



Evaluating and improving the beef quality of freshly dressed cattle carcasses at old – fashioned abattoirs of both Dakahlia and Damietta provinces.

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ARTICLE HISTORY

Received: February 6, 2023

Revised: May 13, 2023

Accepted: May 23, 2023

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ABSTRACT

Objective: To evaluate the quality of beef taken from the freshly dressed cattle carcasses at old – fashioned abattoirs of both Dakahlia and Damietta provinces.

Samples: Sum of 144 swab samples obtained from the external surfaces of freshly dressed 18 healthy cattle carcasses (8 samples each – 4 ones before decontamination trial besides 4 after this trial).

Procedure: All samples were collected from 4 different sites (round- flank- shoulder- neck) of every dressed carcass on one side (right or left) – before any decontamination treatment as well as further 4 samples taken from the same carcass after being decontaminated by hose-spraying of whole carcass with tap water for one minute followed by wiping it entirely with sterile cotton cloth on the other corresponding sites (right or left). All samples were taken at abattoirs then transferred in ice box and investigated bacteriologically for enumerating aerobic mesophilic bacteria, enterobacteriaceae, moulds and yeasts – at the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, wherein the microbiological examination was done without delay.

Results: Plates of count agar medium showed the intensities of aerobic mesophilic bacteria – before a sole decontamination treatment as mean levels of 1.27×10^8 CFU/cm² on rounds, 1.2×10^8 CFU/cm² on flanks, 1.37×10^8 CFU/cm² on shoulders and 1.45×10^8 CFU/cm² on necks, whereas such levels after a sole decontamination treatment were 7.63×10^7 , 7.22×10^7 , 9.01×10^7 and 8.91×10^7 on the same corresponding sites of these carcasses with reduction percentages 40%, 40%, 35% and 39%, respectively. Plates of violet, red bile glucose agar medium showed the mean populations as CFU/cm² of enterobacteriaceae as 1.1×10^5 , 1.03×10^5 , 9.11×10^4 , 1.25×10^5 on the identical sites before decontamination in addition to 7.62×10^4 , 6.33×10^4 , 5.72×10^4 , 8.67×10^4 on the same surfaces after decontamination treatment with reduction percentages 31%, 39%, 38% and 31%, consecutively. On the other hand, dichloran rose bengal chloramphenicol agar plates revealed the aforementioned levels /cm² for both moulds & yeasts as 1.08×10^2 & 4.13×10 on rounds, 7.19×10 & 3.65×10 on flanks, 7.7×10 & 3.76×10 on shoulders and 1.06×10^2 & 4.71×10 on necks – before decontamination treatment plus 5.92×10 & 1.74×10 on rounds, 3.39×10 & 1.36×10 on flanks, 3.49×10 & 1.31×10 on shoulders and 2.12×10 & 4.71×10 on necks after decontamination treatment, with a resultant reduction percentages (45% & 58%) on the surfaces of rounds, (53% & 63%) on flanks, (51% & 66%) on shoulders and (48 % & 55%) on necks, respectively.

Conclusion and clinical relevance: Although the exclusive decontamination treatment applied in the present study – could remove 35-40 % of the aerobic mesophilic bacteria, 31-39 % of enterobacteriaceae, 45-53 % of moulds, plus 55-66 % of yeasts onto the surfaces of tested cattle carcasses, our results obtained in this work indicate the need to replace the old – fashioned abattoirs by the modern ones that possessing the hygienic slaughtering and dressing operations. Also, the recovery of huge numbers of microbes contaminating these carcasses predict the presence of pathogens among them that becoming hazardous for public health.

Keywords: Old fashioned abattoirs, dressed carcasses, decontamination treatment, Egypt.

1. INTRODUCTION

Beef, is a main component of well-balanced, healthy and favourite diet for all Egyptian people due to its higher nutritional value. Beef is a main source of protein, vitamin B complex including vitamin B12 (cyanocobalamin), iron, copper, zinc and selenium for eaters [1]. Freshly dressed cattle carcasses, particularly at our Egyptian unhygienic old-fashioned abattoirs, harboring high intensities of microbes (bacteria, moulds, yeasts) onto their surfaces, coming from diverse sources: dirtiness found on/in floor of slaughter hall, hides of slaughtered cattle, hands and clothes of the workers, as well as contents of accidentally injured rumens and intestines constitute the major sources of such

contamination acquired onto the carcasses' surfaces[2].

