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**MICROBIAL EVALUATION OF FRESH GOAT
MUSCLES SOLD IN A RURAL PLACE
IN UPPER EGYPT**
(With 6 Tables)

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**التقييم الميكروبي لعضلات الماعز الطازجة المباعة
في منطقة قرويه بصعيد مصر**

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اشتمل البحث على الفحص الميكروبي لعدد ٣٠ عضله كامله من ذبائح الماعز الطازجه التى تم ذبحها خارج المجازر فى إحدى قرى مركز أسيوط بصعيد مصر وتحت شروط غير صحيه، حيث تم جمع ١٠ عينات من كل من عضلات الكتف، العضلات القطنيه تحت الظهره وعضلات الفخذ أثناء البيع بعد الذبح مباشرة وذلك للوقوف على مدى التلوث الميكروبي لذبائح الماعز الطازجه المباعه فى منطقة قرويه. كان متوسط العدد الكلى، الأيرومونات هايدروفيليا، الكوليفورم، البكتريا المعديه، الأيشاريشيا كولاي، السيدومونات ايروجنوزا، الفطريات والخمائر ٧٦ X ٦١، ٥١ X ٧١، ٥٠ X ٥١، ٤٦ X ٥١، ٠١، ٢٩، ٢٢ X ٤١، ٥٣ و ٢٢ X ٣١، لكل جرام من عضلات الكتف وبالنسبه للعضلات القطنيه تحت الظهره كانت المتوسطات كالتالى ١٥ X ٦١، ٣٤ X ٧١، ٢٠ X ٥١، ٤٠ X ٥١، ٣١، ٦١، ٨٩ X ٤١، ٣٣ و ١٩ X ٣١ / جرام أما عضلات الفخذ فكانت المتوسطات ٤١ X ٦١، ٣٥ X ٧١، ١٠ X ٥١، ٢١ X ٥١، ٧٠، ٥٦، ١٦ X ٤١، ٧٤، ٢٥ X ٣١ / جرام على التوالي. كما تم عزل وتصنيف فطر الأسبرجيليس فلافس، الأسبرجيليس نيجر، والميوكر والبنسليوم بنسب ١٨، ٧٥٪، ٥٦، ٢٥٪، ١٢، ٥٠٪ و ١٢، ٥٠٪ على التوالي. كما تم مناقشة الخطوره الصحيه على صحة الانسان من ذبح الحيوانات خارج المجازر بالاضافه الى دراسة مصادر التلوث المختلفه لمثل هذه الذبائح.

SUMMARY

Thirty goat muscles were microbiologically examined for selected microorganisms just after slaughter and during selling in a rural place related

to Assiut Governorate. The animals were slaughtered outside the official slaughter houses under unhygienic conditions. The collected samples were 10 muscles of each of the shoulder, psoas and thigh which were obtained from the same owner at different intervals to evaluate the microbial status of fresh goat carcasses as meat is retailed in small quantities from hung carcasses throughout the day. The carcass is exposed to ambient temperature, atmospheric and microbial load due to handling practices as well as surfaces of carcasses are easily contaminated with microorganisms during skinning and evisceration. The average counts of aerobic plate, *Aeromonas hydrophila*, coliforms, Enterobacteriaceae, *E. coli*, *Pseudomonas aeruginosa*, moulds and yeasts were 76×10^6 , 51×10^7 , 50×10^5 , 46×10^5 , 29.01, 22×10^4 , 53 and 22×10^3 /g of examined shoulder muscles, whereas they were 15×10^6 , 34×10^7 , 20×10^5 , 40×10^5 , 61.31, 89×10^4 , 33 and 19×10^3 /g of psoas muscles and they were 41×10^6 , 35×10^7 , 10×10^5 , 21×10^5 , 56.70, 16×10^4 , 74 and 25×10^3 /g of examined thigh muscles, respectively. The incidence of identified moulds species isolated from examined goat muscles was *Asp. flavus* (18.75%), *Asp. niger* (56.25%), *Mucor spp.* (12.50%) and *Penicillium spp.* (12.50%). Public health hazards of slaughtering food animals in unsanitary places outside slaughter houses as well as different sources of contamination of such carcasses were discussed.

