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Magnetic Beads Carried Over in Extracted DNA Elution from Mastitis Cow Milk

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A B S T R A C T

Using the magnetic separation technique in nucleic acid extraction is considered one of the most rabid and easy automated extraction methods that use minimal amounts of equipment. This paper describes some cow-related factors that may affect the carrying of magnetic beads during the extraction process. A total of 85 milk samples were collected from clinically infected cows with mastitis. DNA extraction is done using the Mag MAX™ CORE Nucleic Acid Purification Kit and the King Fisher™ Duo Prime Purification System. Fifty-three (62.4%) samples are extracted normally, while thirty-two (37.6%) have a magnetic bead carried over, and two (6.3%) out of the thirty-two supernatant DNA samples can't be collected. To evaluate the impact of lactation, frequency of mastitis, days in milk, and gestation period on magnetic beads carried over during DNA extraction when using the magnetic separation technique, a Chi-square test of independence was performed, and it showed that there was no significant association between magnetic beads carried over and lactation (Chi square = 0.28, P = 0.60), frequency of mastitis (Chi square = 2.26, P = 0.13), days in milk (Chi square = 0.84, P = 0.66), and gestation period (Chi square = 4.24, P = 0.12). Therefore, more studies need to be conducted to interpret this phenomenon.

Introduction

In general, cow milk is mainly considered a source of macro- and microelements, especially Ca, K, Mg, Na, P, Se, and Zn, for human nutrition, and the content of macro- and microelements in milk is determined by the geochemical conditions of the region and feeding (Voronina *et al.,* 2022). Dairy cow mastitis is an inflammation of the mammary gland that exists on each dairy farm, and it is almost due to a microbial infection (El-Sayed *et al.,* 2017). For its effect on milk production and medicine consumption, it is the costliest disease (Hogeveen *et al.,* 2011). The huge losses were mainly due to reductions in milk production, poor milk quality, discarded milk, and diseased cows' medication (Kuang *et al.,* 2009). There are more than 150 different contagious or environmental microorganisms causing mastitis; those 150 microorganisms are classified into five groups: Gram-positive cocci, Gram-negative bacteria (coliforms), Corynebacterium, Mycoplasma, and other miscellaneous

organisms, which include Nocardia, Prototheca, and yeast (Rebhun, 1995), The most dependable diagnostic test for dairy cow mastitis should be sensitive, rapid, repeatable, and economic (El-Sayed *et al.,* 2017).

Long ago, a lot of studies dealt with the diagnosis of mastitis, most of them based on clinical signs or indirect measurements, such as the somatic cell count (SCC) (Dohoo and Leslie, 1991). Also, we can consider the golden standard California Mastitis Test (CMT) and electrical conductivity (EC) milk measurement, both of which use a hand-held meter (Hillerton and Semmens, 1999).

All the previous studies did not identify the causative agent, which is crucial in disease prevention, treatment, and control. This shortage led to the usage of classical microbiological methods such as the routine identification system (Rudolf and Schleifer, 1995).

Nowadays, polymerase chain reaction (PCR) is a tool of power for its revolutionary rapid, qualitative, quantitative, and reliable identification of mastitis with the availability of commercial kits (Dahm, 2005). So far, the molecular diagnostic techniques developed in the last few years have become the gold standard for mastitis diagnosis. Not only that, but molecular biology techniques can recognize pathogens at the subspecies level, which is very important in epidemiological studies (El-Sayed *et al.,* 2017).

For sure, nucleic acid extraction is the starting point in any molecular biology study, so it is considered an extremely important process (Dahm, 2005). Before the latest revolution in molecular biology, nucleic acid purification had been a time- and effort-consuming process based on several extraction and centrifugation steps, and this long process was limited by small yields and low-purity products. Recently, specifically functionalized magnetic particles were developed, and together with an appropriate buffer system, this new technique allows for quick and efficient purification. Furthermore, in the magnetic beads separation stage, particles with a diameter larger than 1 μm can be easily separated using simple magnetic separators, while separation of smaller particles (magnetic colloids with a particle size ranging from ten to hundreds of nanometers) may require the use of high-gradient magnetic separators (Sonja, 2006).

Because the first DNA extraction was carried out by the Swiss physician Friedrich Miescher in 1869, Friedrich Miescher, by chance, purified DNA from the nucleus while investigating proteins from leukocytes and found that the feature of this substance was structurally different than proteins, consequently coining the term "nuclein" (Dahm, 2005). Since then, molecular biologists have made remarkable progress in designing extraction methods that are more reliable, easier, and faster to perform, more cost-effective, and produce a higher yield.