Beef is nutritious not only for human beings but also for microbial contaminants found onto their carcasses, as it provide all requirements necessary for growth and multiplication of these adherent microbes and implicate in beef spoilage and foodborne illnesses in humans [3]. For providing the Egyptian people with high quality beef, with a minimal microbial contamination, it is imperative to eliminate most of unavoidable numbers of microbial contaminants, found on cattle and buffalo carcasses, slaughtered and dressed at our exclusive old fashioned

abattoirs [4]. Therefore, the present study is planned for quantifying as well as reducing those spoilage and hazardous microbial contaminants adherent onto the cattle carcasses.

2. MATERIALS AND METHODS

2.1. Collection and preparation of samples

The outer surfaces of different sites (round-flank-shoulder-neck) of a total 18 cattle carcasses, slaughtered and dressed at some traditional abattoirs of both Dakahlia and Damietta provinces, were swabbed and tested bacteriologically for estimating aerobic plate counts, enterobacteriaceae counts besides every of moulds and yeasts counts before and after a sole decontamination treatment. All sampled carcasses were derived from excessively dirty animals, and slaughtered then dressed under the unhygienic conditions, where they had been slaughtered by "Halal" method after being lain on a dirty floor, through severing both carotid arteries and jugular veins, trachea and oesophagus, left for bleeding, followed by floor-dressing. The whole samples were taken twice, the first ones were done immediately after skinning and evisceration without rinsing or any decontamination treatment, and the second samples were received from the corresponding another sites of the other carcasses' sides (left or right) after a continuous hose-spraying of the whole carcass with a pressurized tap water for one minute followed by wiping of the whole carcass with a sterile cotton cloth 1.5 x1m.

A limited area (20 cm²) over each surface sample inside a sterilized metal template (4x5 cm) was rubbed repeatedly and successively by three sterilized gauze-cotton swabs (having a size of about 3.5x1.5 cm and attached to flat wooden stick of about 10 cm length), the first swab was moistened by a 0.1% peptone water (the diluent used) while the other two swabs were dry. The 3 swab sticks were broken off below the contaminated handled area into original dilution after thorough homogenization of triplicate swabs. Each swab sample was then marked and subjected to rapid bacteriological examination after preparing decimal (serial) dilutions.

2.2. Decontamination treatment

Hose-spraying with tap water

The whole outer surface of each tested carcass was rinsed, immediately after evisceration, for one minute by a high-pressure spraying of a municipal tap water ejected from the hose after narrowing its nozzle by my fingers, provided that the splashing of neighboring carcasses did not occur.

Sterile wiping cloth

A piece of clean and sterilized cotton cloth having an area of 1.5x1 m, was used by clean hands for wiping carcasses, after being hose-sprayed with a municipal tap water for one minute, up to removal most of visible dirt

from the treated carcass without hand-touching of its surface. Each wiping cloth was sterilized by autoclaving at 121°C for 15 min after being individually wrapped in double layer of aluminum foil. The applied cloth was cleansed then dried after the wiping process, sterilized and used again for decontaminating other carcass.

2.3. Preparation of serial dilutions

A half ml from each of the original dilution (2:1) before and after decontaminating treatment were transferred to a sterile test tube containing 9.5 ml of peptone water (0.1%) for obtaining a dilution of 1:10, from which one ml was transferred to a sterile test tube containing 9 ml of the same diluent to be diluted in a sequential manner preparing a ten fold serial dilutions up to 10⁻⁷, to cover the expected range of samples contamination.

2.4. Bacteriological tests

(1) Aerobic plate counts according to [5]

A tenth ml from each prepared serial dilutions was transferred and evenly spread over a dry surface of duplicated, previously prepared sterile plate count agar medium. The surface of inoculated plate was allowed to dry for 15 minutes before being placed inverted with control plates in the incubator adjusted at 30°C for 2 days. The bacterial colonies in the countable plates (having 25-250 colonies) were enumerated and the total aerobic mesophilic count per cm² of carcass surface was calculated and recorded.

