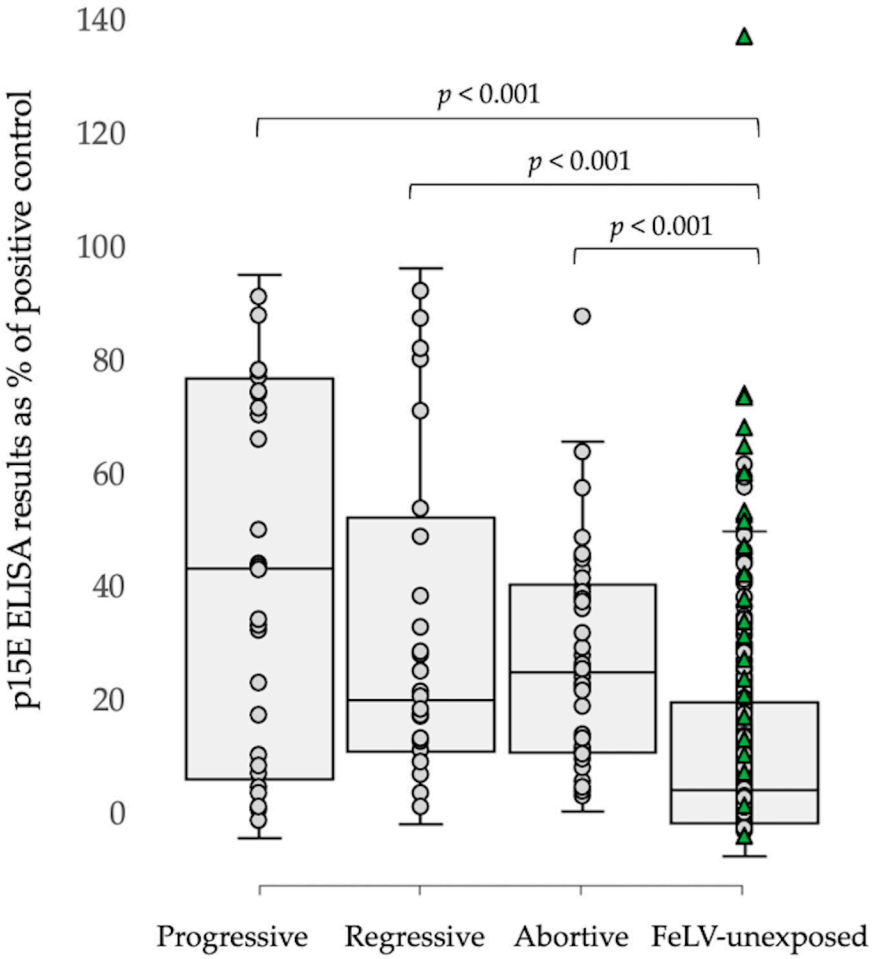

Figure 3. Combined results from p15E laboratory-ELISA testing in both cohorts (i.e., Australia and Germany; $n = 370$). Overall, FeLV-unexposed cats had significantly lower antibody titers than progressively ($p = 0.00002$), regressively ($p = 0.000005$), and abortively ($p < 0.00001$) infected cats. The green triangles represent FeLV-vaccinated unexposed cats.

The v-RetroFel®FIV test kit was more sensitive using samples from Australian cats (18/19 FIV-positive cats) than German cats (3/10 FIV-positive cats) ($p = 0.0005$; Fisher's exact test), while there was no difference in test specificity between countries (178/181 vs. 160/160; $p = 0.25$; Fisher's exact test).

4. Discussion

The present study evaluated a new, commercially available PoC test (v-RetroFel®) in naturally infected cats from Australia and Germany. The test is expected to detect different courses of FeLV infection based on the determination of p27 antigen and anti-p15E antibody status. In addition to FeLV diagnostics, the test is also marketed to detect antibodies against FIV.

The correct FeLV infection status was determined with v-RetroFel®Ag/Ab testing in 271/370 (73%) cases. The v-RetroFel®Ag/Ab PoC test identified most progressively infected cats by detecting the p27 antigen correctly (cohort 1—21/23, cohort 2—4/4). The v-RetroFel®Ag/Ab PoC test, however, was unable to identify regressive and abortive infections in either population of cats (cohort 1—0/55 combined, cohort 2—1/8 combined). Therefore, the version of v-RetroFel®Ag/Ab tested in this study (2020–2021 version) did

not offer any advantages over other available PoC tests that solely detect p27 antigen, and its use cannot be recommended until improvements have been made.

The most significant form of FeLV infection is progressive infection, since these cats are the main source of infection for other, uninfected cats [68]. Progressively infected cats are more likely to develop FeLV-associated diseases, including immunodeficiency; bone marrow suppression (pancytopenias); and neoplasia, resulting in death [9,44,69,70]. In this study, both Australian and German cats were considered progressively infected if they tested positive for the presence of p27 antigen in the blood with a range of commercially available PoC kits and/or a laboratory ELISA. In addition, all progressively infected cats tested provirus PCR-positive. The v-RetroFel®Ag/Ab PoC test was able to identify 25/27 progressive infections within the two cohorts, but of concern were two progressive infections in the Australian cohort that would have been missed with v-RetroFel®Ag/Ab testing alone.

p15E is a transmembrane protein that is expressed on the surface of FeLV-infected cells, and it allows the virus to enter the host cell and inhibit lymphocyte proliferation and T-cell functions, thereby possessing immunosuppressive properties [71,72]. Antibodies directed against p15E rarely have virus-neutralizing properties [73]. Lutz and colleagues analyzed the quality and quantity of antibodies against different FeLV components in naturally infected cats and found that p15E had strong antigenicity. They observed that cats displayed elevated levels of antibodies to p15E, whether they became immune or viremic after infection [48]. In the present study, most progressively infected cats (cohort 1—16/23, cohort 2—3/4) tested positive for anti-p15E antibodies with the p15E laboratory ELISA, as did regressively infected cats (cohort 1—16/23, cohort 2—0/2) and abortively infected cats (cohort 1—25/32, cohort 2—2/6). These results support the hypothesis that most FeLV-infected cats (but not all) produce some antibodies against p15E [49].

FeLV-unexposed cats vaccinated against FeLV in cohort 1 had lower antibody titers against p15E detected by laboratory ELISA testing than unvaccinated cats, suggesting that the presence of anti-p15E antibodies indicates previous infection rather than vaccination. This finding is comparable to previous work, in which it was found that most vaccinated client-owned cats in Switzerland had p15E antibody values lower than the threshold calculated for FeLV-naive cats [49]. No difference in anti-p15E antibody levels was found between FeLV-vaccinated and FeLV-unvaccinated unexposed cats in cohort 2. In cohort 1, vaccination with an IWV FeLV vaccine (Fel-O-Vax® Lv-K or Fel-O-Vax® 5) produced a more reliable p15E antibody response then vaccination with the subunit vaccine (Leucogen®), supporting previous findings that antibody reaction depends on the vaccine administered [49]. In cohort 1, more progressively infected cats (13/23) tested positive with v-RetroFel®Ab than regressively and abortively infected cats (0/55). This finding, however, was not supported by results from p15E laboratory ELISA testing, with no difference in antibody levels between the types of infection found. Further studies are needed to determine to what extent the production of anti-p15E antibodies affects the different possible outcomes following FeLV exposure, how long antibodies are detectable following both FeLV vaccination and infection, and whether p15E antibody testing might predict infection.

Of concern for the p15E laboratory ELISA were the high number of FeLV-unexposed cats that tested antibody-positive (cohort 1—70/122, cohort 2—13/158) using a test cut-off of 16.3% compared to the positive control (pooled serum from experimentally FeLV-infected cats). It is not clear, therefore, to what degree the determination of anti-p15E antibodies is suitable for the determination of FeLV infection status. When the ELISA was first developed, serum samples from 294 cats in Switzerland were used to test the suitability of using the detection of anti-p15E antibodies for the diagnosis of FeLV infection. The sensitivity and specificity of p15E antibodies in experimentally infected cats were 95.7% and 100.0%, respectively. In naturally infected cats, the detection of anti-p15E antibodies showed a sensitivity of 77.1% and a specificity of 85.6% [49]. In this study, conditions of the experimentally infected cats had to be changed to reach an optimal trade-off between

diagnostic sensitivity and specificity (for experimentally infected cats, a ROD cut-off of 0.0495 was used vs. 0.163 for naturally infected cats). Boenzli and colleagues also mentioned that the low specificity would probably have been much higher if the gold standard PCR assay used had been more sensitive and PCR results from organs in the privately owned cats in the present study had been available [49]. In contrast to experimentally infected cats, cats with a natural infection can have multiple organs affected, despite minor bone marrow involvement [16,46].

It is difficult to explain the detection of anti-p15E antibodies in a high number of FeLV-unexposed cats, particularly in the first cohort (Australian cats). One possibility is that endogenous FeLV plays a role. The presence of the transmembrane protein p15E has been described with the subtype FeLV-B [9]. Another possibility is that some of the cats categorized as FeLV-unexposed had been exposed to very low levels of FeLV; too low to cause NAb production, but high enough to be detectable by a sensitive laboratory p15E ELISA. This suggestion is supported by the findings in the FeLV-unexposed Australian cohort that older, male cats were more likely to have p15E laboratory ELISA-positive results than younger, female cats, possibly reflecting increased cumulative risk of low-level FeLV exposure through at-risk roaming behavior [37,74–76]. An Australian study reported FeLV infection or exposure in 13.2% (58/440) of cats tested compared to 7.5% (37/495) of cats tested in Munich, Germany [47,63], suggesting a higher level of FeLV exposure in Australia than Germany. In light of this, the cut-off value of the p15E laboratory ELISA, i.e., the point at which a sample is considered positive, should be critically reevaluated.

In addition, among FeLV-unexposed cats, it was observed that FIV-infected cats (19/19) and FIV-vaccinated cats (25/27) tested positive with the p15E laboratory ELISA. There might be a cross-reaction in the ELISA between p15E and FIV antibodies. This finding needs to be further investigated. In the meantime, especially in countries where vaccination against FIV is currently available (Australia, New Zealand, and Japan), or was previously available (North America), results from testing to detect the presence of anti-p15E antibodies should be interpreted with caution and should not be the sole method used to determine FeLV exposure or non-exposure. Instead, when FeLV infection or exposure is suspected, it is recommended to use other laboratory methods, such as FeLV proviral PCR testing, viral RT-PCR testing, and NAb testing [15].

Overall, v-RetroFel®FIV was able to accurately determine FIV infection status. Interestingly, the sensitivity of v-RetroFel®FIV testing in the present study was significantly higher in Australian cats than in German cats (94.7% vs. 30.0%). Another study reported reduced sensitivity (i.e., false-negative FIV results) in Swiss samples with PoC and laboratory ELISA testing, hypothesizing that the introduction of new FIV field isolates (e.g., due to increased travel) could have been responsible [66]. Similarly, it is possible that the seven FIV-infected cats in Germany that tested falsely negative with v-RetroFel®FIV were the result of genetic virus mutations and altered host antibody production. Little is known about genetic differences between FIV field isolates in Australia and Germany, and this could be an area for future research. It is also possible that the different criteria used to determine FIV infection status in Australian and German cats might have contributed to the different sensitivity rates reported. Due to the difference in test sensitivity between cohorts 1 and 2, v-RetroFel®FIV can be recommended for use particularly by veterinarians in Australia, with caution suggested if used in Germany.

With regards to v-RetroFel®FIV specificity, there was no significant difference when testing Australian cats compared to German cats (98.3% vs. 100%). This was despite the different testing criteria used, and 78 cats in cohort 2 had one band with WB and were categorized as FIV negative at the time of testing. Only p24 reacts in WB in the early acute phase of FIV infection before antibody development occurs, or in the end stage of FIV infection, due to immunodeficiency [66]. It is recommended to retest cats that are only p24-positive in the WB two to three months later [77]. However, there was 100% test agreement between WB and v-RetroFel®FIV in these 78 cases; therefore, discordant results due to early FIV infection did not appear to be a factor affecting test accuracy in the present

study. Of particular interest to Australian vets will be the ability of the v-RetroFel®FIV test kit to differentiate FIV-vaccinated and FIV-infected cats, with all 27 uninfected FIV-vaccinated cats correctly testing negative (i.e., 100% specificity). v-RetroFel®FIV is the first p24/gp40 FIV kit reported in the scientific literature to be capable of DIVA, with three other FIV kits demonstrated to be capable of DIVA all detecting antibodies to gp40 only (Witness®, Anigen Rapid® and RapidSTATUS™, Biotech Laboratories, Rockville, MD, USA) [60,78]. Currently, there is only one commercially available FIV vaccine (Fel-O-Vax® FIV) sold in Australia, New Zealand, and Japan. It was also available in North America from 2003 to 2017, but has never been commercially available in Europe. Every jurisdiction should perform its own testing to determine the accuracy of any FIV PoC test kit, including v-RetroFel®FIV, prior to adopting them for use [61].