In addition to the classic liquid-liquid DNA extraction method that uses organic and inorganic reagents such as phenol-chloroform, which pose a toxic threat to humans, there are new techniques based on physical extraction that have significantly contributed to developing simpler DNA extraction, such as extraction methods using magnetic beads and cellulose-based filter paper (Dairawan and Shetty, 2020). Modern DNA extraction methods are classified into chemical or mechanically processed methods; each category has characteristics that influence their use (Ali *et al.,* 2017).

Basically, DNA extraction is made up of the following steps: first, disruption of cytoplasmic and nuclear membranes; second, separation and purification of DNA from other components of the cell lysate, such as lipids, proteins, and other nucleic acids; and lastly, concentration and purification of DNA (Ali *et al.,* 2017).

It is very important, when selecting a method for DNA extraction, to ensure the quality and quantity of the isolated DNA to carry out the selected downstream applications. In the same way, there are other factors that should be carefully considered to optimize the DNA extraction method, including time, cost, potential toxicities, yield, laboratory equipment and expertise requirements, as well as the required sample amount for the protocol (Chacon Cortes and Griffiths, 2014).

Along with that, magnetic nanoparticles have a high and specific affinity for DNA due to their coat with a DNA-binding antibody or polymer, which can be used to bind DNA to their surface. In general, the core of magnetic beads is composed of magnetite or maghemite, and surface substances that can be used are silica as well as functional groups such as sulphate and hydroxyl groups (Saiyed and Ramchand, 2007). Separation of the DNA-bound magnetic beads from the cell lysate can be performed by applying a magnetic field to the bottom of the tube by using an exterior magnet, and the beads aggregate at the tube bottom. The supernatant can rinse

away, and separation can be part of an automated extraction system (Peterson and Sober, 1956).

On the other hand, using magnetic beads in the separation and purification of nucleic acids and other molecules in a highly efficient and specific manner led to a technological revolution in biological research for its high-quality nucleic acid and low risk of cross-contamination. DNA separation using magnetic technologies is highly recommended; it requires minimal starting material, and it is both beneficial and user-friendly. DNA collected by this method, compared with other conventional methods that can take up to several hours, is much faster. Therefore, MagNa Pure automatic extraction gave a result of a nucleic acid amount of 0.98 µg/µl with a 2.91 purity ratio when compared to other methods, while magnetic beads-based techniques, either the original or the modified one, showed purity ratios of 2.45 and 2.36, respectively, and the amount of nucleic acids recovered was 0.79 µg/µl and 0.76 µg/µl, respectively (Abd El Aal *et al.,* 2010).

Moreover, using magnetic nanoparticles in the extraction of genomic DNA produced up to 1.2 mg per 30 ml of whole blood or 2.0 x 10^5 cultured cells, while it was about 1.8 mg and 2 mg per 30 ml of liver and brain tissue homogenate, respectively (Saiyed and Ramchand, 2007).

In addition to that, there were some factors that can influence nucleic acid purification, such as particle size of MBs, surface groups of MBs, and lysis time (Bo Li *et al.,* 2017).

Along with that, and due to restrictions on producing yields with the highest purity, there is no single procedure that is applicable to all contexts of DNA extraction (Dairawan and Shetty, 2020).

In contrast to that, cow milk fat content increased in early lactation and late lactation and varied little throughout the mid-lactation period, while lactose content levels rose rapidly in the first few days of lactation and then remained constant for several months before declining late in lactation. On the other hand, distinguishing changes were observed in the contents of total protein, of the various protein fractions, and of the major minerals in milk (Rook and Campling, 1964). Furthermore, increasing mammary infection in cows decreased milk production and milk components (Harjanti and Sambodho, 2019).
Thus, due to the magnetic beads carryover phenomenon noted during DNA extraction of mastatic cow milk using the Mag MAX TM CORE Nucleic Acid Purification KIT and King Fisher™ Duo Prime Purification System, this study will demonstrate the relationship between lactation, frequency of mastitis, days in milk, gestation period, and magnetic beads carried over during DNA extraction.

Materials and Methods

During the period from March to April 2019, a total of 85 milk samples were aseptically collected from clinically confirmed mastitis cows from a herd of nine thousand Holstein milking cows. Cows were milked three times a day, with a daily average milk volume of 37 liters. The milk line has The Cleaning in Place System (CIP) consists of three steps: start with normal water rinsing, followed by soda at 65–85 °C for 7–10 minutes, and again, normal water for 10 minutes, then wash with nitric acid for 5–10 minutes at 40–60 °C, followed by normal water rinsing, and sanitization with VT5-paracetic acid at 35–55 °C.

