

The Future of PCR Technologies in Diagnosis of Bovine Mastitis Pathogens

Yasser Mahmmod*

Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt

Bovine mastitis is the most economically important disease affecting dairy cattle worldwide from an economic, diagnostic and public-health related point of view. The disease caused by a wide variety of bacteria, which can be classified as environmental (*Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus* sp. and coagulase-negative staphylococci) or contagious (*Mycoplasma bovis*, *Staphylococcus aureus* and *Streptococcus agalactiae*). Mastitis occurs mainly in two main forms clinical and subclinical, the clinical mastitis form, which is easily visible and diagnosed by farmers because of the characteristic signs on affected udder/quarter and associated changes in milk composition with clots and flakes formation. The subclinical mastitis form, which is hard to be diagnosed visually but characterized mainly reduction of milk production and alteration of milk constituents. The control of mastitis pathogens depends mainly on elimination of existing infections and prevention of new ones within herds through application of sanitary and medical measures as well as maintenance of strict biosecurity. The implementation of the “five-point plan” in dairy herds which was developed in the 1960s is an effective method for control of contagious mastitis.

Monitoring udder health is challenging without reliable and affordable diagnostic methods. Accurate screening tests for the early detection of pathogen-specific subclinical mastitis are essential to promptly initiate the appropriate treatment or culling of infected animals. Therefore, the appreciate measures will be implemented to reduce the risk of new infections within herd or prevent introduction in new herds. There are wide ranges of diagnostic procedures for mastitis with different principles of actions, where some of them are based on detection of abnormalities of the udder and milk, and inflammatory markers. These procedures include physical and clinical examination of the udder, somatic cell counts (SCC), California Mastitis Test (CMT), Electrical conductivity test, pH meter, NaOH test (white side test) and measurement of N-acetyl-b- Dglucosaminidase (NAG-ase), lactate dehydrogenase (LDH). The other type of diagnosis is more specific and based mainly on isolation and identification of the causative pathogen of mastitis or the immune response (antibodies) such as bacteriological culture (BC) of milk, biochemical tests, Milk Elisa, and polymerase chain reaction (PCR). Each diagnostic technique has its own advantages and disadvantages and its performance is dependent on many factors some of which are related to the procedures of sample collection, type, preservation and handling in the laboratory, others include the degree of infection and status of infected udder and infected cow, type of causative pathogen and its virulence and finally those related to the experience of the investigator.

Over the past 10 years, molecular diagnostic techniques have been used intensively for identification of mastitis pathogens, where they may be considered an alternative to conventional microbiological testing. Molecular diagnosis could be the most appropriate technique for the species identification of mastitis pathogens that are difficult to detect and identify by conventional methods. PCR-based diagnostics may offer significant advantages over other diagnostics for its speed and its sensitivity when used for mastitis pathogens detection. PCR methods target the DNA of a specific mastitis pathogen. PCR methods

can be classified into qualitative (inform presence or absence of the pathogen DNA), semi-quantitative and quantitative categories. Some types of PCR targeting one specific pathogen, while others can identify more than one and are therefore known as multiplex PCR tests.

Recently, a commercially available multiplex real-time PCR technique, the PathoProof™ Mastitis PCR Assay (Thermo Fisher Scientific, Vantaa, Finland), has been introduced as a faster and highly accurate alternative to BC [1]. The assay has been evaluated under field conditions for detection of different mastitis pathogens from milk samples of clinical, subclinical cases and spiked samples and showed high accuracy on the quarter-level, the cow-level and the herd-level [1-3]. The assay targets the most common mastitis pathogens (12 mastitis pathogens) with a short throughput time for either freshly collected or preserved milk samples. It has the ability to detect growth-inhibited or dead bacteria and may reduce the number of false negative results. The assay has also been promoted as a suitable tool to detect mastitis organisms from composite milk samples at routine milk recordings. Currently, the assay has been implemented in a number of European countries, where the dairy farmers can order PCR testing of milk samples during routine milk recording. The Cycle threshold (Ct) values obtained for the bacterial DNA targets are used as a scoring for the PCR assay. The PCR assay's thermal cycling protocol involves 40 cycles for the target bacterial DNA. Some recently published the PCR assay results [2,4].

The PCR assay is a promising diagnostic technique for the mastitis diagnosis and control however, its use on a wider scale may be affected by some limitations including: (a) absence of specific guidelines or cut-off point for the definition of sample contamination unlike BC, (b) its use in developing countries is limited comparing to developed countries (economic reasons), (c) possibility of obtaining a false positive results due to milk carryover (defined as transfer of a small amount of milk from one cow sample to the next at the time of collection due to the presence of residual milk in the milking unit, milk meter or milk sampler), (d) applicability of pre-sampling procedures, and (e) the inability to differentiate between viable and non-viable bacterial cells. In my opinion, using the available information such as SCC, history of mastitis, clinical examination of the udder and history of previous treatment side-by-side with the results of PCR will help the dairy advisor to make the right decision regarding treatment or culling or re-sampling.

***Corresponding author:** Yasser Mahmmod, BVSc, MVSc, PhD, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt, Tel: 002-01069900940; E-mail: yasserpcr@gmail.com, yasser@sund.ku.dk

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To summarize, PCR tests based on composite milk samples collected during routine milk recordings can be valuable tools in detection and control of mastitis pathogens due to their high sensitivity and specificity, rapidity, ease of automation, suitability for all types of milk samples (fresh or preserved). The choice of Ct-value cut-off depends on the purpose of the milk sampling, i.e. whether identification of all positive cows or identification of heavily/truly infected cows is of interest. The limitation points which affect the performance of the PCR assay such as carryover and microbial contamination of milk sample can be avoided or at least minimized by disinfection of teats prior to attachment of the milking units, accounting for milking order, repeated tests of positive cows and by considering other inflammation markers. Nevertheless, the PCR assay could serve as a suitable alternative to the current mastitis diagnostics however, further research may be required for more improvements to overcome the reported limitations/drawbacks and subsequent consequences on the diagnostic performance.

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