5. Conclusions

Measuring the antibody response to FeLV in cats with different FeLV outcomes and vaccination scenarios is complex and requires consideration of antibody response to both p15E and SU proteins. Currently, no single antibody test to determine the level of anti-p15E antibodies is completely reliable. FeLV antibody testing should always be carried out together with other laboratory tests, such as p27 antigen, proviral DNA PCR, and/or viral RNA testing, when trying to interpret antibody results. Furthermore, it should be remembered that the determination of FeLV infection status is always a snapshot and can change over time, for example, due to a weakening of the immune system. v-RetroFel®Ag/Ab (in its 2020–2021 version) did not reliably detect different FeLV infection outcomes and, therefore, does not currently offer any advantages over other available PoC tests that solely detect p27 antigen. Thus, its use cannot be recommended until improvements have been made. v-RetroFel®FIV was able to accurately determine FIV infection status, irrespective of a history of FIV vaccination, making it the first p24/gp40 FIV antibody PoC test kit reported to be capable of DIVA. However, of concern, and in need of additional investigation, was reduced test sensitivity in German cats.

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Institutional Review Board Statement: Animal ethics approval for the sampling of the pet cats in Australia was granted by the University of Sydney Animal Ethics Committee (approval numbers N00/1-2013/3/5920, 2017/1130 and 2019/1665). The rescue cats in Australia were sampled and tested at the request of the facility managers following the diagnosis of progressive FeLV infections. Animal ethics approval for the sampling of the pet cats in Germany was approved by the ethical committee (reference number 142-25-08-2018) of the Centre for Clinical Veterinary Medicine of the LMU Munich. Stored samples from cats experimentally infected with FeLV-A/Glasgow-1 and used

as positive controls for the laboratory p15E ELISA had been obtained in an earlier study conducted according to Swiss law and approved by the veterinary office of the Swiss canton of Zurich (approval number TVB 30/2003).

Informed Consent Statement: Informed, written consent was obtained from owners of all cats participating in the studies as part of AEC approval. No animals or people are identifiable within this publication; therefore, additional informed consent for publication was not required.

Data Availability Statement: All data presented in this paper are available upon request.

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Conflicts of Interest: The salary for Juliana Giselbrecht's employment as a research assistant at the Small Animal Medical Clinic, Centre for Clinical Veterinary Medicine LMU Munich was paid by Scil Animal Care Company. However, the funders (including Scil Animal Care Company) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The University of Zurich holds a patent on feline leukemia virus (FeLV) transmembrane protein p15E for the diagnosis of FeLV infection, and R.H-L. is one of the coinventors of this test.

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V. PUBLIKATION 4 (ORIGINALPUBLIKATION)

Evaluation of a revised-point-of-care test for the detection of feline leukaemia p27 antigen and anti-p15E antibodies in cats

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Article

Evaluation of a Revised Point-of-Care Test for the Detection of Feline Leukaemia p27 Antigen and Anti-p15E Antibodies in Cats

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Abstract: The first point-of-care (PoC) test (v-RetroFel®; modified version 2021) determining the presence of FeLV p27 antigen and FeLV anti-p15E antibodies has become recently commercially available to identify different feline leukaemia virus (FeLV) infection outcomes. This study aimed to assess this PoC test's performance concerning FeLV p27 antigen and FeLV anti-p15E antibody detection. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) were assessed after ten minutes (recommended) and 20 min (prolonged) incubation times. The test results were evaluated as either positive or negative. Serum samples from 934 cats were included, originating from Italy (n = 269), Portugal (n = 240), Germany (n = 318), and France (n = 107). FeLV p27 antigen and anti-p15E antibodies were measured by reference standard ELISAs and compared to the PoC test results. The PoC test was easy to perform and the results easy to interpret. Sensitivity and specificity for FeLV p27 antigen were 82.8% (PPV: 57.8%) and 96.0% (NPV: 98.8%) after both, ten and 20 minues of incubation time. Sensitivity and specificity for anti-p15E antibodies were 31.4% (PPV: 71.6%) and 96.9% (NPV: 85.1%) after ten minutes incubation time; sensitivity was improved by a prolonged incubation time (20 min) to 40.0% (PPV: 76.3%), while specificity remained the same (96.9%, NPV: 86.7%). Despite the improved sensitivity using the prolonged incubation time, lower than ideal sensitivities for both p27 antigen and especially anti-p15E antibodies were found, indicating that the PoC test in its current version needs further improvement prior to application in the field.

Keywords: feline leukaemia virus; FeLV; PoC test; test performance; v-RetroFel®; progressive infection; regressive infection; abortive infection; focal infection; antibody detection; in-house testing

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1. Introduction

Feline leukaemia virus (FeLV) is a gammaretrovirus with worldwide distribution and is regarded as one of the most important infectious agents in cats. FeLV can cause different courses of infection, including progressive, regressive, abortive, and focal (atypical) infections. Detection of these infection outcomes can be challenging and several tests are necessary [1–3].

Over the past few decades, FeLV prevalence of progressively infected cats has declined in many countries due to vaccination programs and improved veterinary care, including

better diagnostic techniques and testing and separating programs [4–8]. For example, in Germany, a constant decrease in the FeLV infection rate from 6% to 1% was observed over a period of ten years [7]. However, more recent studies suggest that the decline in prevalence has now plateaued in many countries, potentially due to incomplete vaccination coverage, evolution of the virus itself, and persistence in high-risk groups [8–10]. Therefore, it is crucial not to neglect awareness about this important feline infection and its prevention.

In progressive FeLV infection, the immune system of affected cats is unable to control virus replication, and this results in persistent viraemia. During the viraemic phases, free p27 antigen can be readily detected by commercially available point-of-care (PoC) tests that are based on enzyme-linked immunosorbent (ELISA) or immunomigration principles. These PoC tests detect progressive infection as well as some cats with focal and early regressive infection, but other courses of infection without viraemia (abortive and regressive infection without viraemia) remain undetected. However, abortive and regressive infections also play an important epidemiological role. Knowledge of the presence of a regressive infection is clinically relevant since this form of infection can progress to a progressive outcome with host immunosuppression. This is one reason why a reliable PoC test would be useful for rapid identification of regressively infected cats [1–3]. Furthermore, the easy identification of regressive infection is of particular importance in potential blood donor cats, as they can transmit FeLV provirus to FeLV-naive cats via blood transfusion [11]. Moreover, the detection of cats previously exposed to FeLV (abortive infection) prior to vaccination might be helpful in the decision whether to vaccinate cats against FeLV or not.

The FeLV transmembrane protein p15E is located on the surface of infected cells and enables the virus to enter the host cell. Additionally, it possesses immunosuppressive properties that can inhibit lymphocyte proliferation and T-cell functions [12,13]. Anti-p15E antibodies have limited virus-neutralizing properties. Lutz and colleagues (1980) found that cats that became immune or viraemic after FeLV infection showed elevated levels of anti-p15E antibodies [12,14,15]. Regardless of whether cats develop immunity (regressive and/or abortive courses) or remain viraemic following infection (progressive course), they consistently had elevated levels of antibodies against p15E [14–16]. P15E antibody testing can therefore be useful to identify different courses of FeLV infection. A PoC test for the detection of anti-15E antibodies (and FeLV p27 antigen) has been commercially available since 2018. The performance of this PoC test (in its original version) was evaluated with samples from 370 naturally infected cats in Australia and Germany [17]. The study demonstrated the test's ability to accurately determine the correct FeLV infection status in 271 out of 370 cases (73.2%). However, it was not able to correctly identify most of the regressive and abortive infections. With samples from Australia, the sensitivity and specificity for anti-p15E antibodies were 16.7% and 90.2%, with samples from Germany, only 8.3% and 93.7%, respectively. Most of the progressively infected cats (92.6%; 25/27) were identified correctly by detection of p27 antigen. However, none of the samples from Australia and only one of the samples from Germany (from regressively and abortively infected cats that tested positive in a laboratory-based anti-p15E ELISA) were identified as true-positive by the PoC test. Consequently, the authors advised that use of this PoC test could not be recommended until improvements are made to increase the sensitivity [17].

The aim of the present study was to evaluate the practicability and performance of a modified new version of the commercially available PoC test (version 2021) detecting FeLV p27 antigen and anti-p15E antibodies. The evaluation of such a test prior to application in the field is of importance since clinical decisions including identification of regressively infected cats, consideration of the need for FeLV vaccination, or the suitability of cats as blood donors could rely on these test results.

2. Materials and Methods

2.1. Samples

2.1.1. Experimentally Infected Cats

In total, 30 uninfected specific pathogen-free (SPF) cats and 30 experimentally FeLV-infected SPF cats (16 progressively and 14 regressively infected cats) were included. For each cat, p27 antigen, proviral deoxyribonucleic acid (DNA), and anti-p15E-antibodies in blood were known. All SPF cats included in this study had been part of experimental studies officially approved by the veterinary office of the Swiss Canton of Zurich (11/2011, 160/2010 and 251/2013).

2.1.2. Naturally Infected Cats

In total, 934 cats from four different European countries (Italy n = 269, Portugal n = 240, Germany n = 318, France n = 107) were included in this study. Blood samples were collected from cats that were presented to veterinary clinics for various reasons from 2019 to 2021. The cats needed to have a minimum body weight of 1 kilogram (kg) (inclusion criterium). A subset of the blood samples has been used in a previous epidemiological study [18]. Vaccination status was determined by a survey of the owners and/or veterinarians. The present study was approved by the ethical committee of the Centre for Clinical Veterinary Medicine of the LMU Munich, Germany (reference number 142-25-08-2018).

2.2. Laboratory Tests

From each cat, serum and ethylenediaminetetraacetic acid (EDTA) anticoagulated blood samples were stored at −80 °C for a maximum of 24 months before being sent on dry ice to the Clinical Laboratory at the Vetsuisse Faculty, University of Zurich, where samples were tested for p27 antigen, proviral DNA, and anti-p15E antibodies.

2.2.1. Detection of Free FeLV p27 Antigen in Serum

The presence of free p27 antigen in serum samples was evaluated using a sandwich ELISA as described previously [19]. Each sample was examined in duplicate, and a microplate reader (Synergy H1, Biotek, Winooski, VT, USA) was used to read the absorbances. Any value higher than 4% of the positive control (serum samples from cats naturally infected with FeLV) was classified as positive [20]. The ELISA and the monoclonal antibodies used in this assay have been evaluated and described extensively [21–24]. The ELISA is able to detect between 100 ng and >2000 ng of FeLV p27 per ml of serum in viraemic cats; the lower limit of detection corresponds to an absolute amount in a sample of 1 ng [21]. The ELISA is highly specific due to the monoclonal nature of the three antibodies; they recognized p27 in several hundred FeLV isolates but did not react with any of eight purified leukaemia viruses other than FeLV [22–24].

2.2.2. Detection of FeLV Proviral DNA in Blood

FeLV proviral DNA was determined in all samples by extracting total nucleic acids (TNA) [25] from 100 µL of EDTA anticoagulated whole blood using the MagNA Pure 96 instrument (Roche Diagnostics AG, Rotkreuz, Switzerland) and the Viral NA SV Kit (Roche Diagnostics AG, Rotkreuz, Switzerland), following the protocol as described before [17]. The proviral DNA copy number was quantified by real-time quantitative polymerase chain reaction (qPCR) as described previously [26]. To verify the quality and quantity of the TNA a qPCR was performed for the detection of feline albumin on all 934 TNA samples as previously described [27]. In a previous study, it was shown that FeLV provirus quantitative real-time PCR showed a high analytical sensitivity (detection of 1 copy/PCR) and a high analytical specificity (detection of all three FeLV subtypes, no false-positive results in SPF cats) [28].

