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Effect of Esophageal Sealing and Anal Closure on Microbial Load of Cattle Carcasses

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ABSTRACT

A total of 100 swabs, 50 swabs were taken from non closed esophageal and anal opening cattle carcasses and other 50 swabs taken from closed esophagus and anal opening cattle carcasses, were collected randomly from different places of cattle carcasses (neck, chest, abdomen and pelvic regions) in an moderate abattoir at Elbehaira governorate (abohomos abattoir) were they examined bacteriologically for *total aerobic bacterial, psychrophilic bacterial, Enterobacteriaceae, Enterococcal, Staphylococcal* and *Mould and Yeast*. Microbiological investigations of non closed esophageal and anal opening cattle carcasses revealed that the mean value of *total aerobic bacterial count, psychrophilic bacterial count, Enterobacteriaceae count, Enterococcal count, Staphylococcal count, Mould and Yeast count* were $63.06\times10^5 \pm 2.23 \times10^5$, $60.04 \times10^3\pm1.57\times10^3$, $65.64\times10^4\pm1.72\times10^4$, $58.59\times10^3\pm1.92\times10^3$, $12.32\times10^3\pm0.50\times10^3$ and $7.69\times10^4\pm0.67\times10^4$ cfc/g; respectively. While Microbiological investigations of closed esophageal and anal opening of cattle carcasses was $28.30\times10^4\pm0.63\times 10^4$, $28.78 \times10^2\pm0.70\times10^2$, $28.75\times10^3\pm0.81\times10^3$, $33.25 \times10^2\pm 0.66\times10^2$, $7.58 \times10^2 \pm0.43\times10^2$ and $3.23\times10^3\pm0.26\times10^3$ cfc/g; respectively. The present study indicated significance reduction in all microbiological counts on closed esophageal and anal opening cattle carcasses samples than on non-closed esophageal and anal opening cattle carcasses samples.

INTRODUCTION

Due to the frequent increase in human population and the great progress in many aspects of our life which is associated with great demand of animal protein, recently due to the crises of avian influenza which was widely spread all over the world, the demand of people for red meat was relatively increased as protein source. The meat is consider as an essential food and easily digested, tasty and also it has enough amount of vitamins and mineral. Carcasses contamination does occur during the different stages of the slaughtering process, the beef carcass surfaces are readily subjected to various sources of contamination mainly from hides, dust, water, any inedible material derived in the abattoir in addition to hands and clothes of the workers and the important source of contamination is gastrointestinal tract which include the digesta which contain high number of bacteria **(Gill et al. 1978)** and faces.

Magdy (1995) recorded that the mean values of total bacterial count, Enterobacteriaceae count, Enterococci count, Staphylococcal count, Mould and Yeast count were $1.3 \times 10^5 \pm 3.2 \times 10^4$, $1.6 \times10^3 \pm 3.2 \times10^2$, $6.6 \times 10^2 \pm1.2 \times 10^2$, $6.7 \times10^2 \pm 3.1\times 10^2$, $4.8 \times10^2 \pm 1.0\times10^2$ and $7.1 \times10^2 \pm 1.8\times10^2$ /Cm^2 cattle carcasses surfaces in modern abattoirs, respectively. While, such counts in traditional abattoirs were $1.5\times 10^6 \pm 3.1 \times 10^5$, $1.3 \times10^4 \pm1.9\times 10^3$, $7.1\times 10^3\pm1.5 \times 10^3$, $5.1 \times10^3 \pm 1.2 \times 10^3$, $4.8 \times 10^3 \pm 7.1\times 10^2$ and $3.4\times 10^3 \pm 1.7 \times 10^2$ /Cm^2 of cattle carcasses surfaces, respectively.

Psychrophile bacteria are characterized by fat cell membranes, chemical resistant cooling produced by extremely icy and often produce proteins "antifreeze" to protect the liquid from the interior and their DNA when the average water temperature reaches the frost point **(Siegel, L.J. 2001).**

Calicioglu et al. (1999) recorded that the initial numbers of Enterococci averaged 3.34 log 10 cfc/cm2 (3.07 to 3.79 log 10 cfc/cm^2) using Kanamycin aesculin azide agar. They also stated that the Enterococci may be useful as an indicator of fecal contamination.

Eid (1999) examined bacteriologically 50 samples of precooked meat taken from different butcher shops at Damanhour city and found that the total bacterial count, total Enterobacteriaceae count and Staphlylococci count were $5.8 \times 10^5 \pm 1.1 \times 10^4$, $3.2 \times 10^4 \pm 9.8 \times 10^3$ and $1.4 \times 10^4 \pm 3.1 \times 10^3$ cfc/g of the samples, respectively.