Key words: *Microbial - Goat Muscles*

INTRODUCTION

In rural places, food animals are slaughtered in unhygienic conditions, where meat is retailed in small quantities from hung carcasses throughout the day. There are considerable variations in the microbiological quality as the carcass is exposed to ambient temperature, atmospheric and bacterial load due to handling practices. The source of microbial contamination of the fresh meat may be environment or implements used in slaughtering the animals or through meat-handlers (Gupta *et al.*, 1984 and El-Daly *et al.*, 1988). The significance of such contamination is intensively discussed by many investigators such as Sinha and Mandal (1977) who reported that the average counts of total microbial load and microflora present in market goat chest and thigh muscles were 28.6×10^5 and 6.6×10^5 /g, respectively. Also, Bhagirathi *et al.* (1983) stated that the levels of bacterial contamination in market samples of fresh mutton examined during 1978-1981 ranged from

10^3 to $> 10^5$ /g. There was 22 to 5 log increase in contamination levels on exposure to atmospheric conditions for 6-8 hr.

Aeromonas hydrophila is a common contaminant of numerous foods (Faghri *et al.*, 1984; Hood *et al.*, 1984 and Fathi and Moustafa, 1991). The incidence of *Aeromonas* detected from feces of beef, pig, sheep and turkey was one of 32, none of 22, none of 24 and three of 21, respectively (Stern *et al.*, 1987), while the percentage of *A. hydrophila* isolated from raw pork, beef and chicken was 33, 27 and 24 out of 45 samples of each, respectively (Ternstrom and Molin, 1987).

The mean coliform counts in retail ground beef by using violet red bile surface - overlay plate and pour plate methods were 3.97×10^4 and 3.52×10^4 /g of examined samples, respectively (Pierson *et al.*, 1978), whereas the mean of total aerobic plate and total coliforms counts was 2.3×10^4 and 1.8×10^4 /g of examined imported lean beef pieces, respectively (Oblinger and Jr, 1978). However, the average of total aerobic and coliforms counts from 24 samples of frozen goat meat were 8.3×10^6 and 7.6×10^5 /g, respectively (Srivastava *et al.*, 1981).

The total aerobic and Enterobacteriaceae mean counts in meat collected from open weekly markets and village butcher shops were 17.807×10^6 and 0.919×10^6 and 0.969×10^6 and 0.049×10^6 /g, respectively (Elmossalami *et al.*, 1988), while the mean values of Enterobacteriaceae count were 10^3 , 2×10^4 , 5×10^5 and 8×10^5 organisms/g of examined heart, liver, rumen and intestine, respectively (Khalafala *et al.*, 1989).

Kenneth (1975) reported that the standard limit for *E. coli* is 50/g for fresh or frozen meat products and 10/g for processed meats, however, Fathi *et al.* (1992) pointed out that the presence of *E. coli* in food as agents for foodborne enteritis or as indicators of fecal contamination have resulted in increasing concern and interest for estimation of the organism in selected meat and meat products.

Determination of the numbers and types of moulds and yeasts of the goat muscles are important from the standpoint of public health, for judging effectiveness of sanitary handling during slaughtering and dressing of goat particularly in rural places. Refai and Loot (1969) isolated 186 mould strains out of 96 examined goat meat samples. The isolated strains were identified as *Asp. niger* (52), *Asp. fumigatus* (2), *Asp. flavus* (2), *Penicillium* (21), *Mucor* (2), *Rhizopus* (1), *Cephalosporium* (2), *Scopulariopsis* (1), *Pullularia* (11) and *Streptomyces* (2) strains, whereas Vanderzant and Nickelson (1969) found that the incidence of isolated yeast and mould, and pseudomonas from lamb carcasses was 4(12.5%) and nil, respectively. Also, Koburger and Farhat

(1975) showed that the total yeasts and moulds organisms per gram of the examined lamb shop was 56×10^4 . However, Abdel-Rahman *et al.* (1983) and (1985) stated that the genus *Aspergillus* and *Penicillium* were the most predominant mould genera which were isolated from fresh, cured and processed meat, while Davis (1981) and Misilvic (1981) mentioned that various species of *Aspergillus*, *Penicillium* and *Mucor* have been implicated in production of mycotoxins which occur in levels sufficient to be regarded as significant hazards to animals and human health.