2.2.3. Detection of FeLV Anti-p15E Antibodies in Serum

An in-house developed ELISA was used for the detection of anti-p15E antibodies in serum samples [25]. Each assay included positive and negative controls. Negative controls consisted of sera from SPF cats, while positive controls consisted of pooled serum samples from cats experimentally infected with FeLV-A/Glasgow-1. The relative optical density (ROD) values were determined using the formula ROD = [(sample optical density (OD) − negative control OD)/(positive control OD − negative control OD)]. Samples from experimentally and naturally FeLV-infected cats with ROD values >4.9% and >16.3%, respectively, compared to the positive control [25], were considered anti-p15E antibody-positive. In experimentally infected cats, the p15E ELISA showed a diagnostic sensitivity of 95.7% and a specificity of 100.0%. In naturally infected cats, the p15E ELISA showed a diagnostic sensitivity of 77.1% and a specificity of 85.6% when compared to provirus PCR results [25].

2.3. Classification of Courses of Infection and Vaccination Status

The classification of the 934 cats into the different courses of infection is shown in Table 1.

Table 1. Classification of feline leukaemia virus infection status in 934 naturally infected cats of the present study based on the European Advisory Board on Cat Diseases FeLV diagnostic tool [29].

FeLV Infection Status	p27 Ag ELISA	Proviral DNA PCR *	Anti-p15E Ab ELISA
Progressive (n = 38)	+	+	+/−[2]
Regressive (n = 40)	−	+	+/−[2]
Abortive (n = 108)	−	−	+
Focal (n = 21)	(+) [1]	−	+/−[2]
FeLV unexposed vaccinated [Δ] (n = 72)	−	−	+/−
FeLV unexposed not vaccinated (n = 655)	−	−	−

* FeLV proviral DNA was determined in all samples using a quantitative polymerase chain reaction (qPCR), as described before [17,27]. [Δ] Of the FeLV-unexposed cats, 72/727 (9.9%) were vaccinated against FeLV. Of the vaccinated cats, 16/72 (22.2%) tested positive in the p15E ELISA. Five of these cats were vaccinated with Purevax® FeLV and two cats with Leucogen®; the manufacturer of the other vaccines was unknown (n = 9). [1] Cats with focal infection tested weakly p27 antigen-positive and proviral DNA-negative. [2] Not all cats with a progressive, regressive, or focal infection showed antibodies against anti-p15E. The symbol +/− means that, regardless of the course of the infection, some cats tested positive in the p15E ELISA and others tested negative in the p15E ELISA. The fields highlighted in grey indicate that the mentioned test was not used for the classification to the specific course of infection. FeLV, feline leukaemia virus; Ag, antigen; ELISA, enzyme-linked immunosorbent assay; DNA, deoxyribonucleic acid; Ab, antibody.

2.4. Point-of-Care Test (v-RetroFel®)

The PoC test, an immunochromatographic assay, was coated with antibodies specific to FeLV p27 antigen as well as with an antigen specific for FeLV p15E antibodies. In addition, the test also provided results for FIV (feline immunodeficiency virus) antibodies; evaluation of the detection of FIV antibodies was not part of this study. The PoC test was stored between 18–22 °C and carried out with serum samples according to the manufacturer's instructions. All samples were tested with the same batch of the PoC test (ID 25110011221). Each test was incubated and interpreted according to the manufacturer's specification after ten minutes. Additionally, each test was interpreted after a prolonged incubation period of 20 min. All samples were tested for FeLV p27 antigen and anti-p15E antibodies using the PoC test by the same investigator (J.G.) who was blinded to the results of the p27

antigen and anti-p15E antibodies ELISAs. If the result was uncertain, a second independent person assessed the results (the two investigators always agreed). Once a line was visible, regardless of the intensity of the colour, the test was considered positive.

Practicability, difficulties in test result interpretation, sensitivity (true positive rate), specificity (true negative rate), negative predictive value (NPV) (proportion of predicted negatives that were true negatives), and positive predictive value (PPV) (proportion of predicted positives that were true positives) were calculated.

2.5. Statistical Analysis

Laboratory data from all cats were analysed using Excel (Microsoft Germany GmbH, München, Germany) and R statistical language (version 4.1.2; R Core Team, 2020). To assess the normality of metric variables for the statistical analysis, Shapiro–Wilk normality test was conducted. McNemar test was used to compare the results of the point-of-care test after ten and 20 min of incubation time, concerning anti-p15E antibodies. Kruskal–Wallis test ("ggstatsplot" R package) was used to determine any statistically significant differences between the means of anti-p15E antibody levels in true positive, true negative, false positive and false negative samples in PoC test [30]. Holm–Bonferroni was used for the adjustment of the *p*-values to correct for multiple comparisons. For all analyses, 95% confidence intervals (CI) were calculated. A p-value < 0.05 was used to determine statistical significance.

3. Results

3.1. Performance of the PoC Test

The PoC test can be stored at room temperature and was therefore immediately ready for use. It was easy to perform, without the need for advanced laboratory equipment or trained personnel. After ten minutes of incubation time, 36.2% (21/58) of the samples positive in the p15E antibody PoC test strips showed rather faintly coloured test lines (Figure 1), barely visible and thus difficult to interpret. When the incubation time was prolonged to 20 min, 13.8% (8/58) of the samples positive in the PoC test showed rather faintly coloured lines. In contrast, the p27 antigen test strip was easy to interpret for all samples after both ten and 20 min.

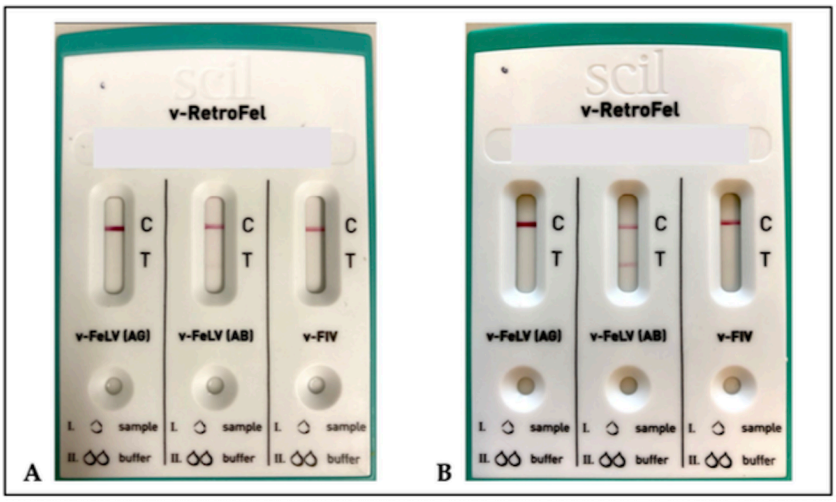

Figure 1. Pictures of a point-of-care test (v-RetroFel®, scil animal care company GmbH, Viernheim, Germany) for the detection of feline leukaemia virus p27 antigen (v-FeLV (AG)), feline leukaemia virus anti-p15E antibodies (v-FeLV (AB)), and feline immunodeficiency virus antibodies (v-FIV). The point-of-care tests were carried out with serum samples with an incubation time of ten minutes each. The pictures show a point-of care tests with a faintly coloured (picture (**A**)) and a clearly visible (picture (**B**)) test line (T) in an enzyme-linked immunosorbent assay anti-p15E antibody-positive sample (v-FeLV (AB)) as well as clearly visible control lines (C).

3.2. FeLV p27 Antigen

Among the 60 SPF cats (30 uninfected and 30 experimentally FeLV-infected), the 16 cat with progressive infection (26.7%) were p27 antigen positive. The prevalence of p27 antigen (determined by reference standard ELISA) was 6.2% (58/934) in naturally infected cats, 10.0% (27/269) in Italy, 5.4% (13/240) in Portugal, 4.7% (15/318) in Germany, and 2.8% (3/107) in France. As per definition, the prevalence of antigenemia was 100.0% (38/38) in progressively infected cats and 100.0% (11/11) in focally infected cats. The prevalence in regressively, abortively, and FeLV-unexposed cats was 0.0%. The p27 antigen performance parameters of the PoC test in experimentally infected cats and in naturally infected cats are summarized in Tables 2 and 3. The p27 antigen performance parameters of the PoC test considering different courses of infection are given in Table 4. All test results remained identical after 20 min of incubation time. Thus, all performance parameters remained unchanged compared to ten minutes of incubation time.

Table 2. Specificity, sensitivity, negative and positive predictive values of the point-of-care test for the detection of feline leukaemia virus p27 antigen in 60 SPF cats (30 uninfected cats, 16 progressively, and 14 regressively infected) compared to the results of an in-house developed p27 antigen enzyme-linked immunosorbent assay as a reference standard, taking experimentally infected cats into account.

p27 Antigen Positive Samples (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
26.7	93.8 (69.8–99.8)	100.0 (92.0–100.0)	100.0 (78.2–100.0)	93.3 (88.2–99.9)

FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives).

Table 3. Specificity, sensitivity, negative and positive predictive values of the point-of-care test for the detection of feline leukaemia virus p27 antigen in comparison to the results of an in-house developed p27 antigen enzyme-linked immunosorbent assay as a reference standard, taking naturally infected cat populations from different countries into account.

Country	Prevalence of p27 Antigen (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	6.2	82.8 (70.6–91.4)	96.0 (94.5–97.2)	57.8 (46.5–68.6)	98.8 (97.9–99.4)
Italy	10.0	92.6 (75.7–99.1)	95.0 (91.5–97.4)	67.6 (50.2–82.0)	99.1 (96.9–99.9)
Portugal	5.4	84.6 (54.6–98.1)	96.9 (93.8–98.8)	61.1 (35.8–82.7)	99.1 (96.8–99.9)
Germany	4.7	60.0 (32.3–83.7)	96.4 (93.6–98.2)	45.0 (23.1–68.5)	98.0 (95.7–99.3)
France	2.8	100.0 (29.2–100.0)	95.2 (89.1–98.4)	37.5 (8.5–75.5)	100.0 (96.3–100.0)

FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives).

Table 4. Specificity, sensitivity, negative and positive predictive value of the point-of-care test for the detection of feline leukaemia virus p27 antigen in comparison to the results of a laboratory-based p27 antigen enzyme-linked immunosorbent assay as a reference standard, taking different courses of feline leukaemia virus infection into account.

Course of FeLV-Infection	Prevalence of p27 Antigen (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	6.2	82.8 (70.6–91.4)	96.0 (94.5–97.2)	57.8 (46.5–68.6)	98.8 (97.9–99.4)
Progressive Infection [1]	100.0	100.0 (90.8.1–100.0)	n. d.	100.0 (90.8–100.0)	n. d.
Regressive Infection [2]	0.0	n. d.	100.0 (91.2–100.0)	n. d.	100.0 (91.2–100.0)
Abortive Infection [3]	0.0	n. d.	95.7 (85.5–99.5)	n. d.	100.0 (92.1–100.0)
Focal Infection [4]	100.0	63.6 (30.8–89.1)	n. d.	100.0 (59.0–100.0)	n. d.
Unexposed	0.0	n. d.	96.1 (94.5–97.4)	n. d.	100.0 (99.5–100.0)

[1] progressively infected cats were defined as follows: p27 antigen-positive and proviral DNA-positive. [2] regressively infected cats were defined as follows: p27 antigen-negative, proviral DNA-positive. [3] abortively infected cats were defined as follows: p27 antigen-negative, proviral DNA-negative, anti-FeLV antibody-positive. [4] focally infected cats were defined as follows: p27 antigen weak positive, proviral DNA-negative. FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives); n. d., could not be determined as none of the samples tested positive or negative in the p27 antigen enzyme-linked immunosorbent assay.