(2) Enterobacteriaceae counts according to [6]

Duplicated sets of sterile Petri dishes were inoculated with 1ml amounts of the chosen range of prepared dilutions. A quantity of about 15 ml of violet red bile glucose agar (oxid CM485B), melted and cooled to 45°C, were added to each inoculated Petri dish, then mixed well. After medium has solidified, 10 ml of the same medium were overlain onto solidified one to ensure anaerobic conditions which suppress the growth of non-fermentative Gram-negative bacteria. Then allowed to be solidified, then incubated "inverted" at 30°C for 2 days. Typical colonies of enterobacteriaceae (round, purple surrounded by precipitation of bile salts in the medium and having 0.5mm or more in diameter) were enumerated in the countable plates (having 25-250 colonies) and the enterobacteriaceae counts per cm² of the examined sample were then calculated and recorded.

(3) Independent mould and yeast counts according to [7]

From the original dilution (2:1), 0.2 ml was evenly spread onto a dry surface every of sterilized duplicate plates of dichloran rose Bengal chloramphenicol agar (oxid CM0727). The inoculated plates as well as the control one were incubated "upright position" at 25°C for 7 days. After the incubation period, the average each of mold and yeast colonies was enumerated over the duplicate plates, and every mold count and yeast count/cm² of the tested surfaces were then calculated and recorded.

2.5. Statistical analysis

The data obtained in this study were statistically analyzed according to the methods described by [8]. The mean value (X) was obtained from the sum of individuals (X) divided on the number of samples (N).

3. RESULTS

Table 1. shows the presence of different microbes in tested swab samples of freshly dressed cattle carcasses at traditional abattoirs, before and after decontamination treatment.

Table (1): Presence of different microbes in tested swab samples of freshly dressed cattle carcasses at traditional abattoirs, before and after decontamination treatment* (n=18)**

Sites of tested surfaces and numbers & percents of contaminated samples	Rounds		Flanks		Shoulders		Necks	
	Before	After	Before	After	Before	After	Before	After
kinds of contaminating microbes								
Aerobic mesophilic bacteria	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)
Enterobacteriaceae	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)
Moulds	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)	18(100%)
Yeasts	18(100%)	14(78%)	17(95%)	13(73%)	18(100%)	12(67%)	18(100%)	15(84%)

*Decontamination treatment was achieved by hose-spraying of whole carcass with tap water for one minute followed by wiping with sterile cotton cloth.

** n= Number of tested swab samples for each site, at every condition (before & after decontamination treatment).

Table 2. shows microbiological quality of freshly dressed cattle carcasses at traditional abattoirs, before and after decontamination treatment.

Table (2): Microbiological quality of freshly dressed cattle carcasses at traditional abattoirs, before and after decontamination treatment*. (n = 18) **

