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Review Article

ORAL FLUID DIAGNOSTICS: A NOVEL APPROACH FOR DISEASE DIAGNOSIS IN VETERINARY SCIENCE

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ABSTRACT

Use of diagnostic tests is a crucial aspect of disease diagnosis in veterinary science since they assist the clinician to establish a confirmatory diagnosis. Diagnostic tests based on fluid generally use blood and urine and less frequently the other fluids such as oral fluid. "Oral fluid" is composed of saliva, gingival crevical fluids contained in the dentogingival sulcus, mucosal transudate, cell detritus, bacteria and food remains. Although saliva is predominantly a watery fluid, it also consists of a complex mixture of proteins, ions and other organic compounds produced mostly by the salivary glands with a small portion originating from the blood. The connection between local (salivary glands) and systemic (blood) sources makes saliva an important fluid for the diagnosis of diseases or to study a physiological status in particular. A huge advantage of saliva over blood is that it is easily accessible and can be obtained non-invasively and relatively stress-free. However, a major barrier in using saliva as a diagnostic fluid could be the fact that many informative analytes are generally present in lower amounts in saliva but with new and highly sensitive technologies, it is no longer a limitation.

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INTRODUCTION

Recognition of disease is the foundation of disease control and prevention. The use of diagnostic tests is a crucial aspect of disease recognition since they assist the clinician to establish a confirmatory diagnosis. Diagnostic tests based on fluid are generally using blood and urine and less frequently the other fluids such as oral fluid primarily saliva. "Oral fluid" is composed of saliva, gingival cervical fluids contained in the dentogingival sulcus, mucosal transudate, cell detritus, bacteria and food remains (De Almeida *et al*., 2008). Although saliva is predominantly a watery fluid, it also consists of a complex mixture of proteins, ions and other organic compounds produced mostly by the salivary glands with a small portion originating from the blood (Pederson *et al*., 2002). The connection between local (salivary glands) and systemic (blood) sources makes saliva an important fluid to search for biomarkers of diseases or to study a physiological status in particular (Kaufman and Lamster, 2002). Often called the 'mirror of the body' or 'a window on health status', oral fluid can be a good medium to be explored for health and disease surveillance.

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Basic Principle

In addition to saliva, the fluid in the oral cavity contains serum transudate that crosses the oral mucosa (oral mucosal transudate) and gingiva (gingival crevicular fluid, GCF) from capillaries located in the oral mucosa and the gingival tissues (Cameron and Carman, 2005). There are several ways by which serum constituents that are not part of the normal salivary constituents (*i.e.*, drugs and hormones) can reach saliva. Within the salivary glands, transfer mechanisms include intracellular and extracellular routes. The most common intracellular route is passive diffusion, although active transport has also been reported. Ultrafiltration, which occurs through the tight junctions between the cells, is the most common extracellular route. Serum constituents are also found in whole saliva as a result of GCF outflow. Depending on the degree of inflammation in the gingiva, GCF is either a serum transudate or more commonly, an inflammatory exudate that contains serum constituents (Eltaz and Era, 2002).

Collection of oral fluid

To obtain the oral fluid, non-invasive collection techniques have been widely used. In cattle, with their continuous flow of saliva, samples can be obtained by simply collecting the mouth flow into a large beaker. Afterwards, the sample is further centrifuged at 3000rpm for 15 min to precipitate the remaining

food particles (Ang *et al*., 2011). However, this method provides an unstimulated sample and will only work with domesticated animals that are used to be handled by man. In swine, the oral fluid samples are collected using cotton rope. Pigs are allowed to chew the rope for about 10 to 15 minutes or until the rope will be sufficiently wet. The wet end of the rope is then cut, placed in a syringe (50 ml), and compressed to recover the oral fluid. An approximate volume of 5 ml oral fluid can be obtained from each animal on each collection day (Lina *et al*., 2013). Alternatively, hold the dry end of the rope with one hand and insert the wet end of the rope into a clean plastic bag. Twist the rope inside the bag to extract the oral fluids. In other farm animals that do not produce large amounts of saliva stimulated saliva collection can be done. In details, under mechanical stimulation the animals chew a cotton roll for some time which is moistened by the saliva. The roll is then centrifuged at 3000rpm for 15 min to release the saliva sample, which is subsequently stored at 80 °C. However, appropriate collection procedures need to be considered for the analysis of different types of salivary analytes (Mau *et al*., 2009).

Diagnostic Applications

There are a number of diagnostic applications of oral fluid in veterinary science. Parida *et al*. (2006) developed an ELISA to detect IgA antibody against structural proteins (SP) in oral fluid under the premise that vaccines used in response to an FMD virus outbreak would not include nonstructural proteins (NSPs). Moreover, oral fluid samples have been used to isolate and culture bacterial pathogens like *Escherichia coli* and *Salmonella spp.* from feedlot cattle (Stanford *et al*., 2005). Kinder *et al*. (1992) conducted a trial in dogs experimentally challenged with *Taenia pisiformis* and detected specific IgG and IgA for 12 weeks post inoculation. He further suggested that oral fluid specimens could serve as an alternate method of detecting intestinal helminth infection. Some zoonotic pathogens are also detectable in canine oral fluid. Rabies virus is commonly transmitted via animal bites and has been demonstrated in canine oral fluid (Kasempimolporn *et al*., 2000). Several strains of *Bartonella spp*. have been detected in canine saliva by PCR (Duncan *et al*., 2007). Diagnostically, equine oral fluid has been used to test for the presence of performance altering chemicals in race horses (Prickett and Zimmerman, 2010). Several swine pathogens are known to be present in detectable levels in oral fluid samples. Vesicular stomatitis virus has been isolated from oral fluid from pigs infected via mechanical vectors or by direct contact with infected pigs (Stallknecht *et al*., 1999). Recently, the detection of porcine respiratory and reproductive syndrome virus (PPRSV) and porcine circovirus type 2 (PCV2) by PCR was reported in swine oral fluid samples collected under both experimental and field conditions (Prickett *et al*., 2008).

Advantages

As a clinical medium, oral fluid has many advantages over serum. It is easy to collect, store, transport and can be obtained at low cost in sufficient quantities for analysis. It can be obtained through a non-invasive collecting technique that dramatically reduces anxiety and discomfort to the animal and simplify procurement of repeated samples for longitudinal monitoring over time. For professionals, oral fluid collection is safer than venepuncture, which could expose health care providers to many zoonotic diseases. It is also easier to handle for diagnostic procedures since it does not clot, lessening the manipulations required.

Conclusion

The analysis of blood and its components has been the mainstay for laboratory diagnostic procedures for several decades. A growing number of examples have been established for using saliva to monitor systemic diseases and conditions. However, a major barrier in using saliva as a diagnostic fluid has been the fact that many informative analytes are generally present in lower amounts in saliva than in serum. With new and highly sensitive technologies, the lower level of analytes in saliva is no longer a limitation. Most of the things that can be measured in blood can also be measured in saliva. Therefore, Oral fluid is now being systematically researched and the results of its analysis are being compared with other diagnostic media such as blood and urine.

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