Murray et al. (2001) examined samples of beef carcasses at 22 and 37 ^{0}C and found that 63 % of samples had yeasts that grow at 22 ^{0}C, while 35 % were positive at 37 ^{0}C. Molds were found in less than 4 % of samples.

MATERIAL AND METHODS

Materials:

Samples:

A total of 100 swabs, 50 swabs taken from non closed esophagus and anal opening of cattle carcasses and other 50 swabs taken from closed esophagus and anal opening of cattle carcasses, were collected randomly from different places of cattle carcasses (shoulder, neck, abdomen and pelvic region) in an moderate abattoir at Elbehaira governorate (abohomos abattoir).

Methods:

Swabbing techniques:

a) Preparation of tampons: Tampons were prepared from 3×6 cm cotton strips, about 150 mg weight their edges were folded inwards so that the width of strip was 2 cm. the tampons were compactly rolled up by hand to take their final shape (drum shape of 1 cm in diameter and 2 cm in length), Proved in a screw capped jar and autoclaved at 121 ^{0}C for 20 minutes.

b) Preparation of template: A template was made from stainless steel having 100 cm^2 (10 ×10) exposed area for sampling. The template was sterilized by using ethyl alcohol and direct flame between each sampling process.

c) Preparation of rinsing fluid peptone water 0.1%) used as a rinsing fluid **(Straka and stokes, 1957).**

Techniques:

For counting the different types of bacteria an area of 100 cm^2 from two examined site of each carcasses swabbed by using forceps, tampon and template as follows. The forceps and tampon were sterilized by immersion in ethyl alcohol and flaming. The template which already become cool was placed firmly against the subcutaneous fascia to limit the examined area by using the sterilized forceps. A sterile tampon was drawn from the tampon jar moistened in the rinsing fluid bottles (peptone water 1 %) pressed against its wall in order to squeeze at the excessive fluid then rolled over the limited surface area of the carcass inside the template opening, rolled in one direction and perpendicularly to such direction to smear completely the required area.

Finally, the used tampon was replaced individually into a sterile screw capped bottle containing 10 ml peptone water 0.1 % representing one of each carcass. The same procedure was repeated with the other carcasses. The screw capped bottles containing swabs were put into ice bags and transferred directly to the

laboratory for bacteria, mould and yeast count.

Preparation of serial dilution:

At the laboratory, the screw capped bottles having the rinsing fluid and tampons were shaken well by using a test tube shaker one ml of the original solution was transferred aseptically with sterile pipette to a test tube containing 9 ml of sterile peptone water. Ten fold serial dilutions up to 10-6 were prepared.

Bacteriological examination:

1. Total aerobic bacterial count:

The number of viable bacteria was done by using the stander pour plating method according to **Cruickshank et al. (1975).**

2. Total psychrophilic bacterial count:

The number of viable bacteria was done by using the stander pour plating method according to **Cruickshank et al. (1972).**

3. Total Enterobacteriaceae count:

The technique recommended by **ICMSF (1978).**

4. Enterococci count according to (mossel et al., 1978 and ICMSF, 1978):

5. Total Staphylococcal count:

Manitol salt agar medium used for enumeration of Staphylococci **(ICMSF, 1978)**.

6. Total mould and yeast count:

Total mould and yeast count was done using sabarouds dextrose agar medium **(Cruickshank et al. 1975)** supplemented with cholormphenicol and chlortetracycline (100 mg of each) as described by **Koburger (1970).**

Statistical analysis

The analysis of variance for the obtained data was performed using Statistical Analysis System **(SAS, 2004)** software to assess significant differences.

RESULTS

Table (1): Statistical analytical results of total aerobic bacterial count (TAB) cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
non closed esophageal and anal opening	28×10^5	80×10^5	$63.06 \times 10^5 \pm 2.23 \times 10^5$ a
closed esophagus and anal opening	19×10^4	36×10^4	$28.30 \times 10^4 \pm 0.63 \times 10^4$ b

Means bearing different superscripts are significant at ($p<0.001$).

Table (2): Statistical analytical results of total Psychrophilic bacterial count cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
Non closed esophageal and anal opening	38×10^3	85×10^3	$60.04 \times 10^3 \pm 1.57 \times 10^3$ a
Closed esophagus and anal opening	18×10^2	39×10^2	$28.78 \times 10^2 \pm 0.70 \times 10^2$ b

Means bearing different superscripts are significant at ($p<0.001$).

Table (3): Statistical analytical results of total Enterobacteriaceae count cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
Non closed esophageal and anal opening	35×10^4	88×10^4	$65.64 \times 10^4 \pm 1.72 \times 10^{4\ a}$
Closed esophagus and anal opening	18×10^3	41×10^3	$28.75 \times 10^3 \pm 0.81 \times 10^{3\ b}$

Means bearing different superscripts are significant at ($p<0.001$).

