PCR APPLICATIONS FOR DIAGNOSIS IN ANIMAL HEALTH

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Viral infectious diseases are a constant risk for livestock production due to the economic and sanitary costs produced by their entrance into a country. Once the causative agent is identified, specific strict sanitary measures must be rapidly implemented to avoid its spread, and to control and eradicate the disease. Classical laboratory diagnosis of viral diseases includes both direct and indirect tools, virus isolation being the "gold-standard" technique to confirm the presence of an etiologic agent. Around 15 years ago, the merging of the PCR completely transformed the diagnostic pathway in the National Reference Laboratories and so became an essential tool. Many benefits prompted its incorporation into routine lab work, high sensitivity, superior to virus isolation, being of most value as it allows the early detection of pathogens even before the appearance of clinical signs in infected animals. The high specificity, the analysis of a great number of any kind of clinical samples within hours, and the chance to test several pathogens in the same reaction (multiplex assays), made PCR a basic tool for screening diagnostic step (figure 1).

The introduction of the real-time PCR changed the workflow once again in lab diagnosis, by reducing analysis time and contamination risk, and increasing the sensitivity and applications in diagnosis. Moreover, real-time PCR accompanied by the appearance of robots in the market for sample preparation and nucleic acid extraction steps, made real a completely automated analysis procedure for a high throughput application (figure 1).

Now, PCR has a wide range of main applications in Animal Health. It is extensively used in surveillance, control and eradication programs of the major viral diseases affecting livestock at national and international levels (figures 1, 2, 3, 5). PCR is a valuable tool in the prevention of new disease entrance, the control of biological products, or the evaluation of vaccines efficacy (figure 4). Generic PCR assays can be developed for detection of new viruses within a genus/family or to determine the subtype/serotype of the identified virus (table 2). Also, PCR methods can be designed to be used as DIVA ("differentiating infected from vaccinated animals") tests, discriminating vaccine strains from field circulating viruses, which can be of great significance when using live attenuated vaccines. Finally, PCR is the starting point in molecular characterization and epidemiology studies of circulating viruses, essential for tracing the origin and evolution of a disease outbreak.

More recently, portable PCR machines and other simple low-cost PCR-based techniques are being launched for on-site application, meaning the analysis of samples can be performed in regional labs or even in the farm/slaughter-house with basic equipment by non-specialized personnel. Due to many viral animal diseases are prone to very rapid spread and the identification of the involved virus is urgently required, these new tools may become useful first line pen-side tests in a short time.