Comparison between PCR, IEM and antibody detection tests regarding canine parvoviral (CPV) enteritis

Abstract:

The subject of this study is the discussion on the optimal use of parvovirus antigen detection assays for use in the general veterinary clinic. The canine parvovirus antigen is generally discharged for 3–12 days. During this time, antigen detection is highly reliable and recommended in practice.
This study is separated into two parts: Part 1 discusses the differences between aspects of two lab assays (PCR and IEM) and antigen detection assays (LFA and ELISA) based on defined fecal samples from dogs (group 1 with acute hemorrhagic diarrhea and group 2 and 3 as control groups). In this comparison, the PCR was too sensitive because it showed 6 positive results from the as negative defined control groups. The IEM can be considered as more sensitive than the antigen detection assays. Due to the uncomplicated, quick test application and cost efficiency of the LFA, this method provides an important diagnostic tool for the veterinary practice, despite of the lower sensitivity in comparison to lab-analyzer based methods. Therefore, the antigen detection tests are reliable on site methods, if the pathophysiology of the antigen shedding is taken into account for the diagnosis.
The second part of the study determines the test performance of the scil v-Parvovirus antigen rapid test in comparison to an ELISA assay (also antigen detection test) and a PCR. A high conformity of results could be observed between the ELISA and the scil v-Parvovirus assay (93.33% accordance in sensitivity and 99.99% in specificity), the results are quite similar. The PCR had a higher sensitivity compared to both antigen detection assays (scil v-Parvovirus and ELISA) as already observed in part one.
In conclusion, the antigen detection tests, incl. the scil v-Parvovirus assays, are useful to diagnose canine parvoviral enteritis on site. Compared to the DNA detection (PCR) a negative test result with antigen detection test does not rule out a Parvovirosis as a differential diagnosis in a dog with hemorrhagic diarrhea. In such cases additional tests based on DNA detection or IEM should be performed. Overall the scil v-Parvovirus is useful tool for an acute, rapid on-site diagnosis for parvovirus.

Canine Parvovirus (CPV)

Canine parvoviral (CPV) enteritis is an acute life-threatening infection. Since the emergence of CPV in the 1970s, continuing genetic variation has resulted in the appearance of novel strains of canine parvovirus 2, with one major variant designated type CPV-2a having replaced the original canine parvovirus 2 strain after which it has been the principal virus found in dogs. Interestingly, the variant CPV-2a and viruses descended from it are more infectious to cats than the original strains of canine parvovirus 2. The epidemiological features of canine parvovirus 2 infections are similar to those of feline panleukopenia. Canine parvovirus (CPV) and feline panleukopenia virus (FPLV) are two closely related viruses, which are known to cause severe disease in younger unvaccinated animals. As well as causing disease in their respective hosts, CPV has recently acquired the feline host range, allowing it to infect both cats and dogs. The virus is highly contagious and very stable in the environment, so most infections result from the exposure of susceptible dogs to virus contaminated feces. Severe disease is most common in rapidly growing pups between 6 weeks and 6 months of age; however, many dogs that are naturally infected with canine parvovirus 2 exhibit only mild or subclinical disease. Canine parvovirus 2 is the cause of an enteritis syndrome analogous to feline panleukopenia, although leukopenia is often less severe in dogs. Intestinal hemorrhage with severe bloody diarrhea is more characteristic of canine parvovirus disease than of feline panleukopenia. The incidence of the enteritis syndrome has fallen since the virus first emerged, due to widespread vaccination, but canine parvovirus 2 is still an important cause of infectious diarrhea in young dogs.

The sudden onset of foul-smelling, bloody diarrhea in young dogs is suggestive, but certainly not diagnostic of canine parvovirus infection. A rapid detection of the virus is possible directly in the clinic by evaluating a fecal sample with an antigen detection assay (LFA as fecal antigen assay). With an enzyme immunoassay (ELISA) the antigen detection can also be done in a lab within one/ two days. Laboratory diagnosis of canine parvovirus infection can further be made by using an Electron microscope (EM), virus isolation or amplification of viral DNA using a PCR assay on fecal samples. Fecal antigen assays and PCR assays are used

most frequently in clinical practice. The sensitivity of immunoelectron microscopy (IEM) and the ELISA is believed to be relatively low due to large quantities of virus required for a positive test result. However, IEM may enhance sensitivity. Polymerase chain reaction (PCR) has been described as both sensitive and specific for the detection of CPV enteritis. For the quick diagnostic directly in the clinic, an antigen detection assay (LFA) is a helpful tool. Several fecal antibody-based antigen tests are available as rapid screening tools in a suspicious patient with a high specificity. Because of the short incubation time, serologic tests are not relevant for the diagnosis of CPV enteritis. Moreover, serum antibodies can persist for months and the serologic response to infection cannot be discriminated from antibody titers induced by vaccination. Thus, serologic tests are not considered useful for the diagnosis of CPV enteritis.