Table (4): Statistical analytical results of total Enterococcal count cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
Non closed esophageal and anal opening	34×10^3	83×10^3	$58.59 \times 10^3 \pm 1.92 \times 10^{3\ a}$
Closed esophagus and anal opening	25×10^2	42×10^2	$33.25 \times 10^2 \pm 0.66 \times 10^{2\ b}$

Means bearing different superscripts are significant at ($p<0.001$).

Table (5): Statistical analytical results of total Staphylococcal count cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
Non closed esophageal and anal opening	7×10^3	28×10^3	$12.32 \times 10^3 \pm 0.50 \times 10^{3\ a}$
Closed esophagus and anal opening	1×10^2	13×10^2	$7.58 \times 10^2 \pm 0.43 \times 10^{2\ b}$

Means bearing different superscripts are significant at ($p<0.001$).

Table (6): Statistical analytical results of Mould and yeast count cfu/g in examined cattle carcasses:

Group	Min	Max	Mean ± SEM
Non closed esophageal and anal opening	5×10^4	12×10^4	$7.69x\ 10^4 \pm 0.67x\ 10^{4\ a}$
Closed esophagus and anal opening	1×10^3	9×10^3	$3.23x\ 10^3 \pm 0.26 \times 10^{3\ b}$

Means bearing different superscripts are significant at ($p<0.001$).

DISCUSSION

The contamination of meat of cattle carcasses has been reported to have a significant effect on the shelf life of meat of cattle carcasses. Moreover, the contamination of meat of cattle carcasses can be directly correlated with the keeping quality of meat. Food hygienists have been attempting to detect and quantify microorganisms of meat of cattle carcasses samples.

1-Total Aerobic bacterial count:

Total bacterial count is described as an important parameter for the sanitation

and hygienic importance of meat carcasses it is evident from table (1) that the total *aerobic* bacterial count cfu/g of non closed oesophagus and anal opening of cattle carcasses ranged from 28 $\times 10^5$ to 80 $\times 10^5$ with the mean value 63.06 $\times 10^5 \pm 2.23 \times 10^5$, while the total *aerobic* bacterial count cfc/g of closed oesophagus and anal opening of cattle carcasses ranged from 19 $\times 10^4$ to 36 $\times 10^4$ with the mean value 28.30 $\times 10^4 \pm 0.63 \times 10^4$, so there is significant difference between the mean values of non closed oesophages and anal opening of cattle carcasses and the mean values of closed oesophages and anal opening of cattle carcasses at $P < 0.001$.

The thigh and both sides of abdomen of alive animals may be heavily soiled with wet faecal matter which come in contact with the carcass either directly or indirectly during processing a wet soiling faecal matter may increase the contamination level by 5-10 folds than dry one **(Patterson and Gibbs, 1978).**

Modernization of old abattoirs in Egypt and the use of the new technology for slaughtering and preparation of carcasses were extensively applied with the goal of lowering microbial load **(Lazarus et al. 1977)** and to minimize the cross contamination and infection. The production of wholesome meat of high keeping quality constitutes one of the main tasks of meat hygienists **(Zlamalova et al. 1978).**

2-Psychrophilic count:

Total *psychrophilic* bacterial count considers as an important indicator for keeping quality of meat through its results. We can detect the predicting period of meat storage and spoilage time occurrence. It used for identifying critical processing steps with respect to the extent of contamination of carcasses by *psychrophilic* spoilage bacteria **(Gustavsson and Borch 1993).**

It is evident from table (2) that the total *psychrophilic* bacterial count cfc/g of non closed oesophagus and anal opening of cattle carcasses ranged from 38 $\times 10^3$ to 85 $\times 10^3$ with the mean value 60.04 $\times 10^3 \pm 1.57 \times 10^3$, while the total *psychrophilic* bacterial count cfc/g of closed esophagus and anal opening of cattle carcasses ranged from 18 $\times 10^2$ to 39 $\times 10^2$ with the mean value 28.78 $\times 10^2 \pm 0.70 \times 10^2$, so there is significant difference between the mean values of non closed esophagus and anal opening of cattle carcasses and the mean values of closed esophagus and anal opening of cattle carcasses at $P < 0.001$.

Spoilage or reduced keeping time of fresh meat can be generally attributed to the presence of very large number of bacteria, these were mainly identified as members of *psychrophilic* bacteria and certain other organisms capable of growing at 0 ^{0}C **(Mousa et al. 1988)** the importance of such types of microorganism is very often due to ubiquitous distribution in the atmosphere in which the meat is handled and stored.

