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DIAGNOSTIC PERFORMANCE OF A RAPID IN-CLINIC TEST FOR THE DETECTION OF FELINE PARVOVIRUS

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ABSTRACT

Feline parvovirus (FPV) is one of the most common causes of acute hemorrhagic enteritis, with high mortalities in kittens. As clinical diagnosis is often indecisive, the aim of our study was to evaluate the diagnostic accuracy of in-clinic rapid tests for the detection of FPV infection in cats. To this end, we contrasted the most reliable diagnostic technique—PCR—with the rapid diagnostic kit for FPV antigen. A total of 100 stool samples were collected from cats suspected of being infected with FPV. A vaccination history was reported for all sampled cats. Every sample underwent both PCR and the rapid diagnostic test, with the results being compared. Anorexia, bloody diarrhea, severe dehydration, hypothermia, and vomiting were the most common clinical findings significantly associated with parvovirus-infected cats. 35 out of 100 clinically ill cats were FPV positive (35%) using the rapid screening test, while 43 (43%) of the tested samples were PCR positive. Overall, the two tests found FPV infection in 32 cases and ruled it out in 54. The PCR technique confirmed the infection in 11 cases that their rapid testing were negative. Rapid antigen-based screening assays demonstrated sensitivity and specificity of 74.42% and 94.74%, respectively. In conclusion, a positive result certainly indicates FPV infection, while a negative result does not rule out parvoenteritis from the differential diagnosis, especially in cats exhibiting clinical symptoms. It is possible to anticipate increased sensitivity if the test is run right following sample collection.

Keywords: Feline parvovirus, sensitivity, specificity, rapid test, PCR

INTRODUCTION

Feline parvovirus infection is a common disease that causes diarrhea in cats and can be life-threatening in severe cases, resulting in great financial and emotional losses to cat breeders (Raj and Haryanto, 2020; Abdel-Baky *et al.*, 2022). Domestic cats harbor several viruses that infect the feline population and other carnivores worldwide (Driciru *et al.*, 2006 and Nishimura *et al.*, 1999). Feline parvovirus was first identified at the beginning of the 20th century and subsequently spread around the world (Barrs, 2019), is regarded as one of the most significant viruses that affect cats (Hellard *et al.*, 2011). Feline parvovirus (FPV) and canine parvovirus-2 (CPV-2) are closely related viruses that cause severe viral disease in kittens. They are the causes of this highly fatal infectious disease that affects both domestic and wild cats (Nakamura *et al.*, 2001). The Parvoviraidae family of viruses includes the two viruses currently known as

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carnivore protoparvovirus-1, a non-enveloped, single-stranded DNA virus (Cotmore *et al.,* 2014).

Anorexia, leukopenia, vomiting, diarrhea, dehydration, fever, and, in the majority of cases, death are the symptoms of the disease (Stuetzer and Hartmann, 2014). Kittens who have not received vaccinations exhibit higher susceptibility to the virus, in contrast to older cats who have been vaccinated and have had prior subclinical infection. Cats of all ages, genders, and breeds are susceptible (Kruse *et al.,* 2010).

Given the highest risk of parvovirus infection, rapid viral diagnosis is essential as a preventive measure (Esfandiari and Klingeborn, 2000). Recently, many different simple, rapid, and sensitive diagnostic tests, also called in-house tests, are commercially available based on either enzyme-linked immunosorbent assays (ELISA) or immunochromatographic technology, which are suitable to screen cats for parvovirus antigen, either FPV or CPV-2, due to the close structural and antigenic relationship between them (Neuerer *et al.,* 2008). Polymerase chain reaction (PCR) is considered the gold standard technique used for diagnosis of FPV infection through genetic detection of the virus, even when viral quantities are low. It is valuable when negative test results are obtained with the rapid commercially available in-clinic kits, but there are obvious clinical signs consistent with the disease (Sykes, 2014).

Although PCR techniques are used to confirm if CPV-2 is present in clinical samples, their usage requires specialized laboratories and individuals with sufficient experience because of the potential for carryover contamination, especially when high sample throughput is involved (Desario *et al.,* 2005 and Decaro and Buonavoglia, 2012). The virus can be identified over a longer period by using a high-sensitivity PCR technology. It is possible to use molecular techniques for subclinical detection on paraffin-embedded, formalin-fixed tissues. Carnivore parvoviruses can infect multiple species, hence identifying a specific viral strain is possible by genetic detection (Greene, 2012).

