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An overview of the use of point-of-care polymerase chain reaction testing in the veterinary clinic

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ABSTRACT: This article describes the advantages of polymerase chain reaction (PCR) testing as a point-of-care (POC) diagnostic tool in the veterinary clinic. Different variations of the test are discussed. Examples of the use of PCR in the veterinary clinic are presented. Despite its high specificity and sensitivity, it is emphasised that the PCR test does not stand alone when making a final diagnosis and must be used in combination with the overall clinical picture. The pitfalls when interpreting PCR results are also discussed.

Introduction

Point-of-care (POC) testing has become an integral and important aspect of veterinary practice for the detection of infectious diseases (Clerc & Greub, 2010). In general, the two principle methods for detecting infectious diseases are based on detection of the pathogen and revealing antibodies associated with the organism. Confirming the presence of the organism gives the most important evidence for a diagnosis of an infectious disease. On the other hand, the existence of antibodies would indicate exposure to the agent at some point in time and not necessarily an active infection.

Over the last 20 years, probably due to a lack of advances in technology and knowledge, antibody detection has predominated, with the development of a wide range of POC ELISA (enzyme-linked immunosorbent assay) tests (Waner, Mazar, Nachmias, Keren-Kornblatt, & Harrus, 2003; Waner, Nachum-Biala, & Harrus, 2014; Waner, Strenger, & Keysary, 2000). These tests, which previously were only available in sophisticated laboratories, have become available to clinicians both in the clinic and in the field (Waner et al., 2014). The tests are easy to use; they do not require either expensive equipment or trained personnel, are rapid and give results within minutes.

Until now, the detection of the infectious agents themselves involved laborious procedures, many of which could only be carried out in the laboratory. These included cytology, histopathology, electron microscopy and culture. These tests are expensive, not available for all the infectious agents and results are only obtainable, at best, in a few days.

Molecular diagnostic tests, based on analysis of nucleic acids (DNA and RNA), were first available only in the laboratory setting and later in the clinic. This development has opened new horizons for veterinary diagnostics. Conventional laboratory polymerase chain reaction (PCR) testing has provided veterinary clinics with highly sensitive tests, but the procedure requires multiple steps and uses expensive and complicated equipment manned by trained personnel. The procedure, under laboratory conditions, may take a number of hours in addition to the time taken to present the samples to the laboratory.

Recently, rapid POC PCR molecular detection kits have been developed for use

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in the veterinary clinic for the detection of infectious diseases (Waner et al., 2014). The tests are based on an isothermal chain reaction process and can provide results in less than 75 min. The equipment and kits are relatively inexpensive and easy to run without trained personnel. The tests are sensitive and specific and in some instances may even provide semi-quantitative results.

The methods of molecular diagnostic testing employed by diagnostic laboratories include:

- end-point PCR
- real-time PCR (RT-PCR)
- reverse transcription PCR, used for the detection of RNA
- multiplex PCR, used to detect a number of potential pathogens simultaneously

PCR testing

The significant advantage of PCR testing compared to serological antibody detection is that, in many cases, PCR-positive results can precede the development of antibodies (Schmitz, Coenen, Matthias, Heinz-Jurgen, & Neiger, 2009). Under these circumstances, PCR will be of advantage to prove the existence of infection in acute cases. Treating a patient when the aetiological agent is known provides the great advantage of being able to select the appropriate medication, thus ensuring a more successful treatment outcome.

By and large, PCR has the advantage of increased sensitivity and significantly shorter turn-around time compared to the common diagnostic tests used today in laboratories. The high sensitivity allows for the test to detect only a few copies of target pathogen nucleic acid in a given sample by means of amplification to millions of copies (Daniels, 2013). The amplification of only a few copies of DNA is carried out using a polymerase enzymatic reaction which enables the generation of a multitude of copies of a DNA molecule in a very short time.

Sources of error

Interpretation of the test requires that the user be aware of a number of pitfalls and drawbacks regarding this technology:

- false-positive results may occur in animals which have been recently vaccinated, although some tests may be specific enough to differentiate the vaccine strain from the wild-type virus (Daniels, 2013)
- PCR reactions are designed to detect DNA associated with the causative agent, so a positive PCR result may not always correlate with the presence of *viable* organisms

As with all laboratory test results, it is imperative to evaluate each case in relation to the patient's history, presenting clinical signs and the interpretation of cell counts, serology and biochemical tests, prior to diagnosis of the disease.

Examples of the use of PCR in clinical practice

Canine parvovirus

Canine parvovirus (CPV) infection is one of the most common viruses, causing acute and often fatal haemorrhagic gastroenteritis in dogs (Marulappa & Kapil, 2009). A rapid and accurate diagnosis is important in order to initiate treatment as soon as possible and improve the prognostic outcome, taking into account the young age of the dogs often affected and their sensitivity to fluid and electrolyte loss. In kennels and shelters, a quick diagnosis is of great importance in order to isolate infected dogs and prevent infection of other susceptible animals. The clinical diagnosis of CPV cannot always be made with confidence for any given case as there are several other pathogens which also cause diarrhoea in dogs (Desario et al., 2005). Therefore, it is necessary to confirm a diagnosis using laboratory tests. The most common tests used to date include (Desario et al., 2005):

- immunochromatography (IC)
- haemagglutination inhibition (HAI)
- virus isolation (VI)
- PCR

Due to the transitory incubation period, serologic tests are not considered diagnostic for CPV enteritis (Schmitz et al., 2009). Furthermore, previous vaccination against CPV may cause false-positive results to be obtained. HAI and VI can only be carried out in specialised laboratories, requiring trained personnel and specialised equipment and reagents.

