BACTERIOLOGICAL EVALUATION OF BOTH PHYSIOLOGICAL AND PATHOLOGICAL (ICTERIC) YELLOW SHEEP CARCASSES

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SUMMARY

One hundred sheep carcasses were inspected at Qena abattoirs for determination of yellow colored carcasses. From the inspected carcasses 27(27%) were attained yellow coloration and could be differentiated into 18(66.6%) physiological and 9(33.3) pathological yellow colored carcasses. A total of 216 samples consisted of 144 from physiological and 72 from pathological yellow colored carcasses were collected from 8 different sites; peri-nephric fat (P.F.), liver tissue (L.T.), hepatic lymph node (H.Ln.), gall- bladder (GB), biceps muscle (M1), gastrocnemius (M2), prescapular lymph node (Ln1) and prefemoral (Ln2). All samples were examined for aerobic plate count (APC), psychrophilic count (PC) and enterobacteriacae count (EC), as well as, for incidence of some specific microorganisms (*E. coli, Salmonella and coagulase Staph. aureus*).

Higher incidence of some specific bacteria were detected in the examined samples of pathological yellow carcasses than that obtained from physiological ones. Therefore, *E. coli, Salmonella and Cp- Staph. aureus* were detected in percentages of (56.9% and 29.3%), (9.7% and 3.1%) and (16.6% and 0.0%) from all examined samples of both pathological and physiological yellow carcasses, respectively. Wide occurrence of these bacteria were found in the samples of gall bladder G.B. (77% and 16.6%), peri- nephric fat P.F.(66.6 and 33.3%) and hepatic lymh nodes H.Ln.(55.5; 22.2) of the pathological and physiological yellow carcasses, respectively.

Pathological yellow colored carcasses were heavily contaminated with bacteria than physiological ones. The averages of APC were ($2.6x10^5$ ±$1.1x10^3$ and $1.7x10^5$ ±$0.6x10^4$), PC ($5.3x10^3$±$0.8x10^2$ and $2.5x10^3$ ±$0.7x10^3$) and EC ($4.9x10^4$ ±$0.3x10^4$ and $3.9x10^3$ ±$0.9x10^2$) in all examined samples of pathological and physiological yellow colored carcasses, respectively. Higher APC was obtained in liver tissues (L.T.; $4.6x10^5$ ±$0.9x10^4$) of pathological yellow colored sheep carcasses and in hepatic lymph nodes (H.Ln.; $2.7x10^5$ ±$0.8x10^4$) in physiological ones. PC was greater in the samples of prefemoral lymph nodes (Ln2; $7.3x10^3$±$0.6x10^3$) of pathological yellow carcasses and in prescapular lymph nodes (Ln1; $8.6x10^3$ ±$0.5x10^3$) of physiological ones. EC was higher in the samples of gastrocnemius muscles (M2; $8.0x10^4$ ±$0.4x10^3$) of pathological yellow carcasses and in peri-nephric fat (P.F.; $7.6x10^3$ ±$0.3x10^3$) of physiological ones.

The importance of distinguishing between physiological and pathological yellow coloration in sheep carcasses, as well as, public health hazards resulting from consumption of icteric meat were fully discussed.

MATERIALS AND METHODS

One hundred sheep carcasses were inspected and exposed for intensive post-mortem examination and bacteriological analysis.

A- Differentiation between physiological and pathological yellow colored carcasses:

by application of Rapid Phase test and Martin test, as following:

1- Rapid phase test **(Rimingto and Ponira 1986):**
In a test tube two g of fat sample were thoroughly mixed with 5ml NaOH and heated for 5 minutes tell complete melting, then cooled under tap water to be at the comfortable warm to human hand, then 5 ml of diethyl ether were added carefully and thoroughly shaked, then left for separation into two layers. When the upper layer became yellow and the lower one nearly colorless; this indicate physiological yellow coloration.
When the lower layer became yellowish green and the upper one was yellow; this indicate pathological yellow coloration (jaundice).
2- Martin test **(Pearson 1962):**
In a test tube two g of fat sample or connective tissue were cut into small pieces, to which 20 ml of 50 % alcohol were added and thoroughly mixed then filtered. To 8 ml of the filtrate 10-20 drops of conc. sulphuric acid were added. Formation of yellow or brown color was attributed to the presence of bilirubin pigment; this color will be changed to blue by addition of concentrated sulphuric acid with boiling due to further oxidation; this indicates pathological yellow coloration.
B- Bacteriological examination:-
This examination was only carried out on samples collected from yellow colored carcasses, either physiological or pathological. The samples were obtained from eight locations; perinephric fat (P.F.), Biceps muscle (M_1), Gastrocnemius (M_2) liver tissues (L.T.), hepatic lymph node (H. Ln.), gall bladder (GB.), prescapular (Ln1) and prefemoral lymph nod (Ln2).
Samples were packed separately in identified polyethylene sacs and transferred ,as soon as possible, in ice box to the laboratory for bacteriological analysis, as follows:
1- Preparation of the samples (**ICMSF, 1978):**
Under aseptic condition; 10g from each sample were added to 90 ml of sterile buffered peptone water and thoroughly mixed using sterile blender for 2-5 minutes at 1500-2000 r.p.m. to provide a homogenate of 1/10 dilution (original dilution), from which one ml was used for serial dilutions up to $1x10^6$.