The above-mentioned facts provided the motivation for this study, which compared the results of the rapid test and PCR and evaluated the rapid screening test's diagnostic performance for cat parvovirus infection detection by estimating its sensitivity, specificity, positive and negative predictive values, and agreement rate, with PCR serving as the reference test.

MATERIALS AND METHODS

Ethical statement:
Every process was carried out in accordance with the ethical standards established by Assiut University's institutional ethics committee. Every cat owner was informed about the study's goals, methods, voluntary participation, and the privacy of personal data. All cats were handled and cared for in accordance with the guidelines set forth by Assiut University on animal research, and samples were taken with permission from the owners. The study had been approved by the institutional ethics committee at Assiut University.

Animals:
A total of 100 cats of different ages, sexes, and breeds were employed in this study. Only cats with signs suggestive of parvovirus infection (lethargy, anorexia, vomiting, and/or bloody diarrhea) were included in the study.

Clinical examination and sampling:
All animals were examined, and stool samples were collected from suspected clinical cases directly from the rectum by using fecal swabs (Islam *et al.,* 2010). Each sample was divided into two parts. One part was used immediately after collection, for screening suspected cats for the presence of FPV and CPV-2 antigens by using the rapid Ag test kit (Parvo SNAP Test®) or the VDRG®CPV Ag Rapid Kit. The other part of the sample was stored at -20 °C until used for molecular detection of FPV.

Screening of suspected cats for the presence of FPV and CPV-2 antigen using rapid test:

Two rapid screening tests were utilized. The first is a fast enzyme immunoassay for detecting canine and feline parvovirus in feces called the IDEXX Laboratories SNAP Canine Parvovirus Antigen Test Kit (SNAP Parvo, USA). This test detects virus surface protein antigens (including intact virus particles) excreted in infected animals' feces. The second is a lateral flow chromatographic immunoassay called the VDRG®CPV Ag Rapid Kit Median, Korea, which is capable of detecting parvovirus in cat feces. Stool samples were taken from each diseased cat, and according to the manufacturer's instructions, the samples were examined using either the VDRG®CPV Ag Rapid Kit or the SNAP Parvo test.

Molecular detection of FPV and CPV-2 DNA:

DNA was extracted from all stool specimens using a commercial kit (QIAamp®Fast DNA Stool Mini Kit, Qiagen, Germany), according to the manufacturer's instructions. Parvovirus screening was carried out by PCR using primers (Sigma-Aldrich) designed by Stephen Dunham to allow both FPV and CPV detection (felVP2-3820F: 5-TTGARGCRTCTACACAAGGG-3′ and VP2-4247R: 5-TGGTGCATTTACATGAAGTCTTGG-3′). The PCR reactions were carried out in a total 25 µl volume, and the thermocycler was programmed by the following PCR cycling conditions: initial denaturation at 95 ºC for 1 minute; 40 cycles of denaturation of 15 seconds at 95 ºC, followed by primer annealing at 60 ºC for 15 seconds; extension at 72 ºC for 15 seconds; and a final extension of 2 minutes at 72 ºC. The product size of the reaction was 428 base pairs (bp).

Data Analysis:

The PCR test, which served as a reference test, was compared to the results of the rapid in-clinic tests. Test performance was compared using the following metrics: overall accuracy (OA) or agreement percentage (probability that a cat will be correctly classified by the tests; sum of true positives plus true negatives divided by the total number of tested cats), specificity (true negative rate), sensitivity (true positive 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 (Motha *et al.*, 1987 and Walter-Weingärtner *et al.*, 2021). Cohen's Kappa statistic was performed to assess the agreement of the results among the tests. Values < 0 indicated poor agreement, 0.00–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect agreement (Landis and Koch, 1977).

Statistical analysis:

Statistical analysis was carried out using GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA) to analyze the correlation between the results of both tests. The odds ratio and 95% confidence intervals were calculated. A probability value (P-value) of $P < 0.05$ was considered statistically significant.

RESULTS

Anorexia, lethargy, abdominal pain, heat, persistent vomiting, mucoid diarrhea, bloody stools, and dehydration were among the many clinical signs that were recorded. The signs and symptoms observed in cats of different breeds, ages, and sexes. Using rapid antigen tests and conventional PCR, the overall prevalence of parvovirus infection in cats was 35% (35/100) and 43% (43/100), respectively. The size of the PCR product amplified from the FPV VP-2 gene was 428 base pairs, which was similar to that of the positive control.