Ten ml of milk from the affected quarter were collected in a sterile plastic tube and then placed in ice boxes at 5°C. All collected samples were immediately transferred to the laboratory for DNA extraction done by using the Mag MAX™ CORE Nucleic Acid Purification KIT and King Fisher™ Duo Prime Purification System as described in the Vet MAX™ Masti-Type Kit workflow summary. Fifty µl of Mag Max CORE Mastitis Panbacteria Solution was added to 200 µl of milk and mixed for 5 minutes at room temperature after pipetting up and down several times. Ten µl of Mag Max CORE proteinase K (20 mg/ml) were added, and then the automated extraction protocol was run for 37 minutes.

Launch script "Mag MAX_ CORE_ DUO_ Mastitis" after loading the plate into the instrument, and the script paused after approximately 8 minutes. A deep well plate had been taken out of the instrument, and 720 µl of lysis-bending-bead-mix (Mag Max tm core lysis solution 350 µl + Mag Max tm core binding solution 350 µl + Mag Max tm core magnetic beads 20 µl) was added to each well after vortex. To digest the milk samples, the sample plate was loaded back onto the instrument and the run continued.

The lactation number, frequency of mastitis, days in milk, and gestation period were collected from the cow records.

Statistical analysis

The generated data from a cross-sectional study are subjected to a chi-square test. The data was analyzed using the SPSS program.

Results and Discussion

A total of 32 samples out of 85 extracted DNA samples had magnetic beads carried over. Two samples that had magnetic beads carried over showed a sticky nature, and the supernatant DNA couldn't be collected (table 1).

Number of lactations

The average number of lactations is 3.48. According to the number of lactations, samples were divided into two groups: the first group is up to three lactations with a total of 43 samples, and the second group is more than three lactations with a total of 42 samples (table 2).

In detail, the first group carried over samples are 14 with a percentage of 34.9%, while the normal extracted samples are 28 with a percentage of 65.1%. On the other hand, the lactation group has 17 carried-over samples with a percentage of 40.5%, and the normal extracted samples have 25 with a percentage of 59.5%.

According to the Chi square statistical analysis result (Chi square = 0.28, P = 0.60), there is no significant relationship found between lactation and magnetic beads carried over (Table.2).

Frequency of mastitis

The average frequency of mastitis per lactation was 2.54. According to the frequency of mastitis samples, they were divided into two groups: the first group includes cows that get mastitis at least twice (N = 54), and the second group includes cows that are infected more than twice per lactation (N = 29).

In the first group, magnetic beads carried over samples are 24 with a percentage of 44.4% and 30 normal extracted samples with a percentage of 55.6%. In comparison, in the second group, which has 8 carried over samples with a percentage of 27.6% and normal extracted 21 samples with a percentage of 72.4%, statistically there is no significant relation found between frequency of mastitis and magnetic beads carried over according to the Chi square statistical analysis result (Chi square = 2.26, *P* = 0.13). (table 3)

Days in Milk (DIM)

In the same way, days in milk samples are divided into three groups: the first one is equal to or less than 100 days, which are 25 (29.4%) samples in total; the second one includes 101–200 days in milk, which are 29 (34.1%) samples in total; and the third one includes more than 200 days in milk, which are 31 (36.5%) samples in total.

In the first group, there are 10 carried over samples with a percentage of 40.0% and 15 normal extracted samples with a percentage of 60.0%, while in the second group, which includes cows with 100–200 days in milk, there are 9 carried over samples with a percentage of 31.0% and 20 normal samples with a percentage of 69.0%.

Similarly, the third group, which includes cows with more than 200 days in milk, shows 13 carried-over samples with a percentage of 41.9%, and normal extracted ones are 18 with a percentage of 58.1%.

The statistical analysis showed that there is no significant (P = 0.66) relation between days in milk and magnetic beads carried over, according to the Chi square statistical analysis result (Chi square = 0.84, P = 0.66) (Table 4).

Gestation period (GP)

As well as the other parameters that have been classified, the gestation period is also divided into three groups: the first group, which has equal to or less than 100 days of pregnancy and includes 13 samples; the second group, which has more than 100 days of pregnancy and also includes 13 samples; and the third group, which includes 59 samples.

The first group to have magnetic beads carried over samples is composed of 2 with a percentage of 15.4% and 11 normal extracted samples with a percentage of 84.6%, whereas the second group, which has more than 100 days of gestation, has 7 carried over samples with a percentage of 53.8% and 6 normal extracted samples with a percentage of 46.2%, differing from the third empty cows group that has 23 carried over samples with a percentage of 39.0% and 36 normal extracted samples with a percentage of 61.0%.