2- Determination of Aerobic plate count (A.P.C.):

According to **ICMSF (1978)** the surface plate technique was applied using standard plate count agar (Oxoid), by spreading of 0.1 ml of each dilution over agar medium by means of a sterile glass spreader, then left to dry and incubated inverted at 29-31ºC for 3 days. Number of colonies/g was calculated in countable plates (30- 300) colony.

Aerobic Plate count/ml = No. of colonies x dilution x10.

3- Determination of psychrophilic count (P.C.):

The technique recommended by **ICMSF (1978)** was applied by incubation of the inoculated plates at 5 ºC for 7 days. Countable plates (30-300 colony) used for counting of colonies as following:

Psychrophilic count/g = No. of colonies x dilution x10.

4- Determination of Enterobacteriaceae count (E.C):

By applying of the technique of surface plate method using Violet Red Bile Glucose agar (VRBG agar, Oxoid M485), according to (**Gork, 1976).** After 24 h, incubation at 37 ºC. All purple colonies with hallo zone were counted:

Enterobacteriaceae count/g = No. of colonies x dilution x 10.

5- Isolation and identification of ***E. coli*** (**ICMSF, 1978) and FAO (1992):**

Lauryal Sulphate Tryptose broth (L.S.T) tubes containing inverted Durhams tubes were inoculated with 1 ml of meat homogenate and incubated at 37 ºC for 24- 48 hours. Another tubes of LST were also inoculated with a loopfull from each (+)ve LST tubes (with gas) and incubated for 48 hours at 44.5 ºC in thermostatically controlled water bath. A loopfull from each gas (+)ve LST tubes was streaked on Eosin Methylene Blue agar (EMB agar) and incubated at 37 ºC for 24 hours.

Colonies of green metallic with dark purple centers were picked and inoculated into slope nutrient agar then incubated at 37 ºC for 24 hours for further identification. This was carried by using morphological and biochemical examinations according to (**Krieg and Holt 1986);** including Indole production, Methyl red, Vogues proskoure, Citrate utilization, Motility, Hydrogen sulphate production on Triple Sugar Iron agar medium (TSI), Urease production and Fermentation of sugars.`

6. Isolation and identification of ***salmonellae***:

The techniques recommended by **ICMSF (1978)** as follow:

a-Pre-enrichment by incubation of the homogenate in sterile flask at 37 ºC for 18-24 hours.

b-Enrichment:

One ml of the pre-encrichmented meat homogenate was added aseptically to 10 ml of Rapport Vassilidis broth, then incubated at 43 ºC for 48 hours.

c-Inoculation of the selective media:

On Xylose Lysine Desoxychcolate (XLD) agar plate; a loop- full from the enrichment broth was streaked then incubated for 24 - 48 hours at 37 ºC, the

colonies with or without black center were picked up and purified and spread on slope agar tubes for further identification.

d-Identification of the isolated ***Salmonellae*** according to **Krieg and Holt (1986)** by morphological, biochemical and serological examinations.

7- Isolation of coagulase- positive ***Staph. aureus***:

According to the technique recommended by Collins and **Cummins (1986);**

a-Microbiological examination for detection of grapes like cocci on the Gram stained film according to the technique recommended by **Cruickshank et al. (1975).**

b-Catalase test recommended by **Varnam and Evans (1991)** for detection of coagulase (+ve) Staph. aureus.

Table (1): Incidence of yellow colored sheep carcasses with differentiation between physiological and pathological types.