Both tests detected FPV infection in 32 cases and ruled it out in 54 cases, although the PCR approach confirmed the infection in 11 cases that had been tested negative by the rapid tests, whereas there were three cases that showed negative PCR results despite being positive by the rapid test (Table 1).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and agreement rate (overall accuracy) of the two antigen-based rapid tests used were 74.42%, 94.74%, 91.4%, 83% and 86%, respectively, when compared to the PCR used as a reference test. There was at least substantial agreement between the rapid test and the PCR (kappa = 0.7077) (Table 2).

Table 1: Comparison between rapid tests and PCR results.

	Rapid test		Total	Odds ratio	95% CI	P-value
PCR	Positive	Negative				
Positive	32	11	43	52.3623	13.5826-201.8611	0.0000
Negative	3	54	57			
Total	35	65	100			

Table 2: Relative performance of the rapid tests for detection of parvovirus infection in cats, as PCR was used as a reference test

Sensitivity %	Specificity %	PPV%	NPV%	Agreement	
				%	Kappa coefficient
74.42	94.74	91.4	83	86	0.7077

DISCUSSION

FPV infection causes an acute and severe disease with a high mortality rate. Therefore, a quick and accurate diagnosis is extremely essential. The reference standard PCR has to be performed in specialized laboratories, and results are generally available no earlier than after a few days. Thus, rapid in-house testing is an important measure to diagnose infected cats immediately and directly at veterinary clinics. Therefore, this study aimed to evaluate the relative performance of the rapid test regarding sensitivity, specificity, NPV, PPV, and overall accuracy when compared to PCR.

In this study, PCR was used as the gold standard test, to which other rapid screening tests were compared and evaluated. When the results of the rapid test and the PCR were compared, 32 samples were positive and 54 cases were negative. However, 11 samples that tested positive by the PCR were missed by the rapid test, and three cases had positive rapid test outcomes but negative PCR results. These findings might be the result of the PCR's higher sensitivity, in contrast to this rapid screening test (Abd-Eldaim *et al.,* 2009 and Awad *et al.,* 2018a&b). The rapid screening test's sensitivity and specificity were reduced, when compared to the confirmatory PCR assay, yielding 11 false negative and 3 false positive results. On the opposite hand, it was found that the rapid screening test was a simple, rapid, and cost-effective means of detecting FPV infection.

The findings of the screening rapid test and the PCR were analyzed statistically, and there was no significant difference between them (P= 0.3102). As a result, in routine veterinary practice, the quick test could be suggested as a rapid in-clinic test for identifying FPV infections. Unlike other time-consuming laboratory procedures, such as hemagglutination (HA), ELISA, and

electron microscopy, rapid screening immunochromatographic diagnostic tests are quick, sensitive, easy, and reproducible, and may be used easily and immediately by veterinarians for diagnosing parvoviral infections in practice (Esfandiari and Klingeborn, 2000 and Vakili *et al.,* 2014).

Although immunochromatographic (IC) assay is a quick and simple technique, they are less sensitive than other laboratory methods. So, to correctly diagnose parvovirus enteritis, more advanced lab tests with high specificity and sensitivity should be used. These include HA, viral isolation, and PCR. Only specialized laboratories and qualified staff may perform these procedures (Koulath *et al.,* 2017). In comparison to other confirmatory tests, PCR is the most reliable and accurate method for detecting parvovirus in feces samples because it is more sensitive, specific, simple, and efficient. PCR is the gold-standard method for diagnosing FPV infection by detecting the virus genetically, even when viral levels are low. It is useful when rapid, commercially accessible in-clinic kits produce negative results, but there are evident clinical findings consistent with the disease. As a result, it can be used to determine and confirm the presence of FPV (Schunck *et al.,* 1995, Abd-Eldaim *et al.,* 2009 and Mende *et al.,* 2014).

The rapid tests failed to detect 11 positive cases, when compared to PCR, indicating a lower sensitivity than PCR. Abd-Eldaim *et al.* (2009) found that out of 97 cases, 57 were positive for PCR, whereas 54 were false negatives for the rapid test. Awad *et al.* (2018a) also identified FPV in 66 positive cases by rapid test and 75 positive cases by PCR, with 9 false negative cases. The low sensitivity of the test in this study may be due to variations in the consistency of the feces seen throughout the study as well as the timing of the test, whether in the early or late stages of the disease, which affects the quantity and shedding of the virus. 5 of the 11 false negative cases were non-diarrheic, and 6 were diarrheic, either bloody or mucoid diarrhea. Diarrhea would be predicted to increase virus shedding from injured intestinal epithelial cells (Steinel *et al.,* 2001). As a result, the rapid test would only detect non-diarrheic patients in a minority of cases. Furthermore, Walter-Weingärtner *et al.* (2021) reported that some feces samples, even those with high viral loads, came back negative with rapid tests. They said this was because the consistency of these samples' feces was very bloody and had a lot of mucous membrane particles, which could make it hard to detect. According to Mende *et al.* (2014), these rapid assays can only detect FPV in feces for up to 48 hours after infection, by which time the virus may no longer be detectable. Furthermore, the results of these rapid assays differ from one to another, as well as depending on the stage of infection, as virus shedding can be temporary (Abd-Eldaim *et al.,* 2009). The timing of the rapid test is critical to avoid false negative results, as experimental investigations have shown that CPV shedding begins 3–4 days after inoculation, with the highest virus shedding occurring 4–7 days following inoculation (Macartney *et al.,* 1984).