To obtain a reliable test result using an antibody based assay (LFA, ELISA) for the detection of antigens in feces, it is important to understand that the antigen concentration in fecal samples from dogs and cats may vary and therefore should always be viewed in the context of acute typical clinical symptoms. The virus is generally discharged extensively for 3–12 days post infection and usually correlates with the onset of clinical signs. During this period, the CPV antigen can be reliably detected. Taking this into consideration, a LFA allows a quick and reliable on-site diagnosis. In the clinical context, it should be noted that a negative test result with the antigen test does not completely rule out a parvovirus infection. The more sensitive PCR can detect parvoviral DNA for some weeks after infection.

Often test results of antigen assays (LFA, ELISA) are directly compared with test results of PCR. This comparison is actually scientifically incorrect for the calculation of test performance data for antigen assays, which is further explained hereafter.

Part 1: Different methods for the detection of Parvovirus

In the following section, different diagnostic methods for parvovirus infection are introduced. The data of these comparisons originated from an external study. In this context, these external independent ascertainments are used to provide a neutral database for evaluating each method, apart from the evaluation of our rapid test.

First, each individual diagnostic method is briefly explained.

Electron microscopy (EM):
Electron Microscopy (EM) is a tool to investigate the complex structures of the cell and organelles, and also to study the cellular biological processes taking place in the responses to changes in the microenvironment. Virus diagnosis by electron microscopy relies on the detection and identification of viruses on the basis of their characteristic morphology. A major advantage of virus diagnosis by EM is the ability to visualize the virus.

There are two types of EM methods: direct or immunoelectron microscopy (IEM). With direct methods, negative staining is normally used, which requires little special equipment, in contrast to thin sectioning techniques. The IEM may identify the virus. The success of immunolabelling at ultrastructural level depends on various factors, including the initial quantity and quality of antigens, and the preservation of the cellular ultrastructure, to be able to finally achieve an accurate localization of the antigen within the cell. Therefore, it is necessary to attain a correct balance between antigen preservation and a good morphology at the ultrastructural level.
Although EM is highly specific and sensitive, it is often too time consuming and expensive for routine use in a clinic. There must also be a minimum number of virus particles present in the sample and some viruses may give a non-distinct morphological appearance which may make detection very difficult. In summary, EM is a very expensive service to provide and requires highly skilled personnel.

Polymerase chain reaction (PCR):
For detection of CPV DNA by PCR, a conventional nested PCR protocols is normally used. The polymerase chain reaction is a method to amplify the DNA in vitro. For this purpose, the enzyme DNA polymerase is used.

Nested PCR
The nested PCR is a highly sensitive PCR method, in which two PCR reactions are switched one after the other. The special thing about this method is, that an aliquot of the PCR product from the first amplification serves as a template for the second PCR product. As demonstrated in several studies, positive PCR results for CPV may be seen in dogs without signs of gastroenteritis or even in dogs with chronic diarrhea. These findings of uncertain clinical significance can be defined as false positive. In addition, attenuated live vaccine virus can uncommonly be detected in the feces or in the blood with PCR assays for an undefined period after vaccination and indicate also false positive results. The major clinical indication to run a PCR is the suspicion of CPV infection, in the context of a negative fecal antigen result (LFA, ELISA).

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Antigen detection assays:
ELISA and LFA are diagnostic methods, which are based on the same detection principle: antigen-antibody reaction/ detection. There are, however, crucial differences between these two methods. The ELISA is a lab based method, the procedure takes a few hours, more than one sample is required for the application and for the read out of the test results a special reader is necessary. The LFA is a ready to use on-site test. The test procedure takes a couple minutes, single samples can be tested and the test results are visually visible within 10 minutes, a special reader is not necessary.