3.3. *Anti-p15E Antibodies*

The prevalence of anti-p15E antibodies in the reference ELISA, when considering a ROD of >4.9% as positive for experimentally infected cats and >16.3% as positive for naturally infected cats, was 100.0% in experimentally infected cats and 19.8% (185/934) in naturally infected cats, 21.1% (57/269) in Italy, 22.5% (54/240) in Portugal, 14.1% (45/318) in Germany, and 27.1% (29/107) in France. All SPF cats (30/30) tested negative in the anti p15E antibody ELISA as well as negative in the PoC test. The anti-p15E antibody performance parameters of the PoC test in experimentally and naturally infected cats are summarized in Tables 5 and 6. The prevalence of anti-p15E antibodies was 78.9% (30/38) in progressively infected cats, 65.0% (26/40) in regressively infected, 100.0% (108/108) in abortively infected, and 23.8% (5/21) in focally infected. Cats unexposed to and not vaccinated against FeLV showed no anti-p15E antibodies (0.0%; 0/655). In total, 22.2% (16/72) of FeLV-unexposed cats but previously vaccinated against FeLV showed anti-p15E antibodies. Four of the vaccinated cats that tested positive in the p15E ELISA also tested positive in the PoC test (25.0%; 4/16). A summary of the performance parameters of the PoC test in progressively, regressively, abortively, focally infected, and FeLV-unexposed cats is shown in Table 7.

After 20 min of incubation, the sensitivity of the POC test for the detection of anti-p15E antibodies could be increased from 31.4% to 40.0% when considering all serum samples. The sensitivity was significantly higher after 20 min of incubation compared to ten minutes ($p < 0.001$). The specificity remained the same at 96.9%. Performance parameters after 20 min of incubation time for the different countries and the different courses of infection are summarized in Tables 8 and 9.

Table 5. Specificity, sensitivity, negative and positive predictive values of the point-of-care test for the detection of feline leukaemia virus anti-p15E antibodies in 60 SPF cats (30 uninfected, 30 FeLV-infected) in comparison to the results of a laboratory-based anti-p15E antibody enzyme-linked immunosorbent assay as a reference standard.

Anti-p15E Antibody Positive Samples (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
50.0	100.0 (88.4–100.0)	100.0 (88.4–100.0)	100.0 (88.4–100.0)	100.0 (88.4–100.0)

FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives).

Table 6. Specificity, sensitivity, negative and positive predictive values of the point-of-care test for the detection of feline leukaemia virus anti-p15E antibodies in comparison to the results of a laboratory-based anti-p15E antibody enzyme-linked immunosorbent assay as a reference standard, taking cat populations from different countries into account.

Country	Prevalence of Anti-p15E Antibodies (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	19.8	31.4 (24.7–38.6)	96.9 (95.4–98.0)	71.6 (60.5–81.1)	85.1 (82.5–87.4)
Italy	21.2	45.6 (32.4–59.3)	94.8 (90.0–97.4)	70.3 (53.0–84.1)	86.6 (81.6–90.7)
Portugal	22.5	27.8 (16.5–41.6)	96.7 (93.1–98.8)	71.4 (47.8–88.7)	82.2 (76.5–87.0)
Germany	14.2	20.0 (9.6–34.6)	98.2 (95.8–99.4)	64.3 (35.1–87.2)	88.2 (84.0–91.6)
France	27.1	27.6 (12.7–47.2)	98.7 (93.1–100.0)	88.9 (51.8–99.7)	78.6 (69.1–86.2)

FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives).

Table 7. Specificity, sensitivity, negative and positive predictive value of the point-of-care test for the detection of feline leukaemia virus anti-p15E antibodies in comparison to the results of a laboratory-based anti-p15E antibody enzyme-linked immunosorbent assay as a reference standard, taking different courses of feline leukaemia virus infection into account.

Course of FeLV-Infection	Prevalence of Anti-p15E Antibodies (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	19.8	31.4 (24.7–38.6)	96.9 (95.4–98.0)	71.6 (60.5–81.1)	85.1 (82.5–87.4)
Progressive Infection [1]	78.9	86.7 (69.3–96.2)	100.0 (63.1–100.0)	100.0 (86.8–100.0)	66.7 (34.9–90.1)
Regressive Infection [2]	65.0	34.6 (17.2–55.7)	85.7 (57.2–98.2)	81.8 (48.2–97.7)	41.4 (23.5–61.1)
Abortive Infection [3]	100.0	16.7 (10.2–25.1)	n. d.	100.0 (81.5–100.0)	n. d.
Focal Infection [4]	23.8	20.0 (0.5–71.6)	100.0 (79.4–100.0)	100.0 (2.5–100.0)	80.0 (56.3–94.3)
FeLV unexposed vaccinated	22.2	25.0 (7.3–52.4)	98.2 (90.5–99.9)	79.8 (28.4–99.5)	82.3 (70.8–90.4)
FeLV unexposed not vaccinated	0.0	n. d.	97.0 (95.3–98.1)	n. d.	100.0 (99.4–100.0)

[1] progressively infected cats were defined as follows: p27 antigen-positive and proviral DNA-positive. [2] regressively

infected cats were defined as follows: p27 antigen-negative, proviral DNA-positive. [3] abortively infected cats were defined as follows: p27 antigen-negative, proviral DNA-negative, anti-FeLV antibody-positive. [4] focally infected cats were defined as follows: p27 antigen weak positive, proviral DNA-negative. FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives); n. d., could not be determined as none of the samples tested negative in the anti-p15E antibody enzyme-linked immunosorbent assay.

Table 8. Specificity, sensitivity, negative and positive predictive value of the point-of-care test after 20 min incubation time to detect feline leukaemia virus anti-p15E antibodies compared with the results of a laboratory-based anti-p15E antibody enzyme-linked immunosorbent assay in cats from Italy, Portugal, Germany, and France.

Country	Prevalence of Anti-p15E Antibodies (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	19.8	40.0 (32.9–47.4)	96.9 (95.4–98.0)	76.3 (66.6–84.3)	86.7 (84.3–89.0)
Italy	21.2	50.9 (37.3–64.4)	94.8 (90.9–97.4)	72.5 (56.1–85.4)	87.8 (82.8–91.7)
Portugal	22.5	40.7 (27.6–55.0)	96.8 (93.1–98.8)	78.6 (59.1–91.7)	84.9 (79.4–89.4)
Germany	14.2	22.2 (11.2–37.1)	98.2 (95.8–99.4)	66.7 (38.4–88.2)	88.5 (84.3–91.8)
France	27.1	44.8 (26.5–64.3)	98.7 (93.1–100.0)	92.9 (66.1–99.8)	82.8 (73.6–89.8)

FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives).

Table 9. Specificity, sensitivity, negative and positive predictive value of the point-of-care test after 20 min incubation time to detect feline leukaemia virus anti-p15E antibodies compared with the results of a laboratory-based anti-p15E antibody enzyme-linked immunosorbent assay, in progressively, regressively, abortively, focally, and unexposed cats.

Course of FeLV-Infection	Prevalence Anti-p15E Antibodies (ELISA) %	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All cats	19.8	40.0 (32.9–47.4)	96.9 (95.4–98.0)	76.3 (66.6–84.3)	86.7 (84.3–89.0)
Progressive infection [1]	78.9	90.0 (73.5–97.9)	100.0 (63.1–100.0)	100.0 (87.2–100.0)	72.7 (39.0–94.0)
Regressive infection [2]	65.0	42.3 (23.4–63.1)	85.7 (57.2–98.2)	84.6 (54.6–98.1)	44.4 (25.5–64.7)
Abortive infection [3]	100.0	28.7 (20.4–38.2)	n. d.	100.0 (88.8–100.0)	n. d.
Focal infection [4]	23.8	20.0 (0.5–71.6)	100.0 (79.4–100.0)	100.0 (2.5–100.0)	80.0 (56.3–94.3)
FeLV unexposed vaccinated	22.2	31.3 (11.0–58.7)	98.2 (90.5–99.9)	83.2 (35.9–99.6)	83.5 (72.1–91.4)
FeLV unexposed not vaccinated	0.0	n. d.	97.0 (95.3–98.1)	n. d.	100.0 (99.4–100.0)

[1] progressively infected cats were defined as follows: p27 antigen-positive and proviral DNA-positive. [2] regressively infected cats were defined as follows: p27 antigen-negative, proviral DNA-positive. [3] abortively infected cats were defined as follows: p27 antigen-negative, proviral DNA-negative, anti-FeLV antibody-positive. [4] focally infected cats were defined as follows: p27 antigen weak positive, proviral DNA-negative. FeLV, feline leukaemia virus; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; PPV, positive predictive value (proportion of predicted positives that were true positives); NPV, negative predictive value (proportion of predicted negatives that were true positives); n. d., could not be determined as none of the samples tested negative in the anti-p15E antibody enzyme-linked immunosorbent assay.

In total, 127/185 samples that tested positive in the anti-p15E ELISA were not detected in the PoC test, neither after ten minutes of incubation nor after 20 min. Particularly, samples (36/185; 19.5%) whose readings were low in the anti-p15E ELISA (close to the cut-off value; ≤20.0%) of the anti-p15E ELISA (16.3%) were not detected by the PoC test (32/36; 88.9%) (Figure 2). However, there was no significant difference in antibody concentrations in the ELISA (median: 24.6%) between false negative samples and true positive samples (median: 70.4%) in the PoC test.

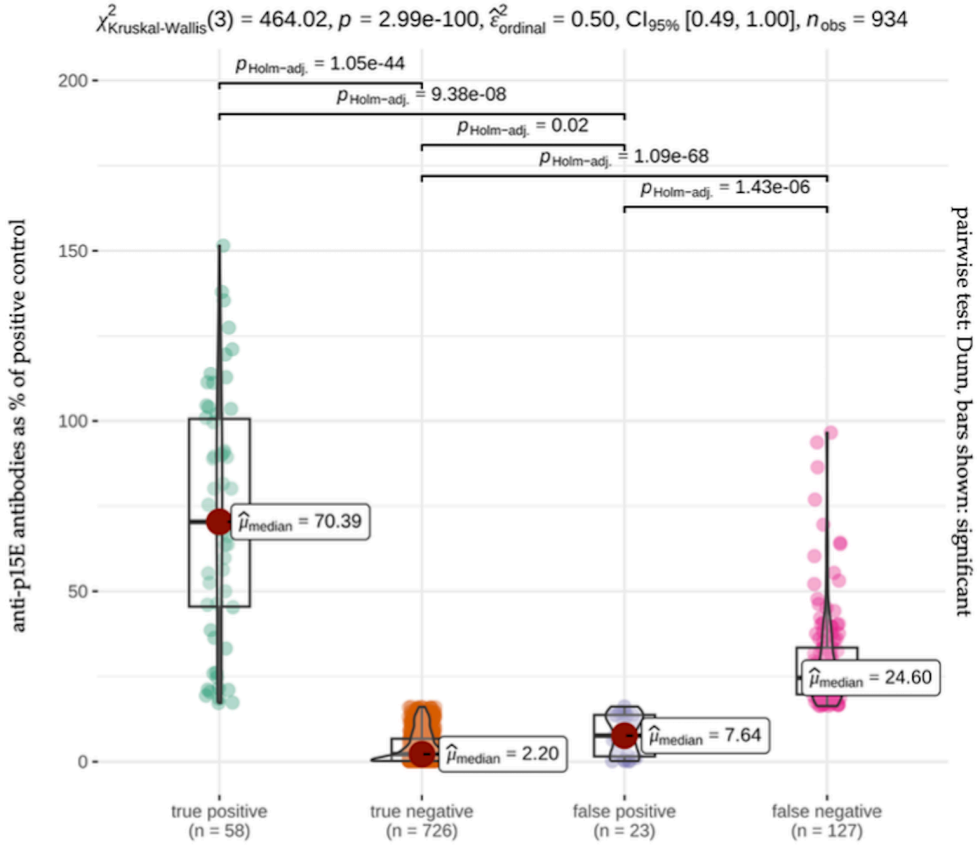

Figure 2. Percent deviations of median (red dot) anti-p15E antibody levels in the reference standard (enzyme-linked immunosorbent assay, ELISA). Violin plots depicting the anti-p15E antibody levels in percent for true positive, true negative, false positive, and false negative results in the point-of-care (PoC) test and the significant differences between the groups. For each box-and-whisker diagram, the solid line within the box represents the median. The lower and upper limits of the box represent the interquartile range (25th and 75th percentiles). The whiskers delimit the range; green, orange, purple, and pink dots represent the single cats. Cats with higher anti-p15E antibody levels in the ELISA (median: 70.4%) had a significantly higher chance to be detected in the PoC test than cats with lower antibody levels (median: 7.6%). In comparison, cats with low anti-p15E antibody levels in the ELISA (median: 2.2%) were significantly more often detected as a true negative in the PoC test compared to cats with higher antibody levels (median: 7.6%).