The low sensitivity of rapid screening tests was explained by Esfandiari and Klingeborn (2000) by the period of fecal viral shedding, which corresponds to the first few days of clinical disease. In the incubation period of four to six days and the late stages of infection (more than 10 days), minimal numbers of virus particles are released, which are hardly detected even by ELISA. One limitation of this test is that the quantity of virus particles can impact the IC test result. An in-house assay was shown to be capable of detecting samples with a viral load of more than 109 DNA copies per mg of feces (Decaro *et al.,* 2009). In addition, whereas rapid tests detected

virions, PCR detected DNA particles, suggesting that infected cells may contain more DNA particles than virions (Decaro *et al.,* 2013). High titers of interfering antibodies, which sequester viral antigens, could also cause false negative results. As a result, rapid screening assays may miss the antigen (Proksch *et al.,* 2015).

The three cases in which the rapid tests yielded positive results even though the PCR was negative are referred to as false-positive cases. One of the three cases had been vaccinated four days earlier. Abd-Eldaim *et al.* (2009) reported a similar result, as one case exhibited a positive result with the rapid test despite the fact that the PCR was negative. False positive results occur occasionally and do not always indicate infection, especially in kittens that have recently been immunized against FPV with modified live vaccines for at least 14 days after vaccination (Patterson *et al.*, 2007).

Screening rapid antigen-based assays had a sensitivity and specificity of 74.42% and 94.74%, respectively, in the current investigation. These findings are consistent with Tinky *et al.* (2015), who indicated that the IC test's sensitivity was 72.22% and its specificity was 92.86%, when compared to PCR. When compared to real-time PCR, the in-clinic test had a relative sensitivity and specificity of 65.3% and 100%, respectively (Decaro *et al.,* 2013). The IC assay was found to be very specific (98.8%) and sensitive (100%) by Esfandiari and Klingeborn (2000). However, the results of this paper should be taken with caution because three different ELISAs were used as the "gold standard," and no electron microscopy, PCR, or virus isolation was done. According to Neuerer *et al.* (2008), the SNAP Parvo test has a 60% sensitivity and a 100% specificity, when compared to electron microscopy. In comparison to qPCR, Decaro *et al.* (2010) found that the IDEXX Snap® Parvo has a sensitivity of 80.4%, 78.0%, and 77.0% for detecting CPV 2a, 2b, and 2c. Moreover, Awad *et al.* (2018a) used a rapid test based on ELISA technology called Snap® Parvo, which has a sensitivity and specificity of 88% and 100%, respectively.

Earlier studies have shown that rapid screening tests can be sensitive in different ways. This is mostly because of the brand of rapid tests used and the amount of virus in each sample, since these tests need a lot of viral antigens to make a visible band. Furthermore, when samples are examined shortly after collection, the sensitivity of the in-clinic immunochromatographic assay is improved. In addition, the interpretation of the result is vulnerable to the operator's subjectivity, especially when the virus quantity is low (Kantere *et al.,* 2015). Walter-Weingärtner *et al.* (2021) studied eight different rapid tests and discovered significant differences in sensitivity between them. They stated that variances in the virus load of the fecal samples could cause the variety in sensitivities, as the smaller the number of virus copies/g feces, the lower the detection likelihood of the point-of-care test. The rapid screening test's specificity was low in three cases, one of which had just been vaccinated. In several studies, the specificity was outstanding, with no false-positive test results reaching 100% (Esfandiari and Klingeborn, 2000; Neuerer *et al.,* 2008; Kantere *et al.* 2015; Awad *et al.,* 2018a and WalterWeingärtner *et al.,* 2021). The difference between the observed specificity rate (94.74%) and that of the previously mentioned authors could be explained by the fact that these investigations only included animals that had not been vaccinated against parvovirus infection in the previous four weeks. In addition, the brand of CPV-2 fecal antigen testing among vaccinated kittens has a significant impact on their specificity (Meason-Smith *et al.,* 2017).

