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Editorial Open Access

Implications of Carryover on the Diagnostic Indicators for Intramammary Infections in Dairy Herds

Yasser Mahmmod*

Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig City 44511, Sharkia Province, Egypt

***Corresponding author:** Yasser Mahmmod, BVSc, MVSc, PhD, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig City 44511, Sharkia Province, Egypt, Tel: (002)-01069900940; E-mail: yasserpcr@gmail.com; yasser@sund.ku.dk

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Editorial

Carryover is the phenomenon that a milk sample from cow B also contains some fraction of milk from cow A that was milked just before cow B in the same milking unit. Many studies reported that carryover of a small amount of milk from one DHI cow sample to the next at the time of collection is possible due to residual milk in the milking unit, milk meter or milk sampler [1-3]. The use of milk samples obtained from milk meters for PCR testing could be carryover of milk components that will contaminate one or more subsequent samples [4]. Carryover has been reported in both conventional and automatic milking systems (AMS) however, milk samples obtained from AMS are more prone to carryover than traditionally obtained samples because of the greater complexity of AMS including valves, pumps, containers and connecting tubes. It was reported that the degree of carryover in AMS was higher (up to 20%) compared to conventional milking parlours [5]. The routinely collected milk samples of DHI are subjected for analysis of some milk parameters such as fat, protein and somatic cell counts (SCC). Based on the milk fat, a statistically non-significant estimated carryover was 2% in conventional milking systems [1] while in earlier study, carryover degree was 6-7% based on milk fat in the Milko-Tester device [6]. In a recent study, the estimated degree of carryover in conventional milk system using fluorescent tracers (yellow dye) was 3.5% in an experimental setup [5].

In some developed countries, the routinely collected samples at DHI can be examined for detection and identification the presence of the bacterial DNA for some mastitis causing pathogens by real-time PCR. Hence, a question has been raised if the carryover lead to misclassification of cows based on PCR analysis and subsequently, results in unnecessary costs for treatment, culling or repeated sampling of cows. It has been suggested that the correlation between consecutively collected milk samples of cows milked at the same milking unit and milk meter can be regarded as indicator for the presence of carryover. Using a recent PCR technique, the degree of carryover was estimated based on the DNA of *Streptococcus agalactiae (S. agalactiae)* in the milk samples at three cut-offs for PCR cycle thresholds values (<40, ≤37, and ≤34) and it was 13%, 11%, 9%, respectively [7]. Furthermore, the authors found that the type of correlation was first-order autoregressive which mean that samples collected close in time would be expected to be more correlated than those collected further apart. This implies that a sample taken from truly negative cow without IMI collected after an IMI cow may contain the bacterial DNA simply, as a consequence of cross-contamination and turn out false positive. In the same context, another study reported that carryover may interfere with the accurate diagnosis of IMI with Staphylococcus aureus based on PCR testing [2]. The expected effect of carryover is mainly false positive PCR results, which leads to misdiagnosis of individual cows. Therefore, carryover could be a challenge for the accurate diagnosis of IMI based on PCR tests on milk recording samples and may bias the results, leading to overestimation of the prevalence of infected cows and eventually, overtreatment of false positive cows. It may worth to ensure that the PCR act by amplifying the target DNA for the target bacterial pathogen, meaning that the carryover is estimated based on the detectible amounts of DNA, while estimating the carryover based on milk fat% and florescent tracers/dyes involve no such amplification process where, fat% was used as a volume base for estimation the carryover.

In conclusion, carryover in terms of correlation between cows milked at the same milking unit may lead to false positive PCR test results for some bacterial pathogens causing IMI such as *S. agalactiae*. However, PCR test result is only one piece of information that must be interpreted in the context of all of the other information available about the cow such as milking order and results of other IMI indicators (e.g., SCC and California Mastitis Test) for supporting the accuracy of the decision-making with treatment, culling or resampling.

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