4. Discussion

Feline leukaemia virus (FeLV) has a worldwide distribution and is one of the most important infectious agents in cats. Due to the complex pathogenesis and varying courses of FeLV infection, diagnosis is difficult and often not possible with a single test [1–3]. The current commercially available PoC tests for the identification of FeLV-infected cats are based on the detection of p27 antigen and thus only detect progressively infected cats. In previous studies, it has been shown that anti-p15E antibodies, in contrast to p27 antigen, are formed and can be detected in blood in most cases after exposure to FeLV regardless of the course of infection

(progressive, regressive, abortive, focal) [14,18,26]. However, the reference standard (ELISA) for the detection of anti-p15E antibodies is only performed in specialized laboratories and is currently offered by only a few institutes, and ELISA results are usually only available after a few days. Therefore, a PoC test for in-house testing would be an important tool to diagnose FeLV-exposed cats immediately at the visit at the veterinarian. For potential blood donor cats, easy identification of regressively infected cats is of particular importance, as they can transmit FeLV provirus to FeLV-naive cats via blood transfusions [11]. Identification of regressively infected cats is also important in multi-cat households, as regressive infection can be reactivated to progressive infection with virus shedding, especially after suppression of the immune system. In addition, identification of FeLV-exposed cats (abortive infections) prior to vaccination might be helpful in deciding whether or not to vaccinate cats against FeLV [1–3]. A PoC test (v-RetroFel®, scil animal care company GmbH, Viernheim, Germany) for simultaneous detection of p27 antigen and anti-p15E antibody was recently evaluated in naturally FeLV-infected cats from Australia and Germany [17]. Unfortunately, the results in this previous study were not very promising concerning the detection of anti-p15E antibodies within this PoC test. Therefore, modifications were made by the manufacturer to improve the PoC test for the detection of anti-p15E antibodies.

In the present study, the modified version of the PoC test (v-RetroFel®; modified version 2021) was easy and quick to perform under practical conditions due to storage at room temperature and easy and few steps required during performance. However, in approximately one third of the samples (21/58; 36.2%) that tested positive in the PoC test, the anti-p15E antibody testing resulted in only a faint colour line. A faint colour line can lead to uncertainty in result interpretation, making it challenging to determine whether the test is truly positive or negative. Faint colour lines could potentially affect the test's accuracy. It is unlikely that the storage time and freezing/thawing of the samples could have influenced the test performance. The results of a human study showed the stability of polyclonal antibodies in serum samples during long-term storage at −65 °C and −20 °C, during multiple freeze/thaw cycles, and during shipping [31].

Regarding the detection of p27 antigen, the PoC test showed a lower sensitivity (82.8%) when considering all samples compared to the first version of the PoC test (91.3%) [17]. According to the manufacturer, no changes were made in the p27 antigen test components; nevertheless, a low sensitivity in p27 testing is a disadvantage. Especially in countries with a high prevalence of FeLV, test performance, particularly sensitivity (the ability to correctly identify serum samples as positive), is the most important parameter for preventing the spread of infection by infected cats. Furthermore, cats with false negative results do not receive the appropriate medical attention and supportive care (e.g., isolation from other cats, treatment of secondary diseases). In animal shelters and adoption programs, poor test sensitivity can lead to unrecognized adoption of FeLV-infected cats. This not only puts the newly adopted cat at risk (higher risk for secondary infections due to immunosuppression) but can also lead to the unintentional spread of the virus to other cats within the shelter or adoptive homes. Specificity for p27 antigen was high (96.0%) and thus comparable to the one of the first version of the PoC test (98.3%) [17].

Regarding anti-p15E antibodies, the sensitivity of the PoC test was increased to 31.4% compared to the first version of this test, where the sensitivity for Australian samples was 16.7% and for German samples was 8.3% [17]. All samples were interpreted both after ten minutes (according to the manufacturer's specification) and after 20 min of incubation time to see whether an increase in sensitivity regarding anti-p15E antibodies is possible with a prolonged incubation time. With an extension of the incubation time to 20 min, the sensitivity regarding the detection of anti-p15E antibodies was increased from 31.4% to 40.0%, which is still low.

The PoC test was not able to detect most regressively, abortively, and focally infected cats that tested positive in the anti-p15E antibody ELISA (sensitivity 34.6%, 17.0%, and 20.0%). At least in samples from progressively infected cats, the PoC test showed a high sensitivity of 86.7%. However, detection of progressively infected cats through anti-p15E

antibody detection is not essential as progressive infection is already detected through the presence of p27 antigen. One reason for the high sensitivity with respect to anti-p15E antibodies in progressively infected cats could be that many of these cats had high antibody concentrations (median 78.6% of positive control) in the reference standard (laboratory-based ELISA). In the present study, it was shown that cats with higher ELISA antibody concentrations (median 70.4% of positive control) were detected more reliably in the PoC test in contrast to cats with low ELISA antibody concentrations (median 24.6% of positive control). Of the 127/185 false negative samples, 116/127 samples had an ELISA antibody concentration < 50.0% of the positive control. At this point, it should be mentioned that experimentally infected cats that tested positive in the anti-p15E antibody ELISA were also all identified as positive in the PoC test. One possible reason for this could be that the experimentally infected cats all showed a quite high antibody level of over 45% of the positive control in the anti-p15E antibody ELISA. Such a high sensitivity (95.7%) and specificity (100.0%) of the p15E ELISA was already shown in a previous study [25] and was confirmed again in the present study, in which all experimentally infected cats tested positive for the presence of anti-p15E antibodies in the p15E ELISA (sensitivity: 100.0%, specificity: 100.0%). In addition, naturally infected cats could be infected with FeLV subtypes that are not detected by the PoC test. Both the laboratory-based ELISA and the PoC test used the transmembrane of FeLV subtype A (GenBank accession no. AAA93093.1) [25]. In ELISA, a cut-off of >16.3% was used as the reference standard for the detection of anti-p15E antibodies in naturally infected cats. The same applies to the PoC test, which is designed to detect samples with an anti-p15E antibody level of >16.3%. The determined cut-off values of the laboratory-based ELISA might not be appropriate for all populations or conditions, including samples from different geographic regions and different breeds or age groups of cats, factors that might have affected the applicability of the test. Westman and colleagues already suggested that the cut-off value, which determines whether a sample is considered positive, should be carefully reevaluated [17].

The specificity of anti-p15E antibody testing was 96.9% in the present study using the modified new version of the PoC test, comparable to the first version of the PoC test with a specificity of 90.2% (Australia) and 93.7% (Germany), respectively [17]. In the present study, a total of 23/934 samples were considered false positive. Contamination of the sample with substances that mimic anti-FeLV antibodies could result in false positive results. Another reason for a false-positive result in a PoC test can be the presence of antibodies against antigens that mimic the FeLV p15E epitopes and therefore cause cross-reactivity. In addition, previous vaccinations against FeLV can lead to positive results [25]. For this reason, it is important to consider the cat's vaccination history when interpreting test results. Nevertheless, it can be concluded that the detection of anti-p15E antibodies more likely indicates a previous infection rather than vaccination. Similar results were reported in previous studies, where the majority of vaccinated cats in Switzerland had lower levels of anti-p15E antibodies than cats previously exposed to FeLV [25]. A recent study showed that the response of p15E antibodies depended on the type of FeLV vaccine administered. Cats vaccinated with an inactivated whole-virus FeLV vaccine (Fel-O-Vax® Lv-K or Fel-O-Vax® 5) more likely had an anti-p15E antibody response compared to those vaccinated with a subunit vaccine (Leucogen®) [17].

Further research is necessary to understand the clinical relevance of anti-p15E antibodies. Cats with high anti-p15E antibody levels might be protected from FeLV infection. In a previous study, it was shown that cats immunized with the FeLV transmembrane protein p15E developed neutralizing anti-p15E antibodies [32]. An experimental study attempted to include p15E in FeLV vaccines. In three of six cats immunized with p15E and experimentally infected with FeLV, protection against FeLV antigenemia was observed at day 960. The remaining three cats immunized with p15E were unprotected and developed progressive FeLV infection after experimental infection with FeLV [33].

5. Conclusions

The PoC test (v-RetroFel®; modified version 2021) provides results quickly. Especially when considering situations such as testing cats before vaccination against FeLV or screening cats for FeLV before introducing them into a new household with other felids, a quick and easy performance of the test would be essential. Sensitivity and specificity of the PoC test for FeLV anti-p15E antibody detection was improved by modifications by the manufacturer when compared to the first version of the PoC test. Nevertheless, the sensitivity was still too low. A high sensitivity and a high positive predictive value are however necessary to correctly identify cats that had contact with FeLV. Therefore, further modifications of the PoC test should be made to improve sensitivity regarding detection of anti-p15E antibodies before the test can be recommended for use under field conditions. Moreover, it would be important to further explore the clinical relevance of anti-p15E antibodies and to evaluate whether anti-p15E antibody-positive cats are protected against FeLV infection.

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VI. DISKUSSION

Das FeLV ist weltweit verbreitet und gehört zu den wichtigsten Infektionserregern der Katze. Aufgrund der komplexen Pathogenese und der unterschiedlichen Krankheitsverläufe ist die Diagnose schwierig (HARTMANN & HOFMANN-LEHMANN, 2020; HOFMANN-LEHMANN & HARTMANN, 2020; LITTLE et al., 2020). Die Entwicklung neuer diagnostischer Verfahren trägt heutzutage dazu bei, unterschiedliche Verlaufsformen einer FeLV-Infektion nachweisen zu können. Gängige Untersuchungsmethoden konzentrieren sich auf den Nachweis von FeLV-Antigen. Durch diesen Nachweis werden hauptsächlich progressiv (in einigen Fällen auch fokal (atypisch)) infizierte Katzen und Katzen in der Anfangsphase einer regressiven Infektion erfasst. Die Bestimmung der Prävalenz aller Verlaufsformen (progressiv, regressiv, abortiv und fokal (atypisch)) ist von besonderer Bedeutung, da eine ganzheitliche Bewertung des FeLV-Status eine umfassende Einschätzung des Infektionsgeschehens in Katzenpopulationen ermöglicht und dazu beiträgt, geeignete Maßnahmen zur Prävention, Behandlung und Kontrolle der Infektion zu ergreifen.

Der Übersichtsartikel (**Publikation 1**) gibt einen umfassenden Überblick über die verschiedenen Verlaufsformen einer Infektion mit dem FeLV sowie über die diagnostischen Methoden zur Identifizierung infizierter Katzen. Außerdem soll er als diagnostischer Leitfaden dienen, um Katzen den unterschiedlichen Verlaufsformen zuteilen zu können und dementsprechende Maßnahmen treffen zu können.

Die Studie zu **Publikation 2** ist die erste Studie, die die FeLV-Prävalenz in vier Ländern Europas unter Berücksichtigung aller Verlaufsformen untersuchte. Insgesamt wurden 934 Katzen aus vier Ländern Europas in diese prospektive Studie eingeschlossen. Davon kamen 509 Katzen aus Ländern mit einer hohen FeLV-Prävalenz (Italien: 269 Katzen, Portugal: 240 Katzen) und 425 Katzen aus Ländern mit einer niedrigen FeLV-Prävalenz (Deutschland: 318 Katzen, Frankreich: 107 Katzen) (STUDER et al., 2019). Es wurden gezielt Länder mit hoher *versus* niedriger FeLV-Prävalenz ausgewählt, um mögliche Unterschiede in der Verteilung der Verlaufsformen in den jeweiligen Ländern aufzuzeigen. Die Auswahl der Länder basierte auf einer europaweiten Studie, die zeigte, dass die

Prävalenz von FeLV-ausscheidenden (somit progressiv infizierten) Katzen in Italien bei 5,7 %, in Portugal bei 8,8 %, in Deutschland bei 0,3 % und in Frankreich bei 1,0 % lag (STUDER et al., 2019). In der vorliegenden Studie (**Publikation 2**) waren ebenfalls mehr Katzen aus Südeuropa (unter Berücksichtigung aller Verlaufsformen Italien 21,2 %, Portugal 20,4 %) mit FeLV infiziert als Katzen aus Westeuropa (unter Berücksichtigung aller Verlaufsformen Deutschland 9,5 %, Frankreich 9,3 %). Das zeigt, dass die FeLV-Gesamtprävalenz vor allem in südeuropäischen Ländern, wie Italien und Portugal, interessanterweise aber auch in Westeuropa (Deutschland und Frankreich) noch immer sehr hoch ist. Ein direkter Vergleich mit zuvor durchgeführten Prävalenzstudien gestaltet sich schwierig, da diese fast ausschließlich auf dem Nachweis von progressiv infizierten Katzen beruhen.