The IC test used to detect CPV antigen in the faeces is the most common rapid laboratory test used in the clinic (Desario et al., 2005). The test procedure is simple, rapid, easy to read and can be performed without difficulty. However, the test has limitations: a large amount of viral antigen is required to produce an unmistakably visible band to conclude a diagnosis. Furthermore, false-positive results are possible 3–10 days post-vaccination with a modified live CPV vaccine, as the vaccine virus is shed in the faeces. The presence of antibodies in the faeces interferes with the availability of antigenic sites on the virus particles, resulting in false-negative results (Desario et al., 2005).

Of all the tests, PCR using blood or faeces has been demonstrated to be more sensitive than other techniques (Desario et al., 2005). A positive PCR result is considered to be strong evidence of an ongoing infection; however, the test results must always be taken together with the history, vaccination history, clinical signs and clinical pathological findings in order to make a final diagnosis.

Leptospirosis

Leptospirosis is a zoonotic bacterial disease with a worldwide distribution, and is an emerging infectious disease in people and in dogs (Greene, Sykes, Moore, Goldstein, & Shultz, 2012).The microscopic agglutination test (MAT) is considered the gold standard for detection of leptospiral antibodies and is performed only in large central laboratories which are able to maintain live cultures of many leptospiral serovars (Greene et al., 2012). The MAT test is insensitive during the first week of the disease, however, thus leaving infected patients without a diagnosis. Furthermore, the risk to humans handling ill and potentially infective dogs is a substantial hazard.

The POC PCR assay (PCRun™; Biogal, Israel) has been designed to detect only pathogenic leptospiral serovars. In the first 10 days of infection, organism numbers are highest in the blood, and thus blood is the sample of choice during the first week of illness (Greenlee, Alt, Bolin, Zuerner, & Andreasen, 2005). After that time, organisms are present in the highest concentration in urine. When the length of time of infection is unknown, simultaneous testing of blood and urine may increase diagnostic sensitivity. Recent antimicrobial treatment can result in false-negative test results for both culture and PCR, although multiple doses of antimicrobials may be required before PCR becomes negative, because PCR detects both viable and non-viable organisms (Sykes et al., 2011).

Because apparently healthy dogs may shed leptospires, a positive PCR test result on urine may not necessarily correlate with illness but with a chronic carrier state (Rojas et al., 2010). It is clearly important to identify these dogs in order to prevent further infection of other dogs or people. Furthermore, in horses, and in people with uveitis, PCR has been used to detect *Leptospira* in the aqueous humour (Chu, Rathinam, Namperumalsamy, & Dean, 1998; Faber et al., 2000).

Figure 1. PCRun™ reader used to detect a semi-quantitative result

Haemotropic *Mycoplasma* in cats

Mycoplasma haemofelis (previous nomenclature *Haemobartonella felis*) is considered to be the agent of feline infectious anaemia (Messick & Harvey, 2012). Transmission occurs through arthropod vectors and the disease is usually manifested as a regenerative haemolytic anaemia, unless the regenerative response is suppressed by an underlying disease such as feline leukaemia virus (Messick & Harvey, 2012).

If untreated, up to about one-third of cats may die quickly due to a precipitous decline in their haematocrit early in the course of the disease from a massive parasite burden and consequent anaemia. However, most cats infected with *M. haemofelis* become asymptomatic carriers; in these cats, the disease may reappear in a milder form during stressful situations.

The diagnosis is usually made by a subjective visualisation of the organism in blood smears (Messick & Harvey, 2012). However, parasitaemia is episodic, thus severely limiting the sensitivity of this diagnostic test. Because of the cyclic nature of the parasitaemia, the absence of *M. haemofelis* in blood smears does not rule out a diagnosis of haemotropic mycoplasmosis.

It has been determined that PCR can detect about 50 DNA copies of *M. haemofelis*, making the test highly sensitive (Cooper, Berent, & Messick, 1999). In any case, whether diagnosed by identification of the organism in blood smears or using PCR-based assays, the significance of infection by *M. haemofelis* must be interpreted in the light of haematological and clinical findings. The presence of *Mycoplasma* in the blood does not necessarily indicate clinical illness due to the agent because organisms may be incidentally observed in carrier cats with other diseases (Messick & Harvey, 2012).

Figure 2. PCRun™ detection device used to detect a qualitative PCR result

Two examples of POC PCR devices

The PCRun™ Molecular Detection Kit for the PCRun Reader contains reagents for the amplification of the target pathogen DNA and is performed together only with the PCRun Reader. Up to 16 tests can be performed in one run. **Figure 1** shows the semi-quantitative result method with the PCRun Reader.

The PCRun™ Detection Device kit contains reagents for the amplification of the target pathogen DNA. **Figure 2** shows the lateral-flow, qualitative result method with the PCRun Detection Cassette.

Conclusion

PCR tests have opened new avenues for the diagnosis of infectious diseases. With the advent of POC PCR kits available in the veterinary clinic setting, veterinary staff will now be able to make accurate and rapid diagnoses of numerous infectious diseases allowing for a better prognostic outcome.

By analysing the molecular properties of a given infectious agent, we are now in a position to confirm accurately and specifically the presence of a definitive infectious agent. The sensitivity of the PCR test is higher than other diagnostic tests designed specifically for detection of infectious diseases. Positive serological results are indicative of exposure to an organism, whereas a positive molecular diagnostic test is indicative of the presence of the DNA or RNA of the agent.

Serology and PCR have different windows for diagnosis. PCR is most applicable for the acute phase of disease, prior to, and in the early stages of antibody generation, while serology can be useful in diagnosis during the early and late recovery phase. Taking all the factors mentioned above into account, it must be emphasised that PCR and serology tests do not stand alone when making a final diagnosis and must be used in combination with an accurate history, clinical signs and other diagnostic tests such as biochemistry and haematology testing, in order to come to a final diagnosis.

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