Pseudomonas aeruginosa is widely spread in nature and is a soil and water pollutants. Its presence in food items could also be taken as indication of faecal contamination, as it is present in the intestinal tract of animals and human beings (Hoadley and McCoy, 1968 and Bergan, 1975).

Recently, Sierra *et al.* (1995) examined thirty lamb carcasses for selected bacteria just after slaughter, where the frequency of isolation was salmonella (10%), motile aeromonads (33%), *Yersinia enterocolitica* (20%) and cold tolerant *Escherichia coli* (56.6%).

Therefore, the present investigation has been aimed to determine the extent of microbial contamination present in goat muscles being sold to the consumers in rural places.

MATERIAL and METHODS

Collection of samples:

Thirty samples of fresh goat muscles, ten of each of shoulder, psoas and thigh muscles were collected and transferred separately to the laboratory in pre-sterilized polyethylene bags, where they were prepared for microbiological analysis immediately upon receipt in the laboratory.

Preparation of samples:

Twenty five grams of each sample were blended in 225 ml of buffered peptone water for 2 min. in a pre-sterilized blender. Individual serial decimal dilutions were prepared in 90 ml volumes of buffered peptone water up to a 10^{-6} dilution of the original sample (ICMSF, 1978).

Aerobic plate count:

Duplicate 1 ml volumes of suitable dilutions were pipetted into each Petri-dish. Then about 15 ml of Standard Plate Count Agar (Oxoid, U.K.) were poured into each Petri-dish. The inoculated plates were incubated at 35°C for 48 h. (ICMSF, 1978).

Aeromonas hydrophila species count:

The samples were analyzed by using enrichment method, where 25 g sample were aseptically transferred to 225 ml of Trypticase soy broth containing 10 ug ampicillin/ml and blended for 2 min., then incubated at 28°C for 6 and/or 24 h. After incubation the enrichment cultures were serially diluted up to 10^{-6} in Butterfield's phosphate diluent and spreaded on MacConkey manitol ampicillin agar with a bent glass rod and incubated at 28°C for 18-24 h. The typical red colonies were estimated as *Aeromonas hydrophila* species (Okrend *et al.*, 1987). For confirmation, typical colonies were picked to triple sugar iron agar and nutrient agar slants. After overnight incubation at 28°C, few drops of a 1% solution of N, N-dimethyl 1-p-phenylene- diamine monohydrochloride were added to the growth on the nutrient agar slant to determine the oxidase reaction (Palumbo *et al.*, 1985).

Coliforms and Enterobacteriaceae plate count:

Duplicate 1 ml volumes of suitable dilutions were dispensed in Petri-dishes and ca. 15 ml of melted Violet red bile agar (VRBA) in case of coliforms and Violet red bile glucose agar (VRBG) for Enterobacteriaceae were added with thorough mixing. After solidification, the plates were overlaid with a second layer of 15 ml of VRBA and VRBG, respectively. Plates were incubated at 35°C for 18-24 h. The red or purple colonies with a diameter >0.5 mm surrounded by a zone of precipitated bile were counted. Colonies judged to be border-line were also counted (Murthy, 1984).

Determination of *E. Coli* (ICMSF, 1978):

One ml from the previously prepared dilutions was inoculated separately into each of three Lauryl Sulphate Tryptose (LST) broth fermentation tubes with inverted Durham's tubes. The tubes were incubated at 35-37°C for 24 and 48 hours. Tubes showing gas production were considered positive. A loopful from each positive tube was transferred separately into each of three EC broth tubes with inverted Durham's tubes. The tubes were incubated at 45.5°C for 48 hours. Tubes showing gas production were considered positive. From each gas positive tube of EC broth, a loopful was streaked on Levine's Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 35-37°C for 24±2 hours. Plates showing typical colonies or colonies most likely to be *E. coli* were recorded for determination MPN/g.