Table.1 Sample Distribution

Description	Number of Samples	%
Normal extracted samples	53	62.4
Magnetic beads carried over the sample.	32	37.6
Non-Extracted	2	6.3

Table.2 The relation between magnetic beads carried over and lactations (Chi square = 0.28, P = 0.60)

	lactations	Description	Carry Over	Normal	Total
lactations	up to 3 lactations	Count	14	28	43
		% within lactations	34.9	65.1	100.0
	> 3 lactations	Count	17	25	42
		% within lactations	40.5	59.5	100.0
Total		Count	32	53	85
		% within lactations	37.6	62.4	100.0

Table.3 The relation between magnetic beads carried over and frequency of mastitis (Chi square = 2.26, *P* = 0.13)

	Frequency of mastitis	Description	Carry Over	Normal	Total
Frequency of mastitis	≤ 2	Count	24	30	54
		% within Infect times	44.4	55.6	100.0
	>2	Count	8	21	29
		% within Infect times	27.6	72.4	100.0
Total		Count	32	51	83
		% within Infect times	36.6	61.4	100.0

Table.4 The relation between magnetic beads carried over and Days in Milk (Chi square = 0.84, P = 0.66)

	Days in Milk	Description	Carry Over	Normal	Total
Days in milk	≤ 100 days	Count	10	15	25
		% within days in milk	40.0	60.0	100.0
	101-200 day	Count	9	20	29
		% within days in milk	31.0	69.0	100.0
	> 200 days	Count	13	18	31
		% within days in milk	41.9	58.1	100.0
Total		Count	32	53	85
		% within days in milk	37.6	62.4	100.0

Table.5 The relation between magnetic beads carried over and gestation period (Chi square = 4.24, *P* = 0.12)

	Gestation Period	Description	Carry Over	Normal	Total
Gestation period	Less than 100 days	Count	2	11	13
		% within gestation	15.4	84.6	100.0
	More than 100 days	Count	7	6	13
		% within gestation	53.8	46.2	100.0
	Empty	Count	23	36	59
		% within gestation	39.0	61.0	100.0
Total		Count	32	53	85
		% within	37.6	62.4	100.0

Statistically, there is no significant (P = 0.12) relationship found between gestation period and magnetic beads carried over, according to the Chi square statistical analysis result (Chi square = 4.24, P = 0.12) (Table 5).

Statistical analysis showed that there is no significant effect due to the number of lactations, frequency of mastitis, days in milk, and gestation period on magnetic beads carried over. This result reflects that milk composition in relation to the above-described parameters has no effect on the extraction procedure, despite the well-known relationship between lactation, frequency of mastitis, days in milk, and gestation period in milk composition (Rook and Campling, 1964) and (Harjanti and Sambodho, 2019).

On the other hand, the result comes along with the state of (Dairawan and Shetty, 2020) that magnetic bead extraction is a physical extraction process, similar to the classification of DNA extraction procedures that classifies extraction processes as chemical or mechanically processed methods, as mentioned by Ali *et al.,* (2017).

Besides that, it showed the importance of the extraction process and reflected its effect on DNA products, as mentioned by (Dahm, 2005). Furthermore, the result highlighted the importance of DNA extraction method selection procedures to ensure the quality and quantity of the extracted DNA when carrying out any downstream applications (Chacon Cortes and Griffiths, 2014).

Along with that, the experiment showed that the magnetic beads extraction method is in line with (Chacon Cortes and Griffiths, 2014) about the factors that should be carefully considered when optimizing a DNA extraction method, which include time, cost, potential toxicities, yield, laboratory equipment and expertise requirements, as well as the required sample amount.
Bo Li *et al.,* (2017) noted that there are some factors that influence nucleic acid purification, such as particle size of magnetic beads, surface groups of magnetic beads, and lysis time.

These factors need to be checked in further studies. Similarly, Sonja (2006) reported that particles with a diameter larger than 1 μm can be easily separated using simple magnetic separators, while separation of smaller particles (magnetic colloids with a particle size ranging from ten to hundreds of nanometers) may require the use of high-gradient magnetic separators.

Hence, there is no single procedure that is applicable to all contexts of DNA extraction (Dairawan and Shetty, 2020). The use of magnetic beads in the separation and purification of nucleic acids surely led to a technological revolution in biological research for its high-quality nucleic acid, low risk of cross-contamination (Abd El Aal *et al.,* 2010), safe time and effort, and human health in comparison with conventional methods, which has significantly contributed to developing simpler DNA extraction (Dairawan and Shetty, 2020), quick and efficient purification product (Sonja, 2006), which is noted very clearly in this work.

We concluded that there is no significant impact of lactation, frequency of mastitis, days in milk, or gestation period on magnetic beads carried over during DNA extraction when using the magnetic separation technique.

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Author Contribution

Mohammed Farah Mohammed Abdelhamed: Investigation, formal analysis, writing—original draft. Rania Hassan Zaid: Validation, methodology, writing—reviewing. Mohamed T. Ibrahim:—Formal analysis, writing—review and editing. Ahmed Eltigani Almansoori: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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