In terms of test quality standards, the PPV and NPV are the most important for a screening test since they predict whether a cat is infected or not with parvovirus, and hence whether it will or will not potentially shed the organism and infect other cats (Neuerer *et al.*, 2008). The rapid antigen-based test utilized in this investigation has a PPV and NPV of 91.4% and 83%, respectively. Tinky *et al.* (2015) observed nearly identical results when they calculated the positive and negative predictive values of the IC strip test, which were 88.95% and 81.25%, respectively. Compared to PCR, the antigen-based rapid screening test utilized in this investigation had an overall accuracy of 86% and a high agreement rate (kappa = 0.7077). This finding was close to that of Walter-Weingärtner *et al.* (2021), who calculated the agreement of eight rapid screening tests and found it practically flawless (Kappa > 0.80), while another study indicated that the one-step test and EM agreed at 85.5% (Esfandiari and Klingeborn, 2000). Awad *et al.* (2018a) observed approximately identical results when comparing the rapid test to the PCR, estimating the accuracy of the rapid test at 94.5%. The in-clinic assay and the PCR reference method, on the other hand, were shown to have a fair agreement (K= 0.203) (Kantere *et al.*, 2015). Desario *et al.* (2005) calculated a 60.04% agreement for the IC test using PCR as a reference method.

CONCLUSION

The screening rapid test could be recommended as a rapid in-clinic test for diagnosing FPV infections in routine veterinary practice as it correlates well with the results of PCR. Rapid screening tests yield false negative results more often than false positives, so a positive test result in a cat with clinical symptoms suggests feline panleukopenia. When suspected cases of feline panleukopenia emerge, the use of a PCR assay as an accurate diagnostic technique is crucial for determining the proper diagnosis and implementing the most effective control strategies.

CONFLICT OF INTEREST:

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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الأداء التشخيصي لاختبار سريع داخل العيادة للكشف عن فيروس بارفو القطط

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فيروس بارفو القطط (FPV) هو أحد الأسباب الأكثر شيوعًا لالتهاب الأمعاء النزفي الحاد، مع ارتفاع الوفيات في القطط. نظرًا لأن التشخيص السريري غالبًا ما يكون غير حاسم، كان الهدف من دراستنا هو تقييم الدقة التشخيصية لاختبار سريع يجري داخل العيادة لاكتشاف عدوى البارفو في القطط. تحقيقًا لهذه الغاية، تمت مقارنة تقنية التشخيص الأكثر موثوقية (تفاعل البلمرة المتسلسل) مع مجموعة التشخيص السريع لمستضد البارفوفيروس. تم جمع ١٠٠ عينة براز من القطط المشتبه في إصابتها بفيروس البارفو. تم معرفة تاريخ التطعيم لجميع القطط التي تم أخذ عينات منها. خضعت كل عينة للاختبار بكل من تفاعل البلمرة المتسلسل والاختبار التشخيصي السريع، مع مقارنة كلتا النتائج. كان فقدان الشهية والإسهال الدموي والجفاف الشديد ونقص الحرارة والقيء أكثر النتائج السريرية شيوعًا المرتبطة بشكل كبير بالقطط المصابة بفيروس البارفو. تم العثور على ٣٥ من أصل ١٠٠ قطة مريضة سريريًا إيجابية %(35) باستخدام اختبار الفحص السريع، في حين أن ٤٣ (٤٣٪) من العينات التي تم اختبارها كانت إيجابية بالاختبار التاكيدي الاخر. بشكل عام، الاختبار السريع أكد عدوى لبارفو في ٣٢ حالة واستبعداها في ٥٤. أكدت تقنية تفاعل البلمرة المتسلسل العدوى في ١١ حالة والتي أظهرها الاختبار السريع أنها سلبية. أظهرت فحوصات الفحص السريعة القائمة على المستضدات حساسية وخصوصية بنسبة ٧٤,٤٢٪ و ٩٤,٧٤٪ على التوالي. في الختام، النتيجة الايجابية بالاختبار السريع تشير بنسبة كبيرة الي وجود عدوى البارفو، في حين أن النتيجة السلبية لا تستبعد وجودها من التشخيص التفاضلي، خاصة في القطط التي تظهر عليها أعراض سريرية. من الممكن توقع زيادة الحساسية إذا تم إجراء الاختبار على الفور بعد جمع العينات.