Mould and yeast count:

From the previously prepared dilutions, duplicate sterile Petri-dishes were pipetted with 1 ml from each suitable dilutions by accurate pipettes which deliver required volumes. Then about 15 ml of acidified malt agar

melted and cooled at 45°C were poured into each Petri-dish. The inoculated plates, after being thoroughly mixed and solidified were incubated at 20°C for 5 days (A.O.A.C., 1975). The colonies were enumerated and recorded according to A.P.H.A. (1978). Isolated moulds were identified according to rapidity of growth, colony morphology, texture of growth, mycelium and pigmentation production as well as microscopically.

Enumeration of *Pseudomonas aeruginosa*:

Surface plating technique for each sample and its dilutions was done using cetrimide agar plates as described by Lowbury (1951). The plates were incubated at 42°C for 48 h., and typical colonies of those formed by *Ps. aeruginosa* were counted. Confirmatory tests were done on each isolate suspected to be *Ps. aeruginosa* as described by Chruickshank *et al.* (1975).

RESULTS

The obtained results are recorded in Tables 1,2,3,4,5 and 6.

DISCUSSION

It is evident from tables 1,2 and 3 that the average aerobic plate counts was 76×10^6 , 15×10^6 and 41×10^6 /g, while the average coliforms and Enterobacteriaceae counts was 50×10^5 and 46×10^5 ; 20×10^5 and 40×10^5 and 10×10^5 and 21×10^5 /g of examined shoulder, psoas and thigh goat muscles, respectively. The presence of numerous mesophilic bacteria which grow readily at or near body temperature often indicated contaminated raw materials or unsatisfactory processing could facilitate the recognized food-borne pathogenic bacteria, furthermore, the presence of Enterobacteriaceae indicates microbiological proliferation when allow multiplication of pathogenic and toxigenic bacteria in meat lead to public health hazard (ICMSF, 1978). The presence of coliforms in meat is frequently a reliable indicator for faulty methods of slaughtering, preparation and handling. Such meat contamination with coliforms by several ways induces undesirable changes and economic losses of meat. Moreover, contamination with great numbers may be associated with increasing the number of *E.coli* and consequently may constitute a public health hazards (Libby, 1975 and ICMSF, 1980). It is concluded that presence of aerobic plate, coliforms and Enterobacteriaceae counts in considerable high average levels in examined goat muscles is attributed to different sources of contamination of carcasses

during slaughtering, evisceration and dressing processes in addition to lack of hygienic circumstances in rural areas, particularly the goats were slaughtered outside the slaughter houses in unsanitary places.

From the results achieved in Tables 1,2 and 3 it is evident that *E.coli* were detected in 5(50%), 6(60%) and 7(70%) of examined shoulder, psoas and thigh goat muscles with average count of 29.01, 61.31 and 56.70/g examined sample, respectively. *Escherichia coli* and related coliform bacteria predominate among aerobic commensal flora present in the gut of man and animals, so, their presence in meat or other meat products is indicative of faecal contamination (ICMSF, 1978). On the other hand, the contamination of meat products with pathogenic bacteria like *E.coli* constitutes a public health hazard in the form of infection or intoxication in human consumers (Mehlman and Romero, 1982).

The results outlined in Tables 1,2 and 3 showed that all examined shoulder, psoas and thigh muscles harboured *A. hydrophila* and *Ps. aeruginosa* with average counts of 51×10^7 and 22×10^4 ; 34×10^7 and 89×10^4 and 35×10^7 and 16×10^4 /g examined samples, respectively. *Aeromonas hydrophila* is capable to grow at refrigerated temperature and has been observed as a part of microflora of fish, milk, poultry and meat (Palumbo *et al.*, 1985). Increasing interest concerning the possible role of *A. hydrophila* species as a cause of human gastroenteritis (Burke *et al.*, 1983). *Pseudomonas aeruginosa* was reported as a causative organism of severe forms of acute gastro enteritis (Sutter *et al.*, 1966 and Pererra *et al.*, 1977). Also, it plays an important role in causing many infections in man and animals such as otitis, meningoencephalitis, pneumonia, pyelitis, eye infections, septicaemia and skin infections (Winso, 1957 and Chernosky and Dukes, 1963).