Lateral Flow Assay (LFA)
The test setup of the assay (sandwich-immunoassay) comprises two different antibodies for the detection of the specific parvovirus antigens in the test sample. The test strip consists of a conjugate pad (with the first monoclonal antibody labeled on gold nanoparticles) and the membrane with a second monoclonal antibody (in the T-line area), which is specific for CPV, furthermore another polyclonal antibody (in the C-line area).
The test procedure can be described as follows: the fecal sample is put with the cotton swab into the reagent of the sample tube. Three drops of the sample reagent, including the diluted fecal sample, are put onto the sample pad. Present antigens in the sample material bind to the gold-labeled monoclonal antibody. Due to capillary force the mixture flows over the test strip and therefore over the immobilized second monoclonal antibody in the test line area. The test line forms by building a sandwich between the gold-labeled antibodies from the conjugate pad, the antigen from the specimen and the immobilized antibody in the test line area. Furthermore, the mixture flows on the strips and passes the C-line area. Within 10 minutes a test line (if specific antigens are present in the sample) and a control line (always) are visible.
If there is no antigen in the specimen, the gold-labeled monoclonal antibody cannot bind to the immobilized second monoclonal antibody in the test line area. After 10 minutes is in such a case only the C-line visible and the test result is negative.

False negative test results can occur due to a decreased or intermittent viral shedding in earlier or later stages of an infection, the binding of serum-neutralizing antibodies with antigen in the intestinal lumen, or the dilutional effect of the diarrhea. It is important to understand the context of antigen concentration in fecal samples from dogs with acute typical clinical signs.

The most cost-effective assays for the virus detection are the rapid point-of-care tests (LFA), including ELISA, using sample material from fecal or rectal swabs. Although their specificity typically exceeds 90%, data on their sensitivity varies substantially, depending on the method utilized as reference/ gold standard (ELISA, PCR or immune-electron microscopy). This statement is discussed now with the help of the results of the external study.

External Study:
In this study a total number of 100 fecal samples, with different clinical symptoms, were compared with three methods (IEM, nested PCR and LFA/ELISA):

Gr.	Characteristic	N	IEM positive	Nested PCR positive	LFA/ ELISA positive
A	Acute hemorrhagic diarrhea	50	10	24	3-5
B	Chronic diarrhea	10	0	1	0
C	No evidence of GI disease	40	0	5	0

GR = group, GI = gastrointestinal

In group A, all fecal samples came from dogs with acute hemorrhagic diarrhea. In 24 cases the PCR was positive. 10 cases were positive by IEM and between 3 and 5 positive results occurred by three different antigen detection assays (dependent on the assay). In the control groups B and C positive results were also obtained by PCR. The dogs from these two groups had no evident parvovirus infection (no clinical suspicion). The results can be interpreted as falsely positive for a parvovirus infection.

The table below summarizes results of the external study, a comparison of three different antigen tests against IEM and PCR. In order to choose which of the antigen tests should be used for the subsequent study, the test data from the external study were compared with their total test performance in a combined manner. The different comparison values of the sensitivities and specificities of the three antigen tests are shown in the table.

Antigen Test No.	Comparing to IEM Sensitivity	Comparing to IEM Specificity	Comparing to PCR Sensitivity	Comparing to PCR Specificity
1	50%	97.8%	18.4%	100%
2	40%	97.8%	15.8%	100%
3	60%	92.2%	23.3%	95.2%

The PCR tested 5 non-infected (healthy) dogs positive and 1 dog with a chronic diarrhea as positive. Reason for these deviating results may be due to

asymptomatic, persistent, subclinical infection or intestinal passage of the virus, but not to an acute infection. It is also possible, that the positive PCR results are related to an earlier vaccination. There is no general gold standard method to detect parvovirus in fecal samples. For this reason the diagnosis must always be accompanied by the observations of clinical symptoms of the patient.

In summary, the theoretical explanations outlined above and the conclusions drawn from the comparisons of the external study exemplify that antigen tests are a useful diagnostic tool for the detection of canine parvoviral enteritis. In case of a positive test result with the antigen test, the diagnosis of parvovirus enteritis is with a very high probability correct. However, a negative test result does not rule out parvovirosis as a differential diagnosis for a dog with hemorrhagic diarrhea. In such cases additional tests should be considered, ideally examination of the fecal samples for parvovirus with IEM or PCR. Overall, antigen tests are very helpful tool for the veterinarian, due to their quick, easy on-site application, with immediate test results.

Part 2: Comparison of LFA (scil v-Parvovirus), ELISA and Nested PCR

The consolidated findings from the described study above are considered in the following evaluation of the scil v-Parvovirus rapid test in comparison to other methods and its application. An alternative antigen detection method (ELISA) is used for a reliable and reasonable evaluation of the scil v-Parvovirus test because the two methods are similar in detecting antigens in fecal samples and thus can be compared well. Therefore, in the second part of this study, the antigen assay (ELISA) from the external study with the best total test performance will be used as second diagnostic assay. The fecal sample examination is extended by the use of a PCR to additionally detect existing Parvovirus DNA in the fecal samples.