In der vorliegenden Studie wurden Katzen mit einer progressiven FeLV-Infektion anhand des Nachweises von p27-Antigen und Provirus-DNA identifiziert (HARTMANN & HOFMANN-LEHMANN, 2020; HOFMANN-LEHMANN & HARTMANN, 2020) (siehe auch Tabelle 1 aus **Publikation 1**). Insgesamt wurden 38 Katzen (4,1 %) einer progressiven Verlaufsform zugeordnet. In Italien, Portugal, Deutschland und Frankreich lag die Prävalenz progressiv infizierter Katzen bei 7,8 %, 3,8 %, 1,9 % und 1,9 %. Eine Zunahme der progressiven FeLV-Infektion war besonders in Italien (7,8 %) festzustellen. So lag die Prävalenz bei streunenden Katzen aus Norditalien zwischen 2008–2010 und 2014 bei 3,8 % und 6,1 % (SPADA et al., 2012a; SPADA et al., 2016). Ein Anstieg dieser Prävalenz könnte auf einen Rückgang der Maßnahmen gegen FeLV zurückzuführen sein, die dazu beitragen, das Risiko einer progressiven FeLV-Infektion zu reduzieren, wie zum Beispiel Testung und Separieren und/oder Impfungen gegen FeLV. Die Wahrscheinlichkeit, dass die höhere Prävalenz in Italien in der vorliegenden Studie auf falsch-positive Ergebnisse zurückzuführen ist, ist gering, da in der vorliegenden Studie alle progressiv infizierten Katzen nicht nur positiv auf p27-Antigen, sondern auch positiv auf virale RNA im Blut getestet wurden; zudem wurden alle progressiv infizierten Katzen, bei denen zusätzlich ein Speichelabstrich (Tupferprobe) zur Verfügung stand, positiv auf virale RNA im Speichel getestet. Vorangegangene Untersuchungen zeigten eine hohe Sensitivität (98,1 %) und Spezifität (99,2 %) für den Nachweis einer progressiven Infektion durch Nachweis der FeLV-RNA (mittels quantitativer reverse Transkriptase-Polymerase-Kettenreaktion (RT-qPCR)) aus Speichel und Blut im Vergleich zum Nachweis von p27-Antigen

(mittels Enzyme-linked-Immunosorbent-Assay (ELISA)) aus Blut; die Übereinstimmung dieser Untersuchungsmethoden war nahezu perfekt (Kappa-Wert: 0,96) (GOMES-KELLER et al., 2006). Im Gegensatz zu dem verzeichneten Anstieg der progressiven FeLV-Infektion in Italien blieb die Anzahl progressiv infizierter Katzen in Deutschland im Verlauf der letzten zehn Jahre weitgehend unverändert (ENGLERT et al., 2012). Im Jahr 2019 wurde in Frankreich eine vergleichbar niedrige Prävalenz von 1,0 % bei FeLV-ausscheidenden Katzen festgestellt (STUDER et al., 2019). Im Vergleich zu einer vorangegangenen Studie hat die Prävalenz progressiv infizierter Katzen in Portugal dagegen abgenommen (STUDER et al., 2019). Forcierte Impfprogramme könnten in Portugal in den letzten Jahren zu einer niedrigeren Prävalenz geführt haben (GROSENBAUGH et al., 2017).

Überraschenderweise konnten in der Studie zu **Publikation 2** bei einer Katze eindeutige Hinweise auf eine progressive FeLV-Infektion (Vorhandensein von viraler RNA im Blut, hohe Proviruslast) nachgewiesen werden, jedoch (wiederholt) kein p27-Antigen. Falsch-negative p27-Antigen-ELISA-Ergebnisse müssen in Betracht gezogen werden. Wahrscheinlicher ist jedoch, dass die Katze sehr früh im progressiven oder regressiven Verlauf getestet wurde und p27-Antigen zu diesem Zeitpunkt noch nicht nachweisbar war. Die Katze zeigte eine hohe Proviruslast im Blut, was auf einen progressiven Verlauf hindeutet. Eine hohe Proviruslast im Anfangsstadium der Infektion schließt jedoch eine regressive Infektion nicht aus (HOFMANN-LEHMANN et al., 2001; HOFMANN-LEHMANN et al., 2007). Wenngleich auch regressiv infizierte Katzen eine relativ hohe Proviruslast aufweisen können, konnte in der vorliegenden Studie ein signifikanter Unterschied zwischen der Proviruslast bei progressiv (hohe Proviruslast) und regressiv (niedrige Proviruslast) infizierten Katzen festgestellt werden. Diese Ergebnisse stimmen mit denen aus einer Studie aus der Schweiz überein (TANDON et al., 2005). Die quantitative Bestimmung proviraler DNA im Blut könnte somit einen Hinweis auf den Verlauf der Infektion geben.

In der vorliegenden Studie wurden Katzen, die p27-Antigen-negativ und Provirus-positiv waren, einer regressiven Verlaufsform zugeordnet (40 Katzen; 4,3 %). In Italien, Portugal, Deutschland und Frankreich lag die Prävalenz regressiv infizierter Katzen bei 4,5 %, 8,3 %, 1,3 % und 3,7 %. Untersuchungen, die die Prävalenz regressiv infizierter Katzen in Italien, Portugal oder Frankreich früher bestimmten, existieren nicht. Es gibt eine Studie aus Süddeutschland, die die Prävalenz regressiv

infizierter Katzen mit 1,2 % ermittelte; die Anzahl regressiv infizierter Katzen in Deutschland blieb in den letzten zehn Jahren also nahezu unverändert (ENGLERT et al., 2012). Die Ursache für die vergleichsweise hohe Anzahl regressiv infizierter Katzen in Portugal ist unbekannt. Möglicherweise ist ein Zusammenhang zwischen der endogenen FeLV (enFeLV)-Viruslast und der Entwicklung verschiedener Infektionsverläufe hierfür verantwortlich. Es ist möglich, dass ein Zusammenhang zwischen der enFeLV-Viruslast und dem Infektionsverlauf besteht. EnFeLV schützt zwar nicht vor einer FeLV-Infektion, verhindert jedoch einen progressiven Infektionsverlauf (POWERS et al., 2018). So könnte eine höhere enFeLV-Viruslast bei portugiesischen Katzen die Ursache dafür sein, dass regressive Infektionsverläufe in Portugal häufiger vorkommen.

Die Zuordnung einer abortiven Infektion erfolgte in der vorliegenden Studie anhand des Nachweises von anti-p15E- und anti-SU-Antikörpern. Insgesamt wurden 47 Katzen (5,0 %) einer abortiven Verlaufsform zugeteilt. In Italien, Portugal, Deutschland und Frankreich lag die Prävalenz abortiv infizierter Katzen bei 6,3 %, 6,7 %, 3,5 % und 2,8 %. In der vorangegangenen Studie aus Süddeutschland lag die Prävalenz der abortiven Infektion mit 9,2 % deutlich höher (ENGLERT et al., 2012). Verschiedene Kriterien zur Klassifizierung eines abortiven Verlaufs in den jeweiligen Studien könnten für diesen Unterschied verantwortlich sein. So wurden von Englert von Kollegen (2012) alle Katzen, die anti-SU-Antikörper-positiv waren, einem abortiven Verlauf zugeordnet. In der vorliegenden Studie hingegen wurde definiert, dass Katzen sowohl anti-SU-Antikörper-positiv als auch anti-p15E-Antikörper-positiv sein müssen, um als abortiv infiziert zu gelten. Experimentelle Untersuchungen haben gezeigt, dass Katzen aufgrund von unspezifischen Reaktionen auf enFeLV anti-SU-Antikörper entwickeln können (H Lutz, E Boenzli, persönliche Kommunikation, Daten nicht veröffentlicht). Die alleinige Untersuchung von anti-SU-Antikörpern könnte also dazu führen, dass die Prävalenz abortiver Infektionen überschätzt wird.

Die vorliegende Studie untersuchte als erste Studie die Prävalenz fokal (atypisch) infizierter Katzen unter Feldbedingungen. In der vorliegenden Studie wurden Katzen, die im p27-Antigen-ELISA schwach positiv und in der Provirus-DNA-PCR negativ waren, einer fokalen (atypischen) Infektion zugeordnet. Insgesamt waren 21 Katzen (2,3 %) fokal infiziert. In Italien, Portugal, Deutschland und Frankreich lag die Prävalenz fokal infizierter Katzen bei 2,6 %, 1,7 %, 2,8 % und 0,9 %. Die vorliegenden Untersuchungen ermöglichen jedoch keine genaue

Festlegung der betroffenen Organe oder Gewebe, in denen sich das FeLV manifestiert hat. Es bedarf weiterer Untersuchungen, um fokal infizierte Katzen genauer zu klassifizieren.

In der Studie zu **Publikation 2** wurde auch das Vorkommen von anti-FeLV-Antikörpern (Ganzvirus, SU, p15E) bei den unterschiedlichen Verlaufsformen untersucht. Progressiv infizierte Katzen hatten signifikant höhere anti-p15E-Antikörpertiter im Vergleich zu abortiv infizierten Katzen. Das p15E-Protein ist ein Transmembranprotein, das auf der Oberfläche von Wirtszellen exprimiert wird, die mit FeLV infiziert sind. Es ermöglicht dem Virus, in Wirtszellen einzudringen und hemmt die Lymphozytenproliferation und die Funktionen der T-Zellen bei der Immunabwehr (ROJKO et al., 1979; HARAGUCHI et al., 2008). Die Unterschiede im anti-p15E-Antikörpertiter könnten darauf zurückzuführen sein, dass FeLV bei progressiv infizierten Katzen aktiv im Körper repliziert, was wiederum zu einer stärkeren Stimulation des Immunsystems und einer vermehrten Bildung spezifischer anti-p15E-Antikörper führt.

Ein weiteres Ziel der Studie zu **Publikation 2** war es, Risikofaktoren für die verschiedenen Verlaufsformen einer FeLV-Infektion mittels uni- und multivariater Regressionsanalyse zu bestimmen. Aufgrund zu geringer Gruppengrößen war es in der vorliegenden Studie jedoch nicht möglich, eine multivariate Analyse durchzuführen. Adulte Katzen zwischen ein und sieben Jahren hatten in der univariaten Analyse ein signifikant höheres Risiko für eine progressive und regressive FeLV-Infektion im Vergleich zu Katzen unter einem Jahr. Dies deckt sich mit den Ergebnissen einer Studie bei 6.005 Katzen aus 32 europäischen Ländern, in der festgestellt wurde, dass erwachsene Katzen im Alter von ein bis sechs Jahren ein signifikant höheres Risiko für eine Virämie aufwiesen als Katzen unter einem Jahr (STUDER et al., 2019). Es gibt mehrere Gründe, warum junge Katzen ein höheres Risiko für eine progressive FeLV-Infektion haben könnten. Junge Katzen sind oft aktiver und erkunden ihre Umgebung mehr, was die Wahrscheinlichkeit erhöht, mit anderen (potentiell FeLV-infizierten) Katzen in Kontakt zu kommen. FeLV wird hauptsächlich durch direkten Kontakt zwischen infizierten und nicht infizierten Katzen übertragen, zum Beispiel durch gegenseitiges Putzen, Kämpfe oder das Teilen von Futter- und Wassernäpfen. Jungkatzen sind aufgrund ihres Spielverhaltens und ihrer sozialen Interaktionen prädisponierter für solche Kontakte (LEVY et al., 2006). Hinzu kommt, dass das

Immunsystem junger Katzen im Vergleich zu älteren Katzen noch nicht vollständig ausgereift ist, weshalb die Katzen weniger in der Lage sind, das Virus abzuwehren (abortive Infektion) oder die Infektion unter Kontrolle zu halten (regressive Infektion). Gerade bei jungen Katzen mit Freigang ist es ist daher wichtig, diese frühzeitig gegen FeLV zu impfen und zu testen und wenn möglich, Risikosituationen, wie den Kontakt zu potentiell infizierten Katzen, zu vermeiden. Das Immunsystem älterer Katzen hingegen ist eher in der Lage, das Virus zu erkennen und effektiv abzuwehren.