Kind of microbes & statistical analysis	Aerobic mesophilic bacteria			Enterobacteriaceae			Moulds			Yeasts			
	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	
Rounds	Before	6.4×10 ⁷	2.12×10 ⁸	1.27×10 ⁸ ± 0.11×10 ⁸	6×10 ⁴	2×10 ⁵	1.1×10 ⁵ ± 0.103×10 ⁵	3.5×10	25×10	1.08×10 ² ± 0.16×10 ²	2×10	7×10	4.13×10 ± 0.35×10
	After	1.5×10 ⁷	1.69×10 ⁸	7.63×10 ⁷ ± 1.15×10 ⁷	2.1×10 ⁴	1.6×10 ⁵	7.62×10 ⁴ ± 0.9×10 ⁴	1.25×10 ⁰	19.25×10	5.92×10 ± 1.47×10	0	4.75×10	1.74×10 ± 0.34×10
Flanks	Before	7×10 ⁷	1.93×10 ⁸	1.2×10 ⁸ ± 0.09×10 ⁸	6.3×10 ⁴	1.47×10 ⁵	1.03×10 ⁵ ± 0.058×10 ⁵	2.75×10 ⁰	17.5×10	7.19×10 ± 1×10	0	7×10	3.65×10 ± 0.39×10
	After	2×10 ⁷	1.5×10 ⁸	7.22×10 ⁷ ± 1.02×10 ⁷	7×10 ³	1.1×10 ⁵	6.33×10 ⁴ ± 0.67×10 ⁴	1.25×10 ⁰	12.75×10	3.39×10 ± 0.61×10	0	3.25×10	1.36×10 ± 0.27×10
Shoulders	Before	7.6×10 ⁷	2×10 ⁸	1.37×10 ⁸ ± 0.092×10 ⁸	5.9×10 ⁴	1.38×10 ⁵	9.11×10 ⁴ ± 0.64×10 ⁴	3×10	18×10	7.7×10 ± 0.83×10	1.5×10	7.5×10	3.76×10 ± 0.36×10
	After	2.8×10 ⁷	1.8×10 ⁸	9.01×10 ⁷ ± 1.09×10 ⁷	1.8×10 ⁴	1.06×10 ⁵	5.72×10 ⁴ ± 0.65×10 ⁴	1.25×10 ⁰	7.5×10	3.49×10 ± 0.49×10	0	5.75×10	1.31×10 ± 0.35×10
Necks	Before	5.5×10 ⁷	2.06×10 ⁸	1.45×10 ⁸ ± 0.11×10 ⁸	8.2×10 ⁴	1.93×10 ⁵	1.25×10 ⁵ ± 0.08×10 ⁵	3.75×10 ⁰	22.75×10	1.06×10 ² ± 0.14×10 ²	1.75×10	10×10	4.71×10 ± 0.59×10
	After	1×10 ⁷	1.99×10 ⁸	8.91×10 ⁷ ± 1.28×10 ⁷	4.9×10 ⁴	1.35×10 ⁵	8.67×10 ⁴ ± 0.6×10 ⁴	1.75×10 ⁰	14.75×10	5.53×10 ± 0.92×10	0	7.5×10	2.12×10 ± 0.45×10

* Decontamination treatment was achieved by hose-spraying of whole carcass with tap water for one minute followed by wiping

with sterile cotton cloth.

**n= Number of tested swab samples for each site, at every condition.

SE = Standard error of the mean levels Min = minimum Max = maximum

Table 3. shows the reduction percentages of the microbial contaminants on cattle carcasses after decontamination treatment.

Table (3): Reduction percentages of the microbial contaminants on cattle carcasses after decontamination treatment

Types of carcasses & Sites of contaminated surfaces	Cattle carcasses (n=18)**			
	Rounds	Flanks	Shoulders	Necks
Kinds of contaminating microbes				
Aerobic mesophilic bacteria	40 %	40%	35%	39%
Enterobacteriaceae	31%	39%	38%	31%
Moulds	45%	53%	51%	48%
Yeasts	58%	63%	66%	55%

**Decontamination treatment was achieved by Hose-spraying of whole carcass with tap water for one minute followed by wiping with sterile cotton cloth.

*** n= Number of tested swab samples for each site, at every condition.

Table 4. shows the numbers and percents of swab samples on cattle carcasses, contaminated with microbes by populations exceeded the recommended limits - before and after the applied decontamination treatment.

Table (4): Numbers and percents of swab samples on cattle and bufflo carcasses, contaminated with microbes by populations exceeded the recommended limits* - before and after the applied decontamination treatment.**

Types of carcasses and kinds of microbes		Cattle (n=18)***	
Sites of contaminated surfaces before and after decontamination treatment		Aerobic mesophilic bacteria	Enterobacteriaceae
Rounds	Before	18 (100 %)	18 (100 %)
	After	18 (100 %)	18 (100 %)
Flanks	Before	18 (100 %)	18 (100 %)
	After	18 (100 %)	18 (100 %)
Shoulders	Before	18 (100 %)	18 (100 %)
	After	18 (100 %)	18 (100 %)
Necks	Before	18 (100 %)	18 (100 %)
	After	18 (100 %)	18 (100 %)

*Recommended limit for APCs is 106 /cm² according to **ICMSF (1986) & Gracey et al. (1999)** and for enterobacteriaceae is 3.2x10²/cm² established by European Commission decision 2001/471/EC reported by Anonymous (2001).

**Decontamination treatment was achieved by Hose-spraying of whole carcass with tap water for one minute followed by wiping with sterile cotton cloth.

*** n= Number of tested swab samples for each site, at every condition.

****Unfortunately, we could not find a legal (maximum) limit for fungal contamination levels onto meat / carcasses surfaces.