3-Enterobacteriaceae count:

Enterobacteriaceae detection may prove to be useful for the simultaneously examining a variety of foods for their sanitary quality and / or the presence of pathogenic organisms. Members of the family *Enterobacteriaceae* contain many species, which have been reported to cause health hazard for consumer from the public health point of view. Other species are important from the economic point of view as they cause spoilage and deterioration of meat and meat products **(National academy of science, 1985).**

Determination of any or all members of the family *Enterobacteriaceae* as indicators of food sanitary quality has received the attention of more and more

food scientists. The occurrence of *Enterobacteriaceae* shows microbiology and toxigenic bacteria in meat and lead to public health significance **(Mira, 1989).**

It is evident from table (3) that the total *Enterobacteriaceae* count cfc/g of non closed esophagus and anal opening of cattle carcasses ranged from 35 $\times 10^4$ to 88 $\times 10^4$ with the mean value 65.64 $\times 10^4$ ± 1.72 $\times 10^4$, while the total *Enterobacteriaceae* count cfc/g of closed esophagus and anal opening of cattle carcasses ranged from 18 $\times 10^3$ to 41 $\times 10^3$ with the mean value 28.75 $\times 10^3$ ± 0.81× 10^3, so there is highly significant difference between the mean values of non closed esophagus and anal opening of cattle carcasses and the mean values of closed esophagus and anal opening of cattle carcasses at $P < 0.001$.

From the above results we observed that *Enterobacteriaceae* count seems to be high and this is attributed to the contamination from enteric sources and can be used as an index of enteric contamination **(Mercuri and Cox, 1979).**

This confirmed what was reported by **Mira (1989)** that the presence of high *Enterobacteriaceae* count indicated that cattle has been slaughtered, prepared and handled under poor sanitary conditions.

4-Enterococci:

The presence of *Enterococci* is known as an index for faecal contamination. *Enterococci* can induce undesirable changes in meat and meat products and when found in large numbers may be implicated in cases of food poisoning **(Libby, 1975).**

It is evident from table (4) that the total *Enterococcal* count cfc/g of non closed esophagus and anal opening of cattle carcasses ranged from 34 $\times 10^3$ to 83 $\times 10^3$ with the mean value 58.59 $\times 10^3$ ± 1.92 $\times 10^3$, while the total Enterococci count cfc/g of closed esophagus and anal opening of cattle carcasses ranged from 25 $\times 10^2$ to 42 $\times 10^2$ with the mean value 33.25 $\times 10^2$ ± 0.66× 10^2, so there is highly significant difference between the mean values of non closed esophagus and anal opening of cattle carcasses and the mean values of closed esophagus and anal opening of cattle carcasses at $P < 0.001$

Calcioglu et al. (1999) reported that the *Enterococci* may be useful as an indicator of faecal contamination.

5-Staphylococci count:

Staphylococci are commonly found on the skin and in the upper respiratory tract of man and animals and can easily contaminated the carcasses. The presence of *Staphylococci* on carcass surface may be due to contamination during dressing and evisceration in slaughter house, contaminated equipment butchers hand with abrasions and wound, slaughter of animal beside dressed one in the same area in the slaughter hall, contaminated air from crowdness of workers and their aerosols which contaminated air with *Staphylococci* during slaughtering. Therefore, some contamination of carcasses with *Staphylococci* can be expected **(Lasts et al. 1992).**

It is evident from table (5) that the total *Staphylococcal* count cfc/g of non closed esophagus and anal opening of cattle carcasses ranged from 7 $\times 10^3$ to 28 $\times 10^3$ with the mean value 12.32 $\times 10^3$ ± 0.50 $\times 10^3$, while the total *Staphylococcal* count cfc/g of closed esophagus and anal opening of cattle carcasses ranged from 1 $\times 10^2$ to 13 $\times 10^2$ with the mean value 7.58 $\times 10^2$ ± 0.43× 10^2, so there is highly significant difference between the mean values of non closed esophagus and anal opening of cattle carcasses and the mean values of closed esophagus and

anal opening of cattle carcasses at $P < 0.001$.

USFDA (2004) reported that *Staphylococci* is ubiquitous and inhabits the mucous membranes. Up to 50 % of humans may carry this organism in their nasal passages and throats and on their hair and skin.

6-Mould and Yeast count:

It is evident from table (6) that the total *Mould* and *Yeast* count cfc/g of non closed oesophagus and anal opening of cattle carcasses ranged from 5×10^4 to 12×10^4 with the mean value $7.69 \times 10^4 \pm 0.67 \times 10^4$, while the total *Mould* and *Yeast* count cfc/g of closed esophagus and anal opening of cattle carcasses ranged from 1×10^3 to 9×10^3 with the mean value $3.23 \times 10^3 \pm 0.26 \times 10^3$, so there is significant difference between the mean values of non closed esophagus and anal opening of cattle carcasses and the mean values of closed esophagus and anal opening of cattle carcasses at $P < 0.001$

The consumption of mouldy and mycotoxins contaminated foods can threaten human health **(Lacey, 1988).**

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