In der vorliegenden Studie hatten 59/146 (40,4 %) Katzen eine Grunderkrankung. Zu den häufigsten Erkrankungen bei FeLV-infizierten Katzen der vorliegenden Studie zählten Gingivitis/Stomatitis (10/59; 16,9 %) und Lymphome (8/59; 13,6 %). Bei progressiv FeLV-infizierten Katzen kann eine Immunschwäche dazu führen, dass die Zusammensetzung der Maulflora gestört ist. Dadurch können sich Bakterien in der Maulhöhle leichter vermehren und eine Entzündung des Zahnfleisches und Zahnhalteapparates verursachen (QUIMBY et al., 2008; KORNYA et al., 2014). Darüber hinaus können progressiv FeLV-infizierte Katzen aufgrund ihrer geschwächten Immunabwehr anfälliger für andere Krankheiten sein, die eine Gingivitis begünstigen, beispielsweise Infektionen mit felinen Caliciviren. Außerdem kann FeLV sich in lymphatischen Geweben vermehren, was wiederum zu einer dysregulierten Zellteilung führen kann. Daraus entstehende abnorme Zellen haben ein erhöhtes Entartungsrisiko, was die Entstehung von Lymphomen fördern kann (KRUNIC et al, 2015).

Zum Nachweis von FeLV-p27-Antigen stehen zahlreiche PoC-Tests für den Einsatz in der Praxis zur Verfügung. Zur Bestimmung des FeLV-Status einer Katze wäre es dringend nötig, zusätzlich zu progressiv infizierten Katzen auch regressiv und abortiv infizierte Katzen zu detektieren. So ist zum Beispiel bei potenziellen Blutspender-Katzen die einfache Identifizierung regressiv infizierter Katzen von besonderer Bedeutung, da sie FeLV über Bluttransfusionen auf FeLV-naive Katzen übertragen können (NESINA et al., 2015). Darüber hinaus kann die Erkennung von FeLV-exponierten Katzen (abortiven Infektionen) vor der Impfung auch bei der Entscheidung, ob Katzen gegen FeLV geimpft werden sollen oder nicht, hilfreich sein. Die Identifizierung regressiv infizierter Katzen ist außerdem von großer Bedeutung, da bei einem regressiven Verlauf das Risiko einer Reaktivierung, insbesondere nach Suppression des Immunsystems, besteht (HARTMANN & HOFMANN-LEHMANN, 2020; HOFMANN-LEHMANN & HARTMANN,

2020; LITTLE et al., 2020).

In den Studien der **Publikationen 3** und **4** wurde ein PoC-Test (v-RetroFel®), der neben p27-Antigen auch anti-p15E-Antikörper nachweist, hinsichtlich seiner Performance (Sensitivität, Spezifität, positiver prädiktiver (PPW) und negativer prädiktiver (NPW) Wert) und hinsichtlich des Nutzens zur Identifikation unterschiedlicher FeLV-Verlaufsformen beurteilt. Die Sensitivität gibt an, wie gut ein Test in der Lage ist, tatsächlich infizierte Katzen zu erkennen. In der Studie der **Publikation 3** wurden Blutproben von Katzen aus Australien und Deutschland getestet. Hinsichtlich der Bestimmung von p27-Antigen wies der v-RetroFel® PoC-Test in seiner auf dem Markt verfügbaren Erst-Version (2020) in Blutproben aus Australien und Deutschland eine Sensitivität von 91,3 % und 100 % auf; er erwies sich somit als sehr effektiv in der Identifikation von progressiv infizierten Katzen. Für anti-p15E-Antikörper lag die Sensitivität zur Identifikation von progressiv, regressiv und abortiv infizierten Katzen bei 16,7 % und 8,3 %. Basierend auf diesen Ergebnissen konnten keine Vorteile gegenüber anderen verfügbaren PoC-Tests festgestellt werden, die ausschließlich FeLV-p27-Antigen nachweisen. Bei der Bestimmung der FIV-Antikörper war die Sensitivität des PoC-Tests im Vergleich zum Goldstandard (Western Blot) bei australischen Proben signifikant höher als bei deutschen Proben (94,7 % gegenüber 30,0 %). Dieser Unterschied könnte auf verschiedene Virusvarianten in den jeweiligen Ländern zurückzuführen sein. Über Unterschiede zwischen FIV-Feldisolaten in Australien und Deutschland ist derzeit noch wenig bekannt, weshalb weitere Untersuchungen wünschenswert sind. Im Gegensatz zur Sensitivität gibt die Spezifität an, wie gut ein Test in der Lage ist, tatsächlich nicht infizierte Katzen zu erkennen. Die Spezifität für p27-Antigen bei progressiv infizierten Katzen lag bei australischen und deutschen Proben bei 99,4 % und 100 %. Die Spezifität für anti-p15E-Antikörper bei progressiv, regressiv und abortiv infizierten Katzen lag bei 90,2 % und 93,7 %, die für FIV-Antikörper bei 98,3 % und 100 %. Hinsichtlich der Spezifität konnte somit kein signifikanter Unterschied zwischen Proben aus Australien und Deutschland festgestellt werden. Allerdings gilt zu beachten, dass bei der Durchführung der Tests in den Ländern unterschiedliche Chargen des PoC-Tests verwendet wurden. Schlussfolgernd (**Publikation 3**) sind Modifikationen des PoC-Tests dringend notwendig, um die Sensitivität in Bezug auf die Bestimmung von anti-p15E- und FIV-Antikörpern zu verbessern.

Im Jahr 2021 wurde eine modifizierte Version (Version 2021) des PoC-Tests herausgegeben, die in einer weiteren unabhängigen Studie (**Publikation 4**) hinsichtlich ihrer Praktikabilität und Performance evaluiert wurde. Hierfür wurden 934 Serumproben aus Italien, Portugal, Deutschland und Frankreich herangezogen. Der PoC-Test wurde in Bezug auf p27-Antigen und anti-p15E-Antikörper zur Identifizierung der unterschiedlichen FeLV-Verlaufsformen (progressiv, regressiv, abortiv und fokal) evaluiert. Der PoC-Test war unter Praxisbedingungen einfach und aufgrund der Lagerung bei Raumtemperatur und nur wenigen notwendigen Schritten bei der Durchführung schnell durchführbar. Der PoC-Test wurde sowohl nach zehn Minuten (entsprechend der Herstellervorgabe) als auch nach 20 Minuten Inkubationszeit interpretiert. Für den Nachweis von p27-Antigen wurden keine Unterschiede zwischen den Inkubationszeiten von zehn und 20 Minuten festgestellt. Bei etwa der Hälfte (n = 38) der 81 positiv getesteten Proben waren die anti-p15E-Antikörper-Banden nach zehn Minuten jedoch nur schwach gefärbt, was zu Schwierigkeiten bei der Interpretation der Ergebnisse führte; dies kann mit fehlerhaften Diagnosen verbunden sein und in Folge zu falschen Behandlungsentscheidungen führen oder Nachtestungen erforderlich machen. Eine eindeutige Zuordnung der positiven anti-p15E-Antikörper-Ergebnisse war erst nach einer Inkubationszeit von 20 Minuten möglich.

Hinsichtlich der Bestimmung von p27-Antigen hatte der PoC-Test (unter Berücksichtigung der Serumproben aus allen Ländern) überraschenderweise eine niedrigere Sensitivität (82,8 %) im Vergleich zu seiner Erst-Version (91,3 %); 10/934 Proben wurden im p27-Antigen PoC-Test als falsch-negativ gewertet. Laut Hersteller wurden keine Änderungen der p27-Antigen-Testkomponenten vorgenommen; nichtsdestotrotz könnte die Modifikation des PoC-Tests zu einer nunmehr niedrigeren Sensitivität beigetragen haben. Dies ist ungünstig, weil eine niedrigere Sensitivität zu falsch-negativen Ergebnissen führen kann und somit progressiv infizierte Katzen nicht zuverlässig erkannt werden können. Die Spezifität für p27-Antigen war mit 96,0 % vergleichbar mit jener der Erst-Version des PoC-Tests (98,3 %).

Hinsichtlich der anti-p15E-Antikörper konnte die Sensitivität der modifizierten Version des PoC-Tests (Version 2021) im Vergleich zur Erst-Version des PoC-Tests (Version 2020) von 16,7 % auf 31,4 % gesteigert werden. Bei der modifizierten Version des PoC-Tests wurden insgesamt nach zehn Minuten Inkubationszeit 127/934 (13,6 %) Proben als falsch-negativ gewertet. Mit einer

Verlängerung der Inkubationszeit auf 20 Minuten wurden noch 111/934 (11,9 %) Proben als falsch-negativ gewertet, die Sensitivität konnte daher auf 40,0 % gesteigert werden. Die Spezifität der anti-p15E-Antikörper lag bei 96,9 %. Insgesamt wurden 23/934 Proben als falsch-positiv gewertet. Die Spezifität (96,9 %) blieb nach 20 Minuten Inkubationszeit unverändert. Mögliche Gründe für falsch-positive Ergebnisse im PoC-Test sind unter anderem Kreuzreaktionen. Des Weiteren können Fehler bei der Durchführung des Tests oder bei der Interpretation der Ergebnisse zu falsch-positiven Resultaten führen. Der positive prädiktive Wert (PPW) für den Nachweis von anti-p15E-Antikörpern lag in der vorliegenden Studie bei 64,3 % in Deutschland und 88,9 % in Frankreich. Der PPW gibt an, mit welcher Wahrscheinlichkeit ein positives Testergebnis tatsächlich positiv ist. So besteht in Populationen mit niedriger Prävalenz eine höhere Wahrscheinlichkeit, dass positive Testergebnisse falsch-positiv sind und Katzen fälschlicherweise als infiziert eingestuft werden. Im Gegensatz dazu sind in Populationen mit hoher Prävalenz positive Testergebnisse zuverlässiger, da die Wahrscheinlichkeit für falsch-positive Ergebnisse geringer ist. Hingegen gibt der negative prädiktive Wert (NPW) an, mit welcher Wahrscheinlichkeit ein negatives Testergebnis tatsächlich negativ ist. Dieser ist besonders in Ländern mit einer hohen Prävalenz (zum Beispiel Italien und Portugal) von hoher Wichtigkeit, da ein niedriger NPW die Wahrscheinlichkeit für falsch-negative Ergebnisse erhöht. Der NPW für den Nachweis von anti-p15E-Antikörpern lag in der vorliegenden Studie bei 86,6 % in Italien und 82,2 % in Portugal.

Ein Grund für die niedrige Sensitivität und zahlreichen falsch-negativen anti-p15E-Antikörper-Ergebnisse (127/185) könnte sein, dass der PoC-Test nicht empfindlich genug ist, um Katzen mit sehr niedrigen anti-p15E-Antikörpertitern zu erkennen. In der vorliegenden Studie (**Publikation 4**) wurde gezeigt, dass Katzen mit hohen Antikörpertitern (>50,0 %) im Gegensatz zu Katzen mit niedrigen Antikörpertitern (≤50,0 %) im PoC-Test zuverlässiger erkannt wurden. Sowohl in **Publikation 4** als auch in **Publikation 2** und **Publikation 3** wurde ein ELISA mit einem festgelegten Grenzwert von >16,3 % als Standardreferenz zur Bestimmung von anti-p15E-Antikörpern verwendet. In **Publikation 3** wurde gezeigt, dass auch Katzen, die keinen Kontakt zu FeLV hatten, anti-p15E-Antikörper-positiv sein können (29,6 %; 83/280). Ein möglicher Grund hierfür könnte das Vorhandensein von Antikörpern gegen Antigene sein, die die FeLV-p15E-Epitope nachahmen und somit eine Kreuzreaktivität verursachen. Zudem können positive Resultate auf

vorherige FeLV-Impfungen zurückzuführen sein.