4. DISCUSSION

Data in arranged [Table 1] reveal contamination of all examined swab samples (100%), taken from outer surface of round, flank, shoulder and neck of cattle carcasses before and after decontamination treatment, with aerobic mesophilic bacteria. These findings agreed with the literature of [9] who emphasized that the transfer of

microfloral contamination from hide and gut to the surface of animals' carcasses during dressing is inevitable thing. Exponential levels of aerobic mesophilic bacteria (aerobic plate counts =APCs) onto swabbed surfaces of 18 cattle carcasses (freshly dressed and sampled at slaughter halls of old-fashioned abattoirs before and after decontamination treatment were estimated as ranges of 6.4x10⁷-2.12x10⁸ CFU/cm² on rounds, 7 x10⁷-1.93x10⁸ CFU/cm² on flanks,

7.6x10⁷ – 2 x10⁸ CFU/cm² on shoulders, 5.5 x10⁷-2.06 x10⁸ CFU/cm² on necks with mean ± SE values 1.27x10⁸ ±0.11x10⁸ , 1.2x10⁸ ± 0.09x10⁸, 1.37x10⁸ ± 0.092x10⁸ and 1.45x10⁸ ± 0.11x10⁸ CFU/cm², consecutively. The abattoir decontamination trial (hose-spraying of whole carcass with tap water for one minute followed by wiping with sterile cotton cloth) could reduce these intensities to 1.5x10⁷ -1.69x10⁸ CFU/cm² on rounds, 2x10⁷ -1.5x10⁸ CFU/cm² on flanks, 2.8x10⁷ -1.8 x10⁸ CFU/cm² on shoulders, 1x10⁷ -1.99 x10⁸ CFU/cm² on necks with mean ± SE values 7.63x10⁷±1.15x10⁷, 7.22x10⁷ ± 1.02x10⁷, 9.01x 10⁷ ± 1.09x 10⁷ and 8.91x 10⁷ ± 1.28x10⁷ CFU/cm², consecutively [Table 2]. Our results of APCs agree with [10, 11]. Lower APCs on identical surfaces on cattle carcasses were obtained by [12, 13, 14, 15].

Enterobacteriaceae organisms can be determined onto the tested surfaces of all carcasses sites before and after decontamination treatment (100%), indicating the contamination of all of them with intestinal/ faecal material and agreed with [16, 17] who emphasized that contamination of beef carcasses during dehiding is inevitable due to the passage of knife through faecal material on the hide. Moreover, fault evisceration such as puncture of the visceral contents plays a major role in carcasses contamination [Table 1].

Tabulated findings - obtained in the present work - exhibited the mean ± SE levels of the enterobacteriaceae organisms as 1.1x10⁵ ±0.103x10⁵ - 7.62x10⁴ ±0.9x10⁴ on rounds, 1.03x10⁵ ±0.058x10⁵- 6.33x10⁴ ±0.67x10⁴ on flanks, 9.11x10⁴±0.64x10⁴- 5.72x10⁴ ±0.65x10⁴ on shoulders and 1.25x10⁵ ± 0.08x10⁵- 8.67x10⁴ ±0.6x10⁴CFU /cm² on necks surfaces of cattle carcasses before and after decontamination treatment, respectively, with ranges (minima - maxima) as 6 x10⁴ -2 x10⁵ on rounds, 6.3 x10⁴ -1.47 x 10⁵ on flanks, 5.9 x 10⁴ – 1.38 x10⁵ on shoulders and 8.2 x 10⁴ – 1.93 x 10⁵ CFU/cm² on necks surfaces of cattle carcasses before decontamination treatment. The abattoir decontamination trial could reduce these intensities to 2.1x 10⁴ - 1.6x10⁵ CFU/cm² on rounds, 7x10³ -1.1x10⁵CFU/cm² on flanks, 1.8 x10⁴ -1.06 x10⁵ CFU/cm² on shoulders, 4.9x10⁴ -1.35 x10⁵ CFU/cm² on necks [Table 2]. By comparison the enterobacteriaceae counts - calculated in this work with identical counts obtained by other researchers, nearly similar counts were calculated on cattle carcasses surfaces by [18, 19]. lower intensities of these microorganisms were enumerated on identical carcasses by [20, 21].