Moulds and yeasts are of wide distribution and are regarded more or less as sources of contamination of meat and its products which may lead to spoilage and/or food poisoning. From the results recorded in tables 4 and 5, it is observed that the average mould count was 53, 33 and 74/g, while the average yeast count was 22×10^3 , 19×10^3 and 25×10^3 /g of the examined shoulder, psoas and thigh muscles of fresh goat carcasses, respectively. On the other hand, the incidence of isolated *Asp. flavus*, *Asp. niger*, *Mucor* spp. and *Penicillium* spp. from examined goat muscles was 18.75, 56.25, 12.50 and 12.50, respectively (Table 6). It is noticed that *Asp. niger* was the predominant mould isolated from examined goat muscles, which is in agreement with the findings reported by Refai and Loot (1969) in goat meat. As a result of contamination with mould and yeast, such meat may undergo

spoilage beside they are incriminated in human mycosis as mentioned by Mossel (1977). Moreover, incidence of moulds and yeasts in meat indicates the bad hygienic measures adopted in the slaughtering places as well as during evisceration and handling of goat carcasses.

The effective design and implementation of hazard analysis and critical control point (HACCP) systems for fresh meat is dependent on identification of those process steps that determine most of the contamination on the carcass, and institution of appropriate critical limits (Biss and Hathaway, 1995).

So, it is recommended that slaughtering of food animals outside slaughter houses must be prevented to reduce sources of carcasses contamination and minimize as possible public health hazards.

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Table 1: Average bacterial count/g of examined goat shoulder muscles.

Microorganisms	No. of examined samples	Positive samples		Min	Max	Average
		No.	%			
Aerobic plate count	10	10	100	35×10^5	23×10^7	76×10^6
<i>Aeromonas hydrophila</i>	10	10	100	20×10^7	85×10^7	51×10^7
Coliforms	10	10	100	13×10^5	15×10^6	50×10^5
Enterobacteriaceae	10	10	100	80×10^4	19×10^6	46×10^5
<i>E. coli</i>	10	5	50	9.1	120	29.01
<i>Pseudomonas aeruginosa</i>	10	10	100	70	70×10^4	22×10^4

Table 2: Average bacterial count/g of examined goat psoas muscles

Microorganisms	No. of examined samples	Positive samples		Min	Max	Average
		No.	%			
Aerobic plate count	10	10	100	55×10^5	24×10^6	15×10^6
<i>Aeromonas hydrophila</i>	10	10	100	16×10^7	61×10^7	34×10^7
Coliforms	10	10	100	50×10^4	41×10^5	20×10^5
Enterobacteriaceae	10	10	100	68×10^4	14×10^6	40×10^5
<i>E. coli</i>	10	6	60	9.1	150	61.31
<i>Pseudomonas aeruginosa</i>	10	10	100	40	80×10^5	89×10^4

Table 3: Average bacterial count/g of examined goat thigh muscles.

Microorganisms	No. of examined samples	Positive samples		Min	Max	Average
		No.	%			
Aerobic plate count	10	10	100	35×10^5	90×10^6	41×10^6
<i>Aeromonas hydrophila</i>	10	10	100	14×10^7	60×10^7	35×10^7
Coliforms	10	10	100	25×10^4	22×10^5	10×10^5
Enterobacteriaceae	10	10	100	26×10^4	80×10^5	21×10^5
<i>E. coli</i>	10	7	70	3	150	56.70
<i>Pseudomonas aeruginosa</i>	10	10	100	370	78×10^4	16×10^4

Table 4: Average mould count/g of examined goat muscles

Muscles	No. of examined samples	Positive samples		Min	Max	Average
		No.	%			
Shoulder	10	7	70	10	140	53
Psoas	10	5	50	10	110	33
Thigh	10	7	70	20	300	74

Table 5: Average yeast count/g of examined goat muscles

Type of muscles	No. of examined samples	Positive samples		Min	Max	Average
		No.	%			
Shoulder	10	10	100	30×10^2	60×10^3	22×10^3
Psoas	10	10	100	40×10^2	50×10^3	19×10^3
Thigh	10	10	100	60×10^2	40×10^3	25×10^3

Table 6: Frequency distribution of identified mould species isolated from fresh goat muscles.

Isolates	No. of isolates	%
Aspergillus flavus	3	18.75
Aspergillus niger	9	56.25
Mucor spp.	2	12.50
Penicillium spp.	2	12.50
Total	16	100.00