Die klinische Relevanz des Vorhandenseins von anti-p15E-Antikörpern ist nicht genau geklärt. Katzen mit hohen anti-p15E-Antikörpertitern sind möglicherweise vor einer FeLV-Infektion geschützt. In einer Studie wurde gezeigt, dass Katzen, die mit dem FeLV-Transmembranprotein p15E immunisiert wurden, neutralisierende anti-p15E-Antikörper entwickelten (LANGHAMMER et al., 2006). Daraufhin versuchten die Autoren in einer experimentellen Studie, p15E in FeLV-Impfstoffe einzubeziehen. Bei 3/6 Katzen, die mit p15E immunisiert und anschließend experimentell mit FeLV infiziert wurden, konnte am Tag 960 ein Schutz vor einer FeLV-Antigenämie beobachtet werden. Die restlichen drei mit p15E immunisierten Katzen waren ungeschützt und entwickelten nach der experimentellen Infektion mit FeLV eine progressive FeLV-Infektion (LANGHAMMER et al., 2011). Durch eine Impfung mit p15E konnte daher kein verlässlicher Schutz vor einer progressiven Infektion hervorgerufen werden. Leider kann also auch keine Aussage darüber getroffen werden, ob anti-p15E-Antikörper-positive Katzen von weiteren Impfungen gegen FeLV profitieren oder nicht.

Derzeit ist der v-RetroFel® PoC-Test der einzige kommerziell erhältliche und unter Praxisbedingungen durchführbare Test zum Nachweis von anti-FeLV-Antikörpern. Aufgrund der Ergebnisse sollte der Test erst nach 20 Minuten Inkubationszeit interpretiert werden. Ein negatives Ergebnis schließt jedoch eine vorangegangene Exposition mit FeLV nicht aus, während ein positives Ergebnis sehr wahrscheinlich auf eine Auseinandersetzung mit dem Virus hindeutet. Der PoC-Test kann helfen, Katzen mit einem hohen anti-p15E-Antikörpertiter zu identifizieren. Nichtsdestotrotz ist es ratsam, das Ergebnis des PoC-Tests kritisch zu bewerten und die Ergebnisse durch weitere labordiagnostische Tests zu bestätigen. Außerdem ist eine weitere Verbesserung der Sensitivität zum Nachweis regressiver und abortiver Infektionsverläufe unbedingt notwendig.

VII. ZUSAMMENFASSUNG

Die vorliegende Arbeit enthält eine Übersichtspublikation und drei Originalarbeiten. Teil 1 der Arbeit ist ein Übersichtsartikel (**Publikation 1**), der einen umfassenden Überblick über die verschiedenen Verlaufsformen einer Infektion mit dem FeLV und die diagnostischen Methoden zur Identifizierung infizierter Katzen gibt.

Teil 2 der Arbeit hatte zum Ziel, die FeLV-Prävalenz unter Berücksichtigung aller Verlaufsformen (progressiv, regressiv, abortiv, fokal (atypisch)), in Ländern mit einer hohen (Italien, Portugal) und niedrigen (Deutschland, Frankreich) Prävalenz zu bestimmen (**Publikation 2**). Blutproben von 934 Katzen (Italien 269, Portugal 240, Frankreich 107, Deutschland 318) wurden auf p27-Antigen sowie anti-FeLV-Ganzvirus-, anti-Surface Unit-(SU-) und anti-p15E-Antikörper mittels Enzyme-linked-Immunosorbent-Assay (ELISA) untersucht und auf Provirus-DNA mittels Polymerase-Kettenreaktion (PCR) getestet. Positive p27-Antigen-ELISA-Resultate wurden mittels reverse Transkriptase-PCR (RT-PCR) auf virale RNA aus Speicheltupfern und/oder Blut verifiziert. Die FeLV-Gesamtprävalenz betrug 21,2 % in Italien, 20,4 % in Portugal, 9,5 % in Deutschland und 9,3 % in Frankreich. Die Prävalenz einer progressiven, regressiven, abortiven und fokalen Infektion lag in Italien bei 7,8 %, 4,5 %, 6,3 % und 2,6 %, in Portugal bei 3,8 %, 8,3 %, 6,7 % und 1,7 %, in Deutschland bei 1,9 %, 1,3 %, 3,5 % und 2,8 %, in Frankreich bei 1,9 %, 3,7 %, 2,8 % und 0,9 %. Wurden alle Verlaufsformen der FeLV-Infektion betrachtet, lag die FeLV-Gesamtprävalenz vor allem in südeuropäischen Ländern (nach wie vor) sehr hoch. Insbesondere in diesen Ländern sollten Katzen daher weiterhin getestet und separiert sowie gegen FeLV geimpft werden.

Zum Nachweis verschiedener Verlaufsformen einer FeLV-Infektion und einer Infektion mit dem felinen Immunschwächevirus (FIV) steht seit kurzer Zeit ein Point-of-Care-(PoC-)Test (v-RetroFel®) zur Verfügung. Teil 3 und 4 der Arbeit hatten das Ziel, die Durchführbarkeit und Qualität des PoC-Tests vor (**Publikation 3**) und nach Modifikationen (**Publikation 4**) zu beurteilen. Zur Evaluation der Erst-Version des PoC-Tests wurden Blutproben aus Australien (n = 200) und Deutschland (n = 170) analysiert, um die Performance-Parameter

(Sensitivität, Spezifität) zu überprüfen (**Publikation 3**). Die Sensitivität für p27-Antigen für Proben aus Australien und Deutschland betrug 91,3 % und 100 %, während die Spezifität bei 99,4 % und 100 % lag. Die Sensitivität für anti-p15E-Antikörper betrug 16,7 % für australische Proben und 8,3 % für deutsche Proben, bei einer Spezifität von 90,2 % und 93,7 %. Die Sensitivität des PoC-Tests für die Bestimmung von Antikörpern gegen FIV war bei australischen Katzen (94,7 %) signifikant höher als bei deutschen Katzen (30,0 %). Im Gegensatz dazu konnte bezüglich der Spezifität für FIV-Antikörper kein signifikanter Unterschied zwischen Proben aus Australien und Deutschland festgestellt werden (98,3 % und 100 %).

Aufgrund der niedrigen Sensitivität bezüglich des Nachweises von anti-p15E-Antikörpern initiierte der PoC-Test-Hersteller Schritte zur Verbesserung der Sensitivität. In Teil 4 der Arbeit wurde die modifizierte Version des PoC-Tests hinsichtlich p27-Antigen und anti-p15E-Antikörper reevaluiert (**Publikation 4**). Hierfür wurden Serumproben aus Italien (n = 269), Portugal (n = 240), Deutschland (n = 318) und Frankreich (n = 107) herangezogen. Die Ergebnisse wurden für die verschiedenen FeLV-Verlaufsformen (progressiv, regressiv, abortiv und fokal) interpretiert. Dabei wurden die Praktikabilität und Performance-Parameter (Sensitivität, Spezifität, negativer und positiver prädiktiver Wert) des PoC-Tests bestimmt. Der PoC-Test wurde sowohl nach zehn Minuten (entsprechend der Herstellervorgabe) als auch nach 20 Minuten Inkubationszeit interpretiert. Bei der Detektion des p27-Antigens zeigte die modifizierte PoC-Test-Version insgesamt überraschenderweise eine niedrigere Sensitivität (82,8 %) im Vergleich zur Erst-Version des PoC-Tests (91,3 %). Die Spezifität war mit 96,0 % vergleichbar mit der der ursprünglichen Version des PoC-Tests (98,3 %). In Bezug auf die anti-p15E-Antikörper konnte die Sensitivität auf 31,4 % gesteigert werden, während die Spezifität bei 96,9 % lag. Durch eine Verlängerung der Inkubationszeit auf 20 Minuten konnte die Sensitivität weiterhin verbessert werden, jedoch lediglich auf 40,0 %, wobei die Spezifität unverändert bei 96,9 % blieb. Es konnte gezeigt werden, dass Katzen mit hohen Antikörpertitern zuverlässiger im PoC-Test erkannt wurden. Ein positives Ergebnis im PoC-Test lässt eine Exposition mit dem FeLV vermuten.

Aufgrund der niedrigen Sensitivität bezüglich des Nachweises von anti-p15E-Antikörpern ist der PoC-Test nicht geeignet, den FeLV-Status der Katzen unter Berücksichtigung aller Verlaufsformen (progressiv, regressiv, abortiv und fokal

(atypisch)) verlässlich zu bestimmen. Grundsätzlich sollte die Bedeutung von anti-p15E-Antikörpern für die Erkennung von (insbesondere) regressiv und abortiv infizierten Katzen in Frage gestellt werden, da nicht jede FeLV-infizierte Katze anti-p15E-Antikörper bildet.

VIII. SUMMARY

In this doctoral thesis, 4 articles are included, one review article and three original publications. The review article (**publication 1**) is aimed to give a detailed overview of the different courses of feline leukaemia virus (FeLV) infection and the diagnostic methods to identify infected cats.

In the second study the FeLV prevalence was determined considering all courses of infection (progressive, regressive, abortive, focal (atypical), in countries with a high (Italy, Portugal) and low (Germany, France) FeLV prevalence (**publication 2**). Blood samples from 934 cats (Italy 269, Portugal 240, France 107, Germany 318) were analysed for p27 antigen, anti-FeLV whole virus, anti-surface unit (SU) and anti-p15E antibodies by enzyme-linked immunosorbent assay (ELISA) and for provirus DNA by polymerase chain reaction (PCR). Positive p27 antigen ELISA results were verified by reverse transcriptase-PCR (RT-PCR) for viral RNA from saliva swabs and/or blood. The overall FeLV prevalence was 21.2% in Italy, 20.4% in Portugal, 9.5% in Germany and 9.3% in France. The prevalence of progressive, regressive, abortive, and focal infection was 7.8%, 4.5%, 6.3%, and 2.6% in Italy, 3.8%, 8.3%, 6.7%, and 1.7% in Portugal, 1.9%, 1.3%, 3.5%, and 2.8% in Germany, and 1.9%, 3.7%, 2.8%, and 0.9% in France. When all courses of FeLV infection were considered, the overall FeLV prevalence especially in southern European countries was (still) very high. Especially in these countries, cats should therefore continuously be tested and separated as well as vaccinated against FeLV.

A point-of-care (PoC) test (v-RetroFel®) is available to detect different courses of FeLV infection and feline immunodeficiency virus (FIV) infection. Aim of the third and fourth study was to assess the feasibility and quality of the PoC test before (**publication 3**) and after modifications (**publication 4**). To evaluate the first version of the PoC test, serum samples from Australia (n = 200) and Germany (n = 170) were analysed to check the performance parameters (sensitivity, specificity) (**publication 3**). The sensitivity for p27 antigen serum samples from Australia and Germany was 91.3% and 100.0%, respectively, while the specificity was 99.4% and 100.0%. The sensitivity for anti-p15E antibodies was 16.7% for Australian serum samples and 8.3% for German serum samples, with a specificity of 90.2% and 93.7%, respectively. The sensitivity of the PoC test for the

determination of antibodies against FIV was significantly higher in Australian cats (94.7%) than in German cats (30.0%). In contrast, no significant difference was noted between serum samples from Australia and Germany regarding the specificity for FIV antibodies (98.3% and 100.0%).

As sensitivity regarding the detection of anti-p15E antibodies was very low, the PoC test manufacturer initiated steps to improve sensitivity. Subsequently, the modified version of the PoC test was evaluated with regard to p27 antigen and anti-p15E antibodies (**publication 4**). For this purpose, serum samples from Italy (n = 269), Portugal (n = 240), Germany (n = 318), and France (n = 107) were included. The results were interpreted for the different FeLV courses (progressive, regressive, abortive and focal). The practicality and performance parameters (sensitivity, specificity, negative and positive predictive value) of the PoC test were determined. The PoC test was interpreted both after ten minutes (manufacturer's specification) and after 20 minutes of incubation time. For the detection of p27 antigen, the modified PoC test version surprisingly showed a lower overall sensitivity (82.8%) compared to the initial version of the PoC test (91.3%). However, the specificity (96.0%) was comparable to that of the original version of the PoC test (98.3%). Concerning anti-p15E antibodies, the sensitivity could be increased to 31.4%, whereas the specificity was 96.9%. By extending the incubation time to 20 minutes, the sensitivity could be improved to 40.0%, while the specificity remained unchanged (96.9%). It was also shown that cats with high antibody titres were more reliably detected in the PoC test and that a positive result in the PoC test suggested exposure to FeLV.

Due to the low sensitivity regarding the detection of anti-p15E antibodies, the PoC test is not suitable to determine the FeLV status of cats considering all courses of infection (progressive, regressive, abortive and focal (atypical)). In general, the relevance of anti-p15E antibodies for the detection of (especially) regressively and abortively infected cats should be questioned, since not every FeLV-infected cat seems to produce anti-p15E antibodies.

IX. LITERATURVERZEICHNIS

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