In the following study fecal samples from dogs and cats are divided into two groups, one group with a suspicion for a parvovirus (PV) infection and the other group as control group from dogs without a suspicion of parvo virus infection. Group 1 consists of 53 samples from cats and dogs and group 2 consists of 47 fecal samples.
All fecal samples were tested in an external lab with a nested PCR. The comparison between the both antigen tests (ELISA and scil v-Parvovirus) was made in a second, different lab.

Results

Group 1 consists of animals suspected of having parvovirus, which also were defined as positive by PCR by the laboratory. There is a high accordance of the test results of both antigen test (ELISA and scil v-Parvovirus). 33 of the 53 fecal samples were tested positive by PCR. 14 of the 33 PCR positive samples were also detected as positive for antigens by ELISA as well as by the scil v-Parvovirus. One other positive PCR sample was only detected by the ELISA.

Group 1: dogs and cats with suspicion of Parvovirosis

	Positive	UN	Negative	UP	∑
Nested PCR	33	0	20	0	53
ELISA	15	18	20	0	53
scil v-Parvo	14	19	20	0	53

UN = unclear negative results, UP = unclear positive results

In case of unclear negative results, it should be confirmed whether really parvovirus antigens are present in the samples, with different/ other tests. As no other tests were used to further define the unclear results, these samples remain unclear negative in this study.

For the other 19 cases of unclear results (PCR positive, ELISA/ scil v-Parvovirus negative) it might be assumed that the amount of parvovirus antigens in the sample was not in a detectable concentration range for antigen tests, nevertheless a parvovirus infection might be present.

Group 2 consists of animals with no suspected parvovirus infection. For these samples, it should be assumed that no sample from the group 2 is tested positively for parvovirus. Nevertheless, the PCR defined 5 fecal samples as positive.

Group 2: control group

	Positive	UN	Negative	UP	∑
Nested PCR	0	0	42	5	47
ELISA	0	5	42	0	47
scil v-Parvo	0	5	42	0	47

UN = unclear negative results, UP = unclear positive results

For these 5 cases of unclear results, it can be assumed that the PCR does not detect a parvovirus infection, but rather shows unspecific results or indicates an asymptomatic, latent infection.

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Summary

In this study a comparison between PCR defined fecal samples, the ELISA and the scil v-Parvovirus test (antigen assays) was performed. The ELISA and the scil v-Parvovirus showed very similar test results, with only one sample differing in the test results. Because of this difference, the sensitivity of scil v-Parvovirus is considered as slightly lower than the sensitivity of the ELISA.

scil v-Parvovirus compared to ELISA

Group	Sensitivity	Specificity	Total Test Performance
1	93.33%	100%	98.11%
2	100%	100%	100%
3	93.33%	100%	99.00%

Overall a high accordance between the ELISA and the scil v-Parvovirus could be observed (93.33% accordance in sensitivity and 99.99% in specificity). The comparison of the PCR and the scil v-Parvovirus (alternatively ELISA) showed the following data:

scil v-Parvovirus compared to PCR

Group	Sensitivity	Specificity	Total Test Performance
1	42% (45%*)	100%	64% (66%)
2	0%	100%	89%
3	37% (39%)	100%	76% (77%)

*Result in brackets from ELISA

The higher sensitivity in group 1 for the PCR compared to the antigen detection methods might be explained by the low amount of antigen in the sample or the rapid development of an intestinal immune response to CPV results in the formation of undetectable immune complexes. The unclear test results for PCR in group 2 are more difficult to explain. It remains unclear whether these results are due to the presence of non-pathogenic viral parvovirus DNA at the tested point in time, or false positive test results due to possible cross reactivity. Some dogs with chronic diarrhea or without gastrointestinal signs may also have positive CPV PCR results.

Conclusion

Parvovirus infections in dogs have become an important problem globally. The clinical signs resemble other enteric diseases and hence rapid and early diagnosis of the condition is important. Conventional methods such as EM and virus isolation are time consuming, less sensitive, and expensive. Hence, these tests are now replaced by molecular methods like PCR, which have higher specificity and sensitivity than the conventional antigen or antibody-based methods. However, the necessity of expensive equipment and reagents restricts its use for on-site rapid testing. The fastest method for diagnosing a parvoviral infection in the practice are lateral flow tests like the scil v-Parvovirus test (fecal antigen test), which is a simple, rapid on-site tool. The test is easy to perform by veterinarians without any specialized equipment. In case of a suspected parvovirus infection, an on-site antigen test should be performed as first diagnostic tool in the practice. In case of a positive test results a treatment can start immediately.

The scil v-Parvovirus is recommended to detect acute cases of enteritis in puppies.

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