Concerning the independent contamination (moulds & yeasts) onto the surfaces of evaluated carcasses, plates of dichloran rose bengal chloramphenicol agar showed the ranges (minima - maxima) and mean ± SE levels of moulds onto all (100%) cattle carcasses [Table 1] before and after decontamination treatment as 3.5x10-25x10 (1.08x10² ±0.16x10²) CFU/cm² on rounds, 2.75x10 -17.5x10 (7.19x10 ±1x10) CFU/cm² on flanks, 3x10 -18x10 (7.7x10 ± 0.83x10) on shoulders, 3.75x10 -22.75x10 (1.06x10² ± 0.14x10²) on necks, After decontamination trial as 1.25x10-19.25x10 (5.92x10 ±1.47x10) CFU/cm² on rounds, 1.25x10-

12.75x10 (3.39x10 ± 0.61x10) CFU/cm² on flanks, 1.25x10-7.5x10 (3.49x10 ± 0.49x10) on shoulders, 1.75x10-14.75x10 (5.53x10 ±0.92x10) on necks [Table 2]. Nearly equal mould counts onto cattle carcasses were detected by [22, 23, 24]. While, higher mould populations were found on identical surfaces by [25].

Yeasts populations as fungal contaminants were also recovered from the evaluated carcasses surfaces 18(100%)-14(78%) on rounds, 17(95%)-13(73%) on flanks, 18(100%) -12(67%) on shoulders and 18(100%)-15(84%) on necks surfaces of cattle carcasses before and after decontamination treatment, respectively, [Table 1], then enumerated by using dichloran rose bengal chloramphenicol agar plates as mean ± SE levels and ranges (minima - maxima) before decontamination treatment 4.13x10 ±0.35x10 (2x10-7x10 CFU /cm² on rounds; 3.65x10 ±0.39x10 (0-7x10 CFU /cm² on flanks; 3.76x10 ±0.36x10 (1.5x10-7.5x10) CFU /cm² on shoulders and 4.71x10 ± 0.59x10 (1.75x10-10x10)CFU /cm² on necks surfaces of cattle carcasses, meanwhile, these values on cattle carcasses after decontamination treatment were 1.74x10 ±0.34x10 (0-4.75x10) CFU /cm² on rounds; 1.36x10 ±0.27x10 (0-3.25x10)CFU /cm² on flanks; 1.31x10 ±0.35x10 (0-5.75x10) CFU /cm² on shoulders and 2.12x10 ±0.45x10 (0-7.5x10)CFU /cm² on necks surfaces [Table 2]. Approximately similar yeasts counts were estimated on beef by [26] , Meanwhile, higher yeast counts onto identical surfaces by[27, 28].

In regard to the decontaminating effects of my trial (hose-spraying of whole carcass with tap water for one minute followed by wiping with sterile cotton cloth), the reduction percentage of APCs was (40%) on the surfaces of both rounds & flanks, (35%) on shoulders and (39%) on necks, for enterobacteriaceae counts was (39%) on the surfaces of flanks, (38%) on shoulders, (31%) on both rounds and necks, for moulds and yeasts was (45% & 58%) on the surfaces of rounds, (53% & 63%) on flanks, (51% & 66%) on shoulders and (48 % & 55%) on necks, respectively [Table 3].

Recommended limit for APCs is 106 /cm² according to [29, 30] and for enterobacteriaceae is 3.2x10²/cm² established by European Commission decision 2001/471/EC reported by [31], data arranged in [Table 4] declared that all tested carcasses contaminated with microbes by populations exceeded this recommended limits - before and after the applied decontamination treatment . Unfortunately, we could not find a legal (maximum) limit for mould contamination levels onto meat / carcasses surfaces, therefore, we could not judge the surveyed carcasses - from a mycological viewpoint - and did not categorize their grades accordingly.

Acknowledgement

This work has received no financial support

Conflict of interest

There is no conflict of interest.

Research Ethics committee permission

The research was conducted according to standards of Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Authors' Contributions:

Asmaa Zaghloul performed the lab work and wrote the first draft, Hanan Zaher designed the study, management and coordination responsibility for the research activity planning and execution, reviewing and editing the article. Mohammed Elgazzar revised the final revision. All authors have read and approved the final version of the manuscript for publication.

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