Total No. of examined carcasses	*No. of yellow colored carcasses*	*%*	*Rapid phase test*				*Martin test*			
			Physiological		**Pathological**		**Physiological**		**Pathological**	
			No. of samples	**%**	**No. of samples**	**%**	**No. of samples**	**%**	**No. of samples**	**%**
100	**27**	**27**	**18**	**66.6**	**9**	**33.3**	**18**	**66.6**	**9**	**33.3**

Table (2) Prevalence of some specific bacteria isolated from the examined physiological (n= 144) and pathological (n=72) yellow colored sheep carcasses.

Samples	**Total No.**	**Physiological yellow carcasses**			**Pathological yellow carcasses**			
		E. coli	**Salmonella**	**CP.Staph. aureus**	**Total**	**E. coli**	**Salmonella**	**CP.Staph. aureus**
		No. (%)	**No. (%)**	**No. (%)**	**No.**	**No.(%)**	**No. (%)**	**No. (%)**
P.F.	**18**	**6 (33.3)**	**0 (0.0)**	**0 (0.0)**	**9**	**6 (66.6)**	**0 (0.0)**	**2 (22.2)**
L.T.	**18**	**5 (27.7)**	**1 (5.5)**	**0 (0.0)**	**9**	**4 (44.4)**	**3 (33.3)**	**4 (44.4)**
H.L.n	**18**	**4 (22.2)**	**0 (0.0)**	**0 (0.0)**	**9**	**5 (55.5)**	**0 (0.0)**	**0 (0.0)**
G.B.	**18**	**3 (16.6)**	**1 (5.5)**	**0 (0.0)**	**9**	**7 (77.7)**	**2 (22.2)**	**0 (0.0)**
M_1	**18**	**4 (22.2)**	**0 (0.0)**	**0 (0.0)**	**9**	**3 (33.3)**	**1 (11.1)**	**0 (0.0)**
M_2.	**18**	**2 (11.0)**	**0 (0.0)**	**0 (0.0)**	**9**	**4 (44.4)**	**0 (0.0)**	**2 (22.2)**
Ln_1	**18**	**5 (27.7)**	**0 (0.0)**	**0 (0.0)**	**9**	**5 (55.5)**	**0 (0.0)**	**2 (22.2)**
Ln_2	**18**	**7 (38.8)**	**1 (5.5)**	**0 (0.0)**	**9**	**7 (77.7)**	**1 (11.1)**	**2 (22.2)**

Total	**144**	**36 (29. 3)**	**3 (2.1)**	**0 (0.0)**	**72**	**41(56.9)**	**7 (9.7)**	**12 (16.6)**

P.F. = perinephric fat **M1 = Biceps muscle**
L.T. = Liver tissue **M2 = Gastrocenemius muscle**
H. Ln.= Hepatic lymph node **Ln1 = Prescapular lymph node**
G.B. = Gall bladder **Ln2 = Prefemoral lymph node**

Table (3) Statistical analysis of bacterial counts contaminated the examined samples of the inspected physiological yellow colored sheep carcasses(n=144).

Sam-ple	Total No.	A.P.C.			P.C.			E.C.		
		Min.	Max.	Mean ±Sd	Min.	Max.	Mean ±Sd	Min.	Max.	Mean ±Sd
P.F.	18	$5.2x10^4$	$8.1x10^5$	$6.7x10^4$ $\pm0.7x10^4$	$3.0x10^3$	$5.1x10^3$	$4.7x10^3$ $\pm0.2x10^3$	$2.0x10^2$	$6.1x10^4$	$7.6x10^3$ $\pm0.3x10^3$
L.T.	18	$9.0x10^3$	$3.1x10^5$	$1.6x10^5$ $\pm1.6x10^4$	$2.0x10^3$	$5.0x10^3$	$3.6x10^3$ $\pm0.6x10^3$	$4.1x10^2$	$8.1x10^3$	$3.4x10^3$ $\pm0.3x10^2$
H.Ln.	18	$3.0x10^4$	$5.2x10^5$	$2.7x10^5$ $\pm0.8x10^4$	$2.1x10^3$	$3.1x10^3$	$2.1x10^3$ $\pm0.2x10^3$	$4.0x10^2$	$8.0x10^3$	$4.2\ x10^3$ $\pm0.7x10^2$
G.B.	18	$3.1x10^4$	$3.2x10^5$	$1.8x10^5$ $\pm0.9x10^4$	$1.0x10^3$	$2.2x10^3$	$1.1x10^3$ $\pm0.1x10$	$3.6x10^2$	$4.2x10^3$	$2.3\ x10^3$ $\pm0.5x10^2$
M_1	18	$3.2x10^3$	$8.0x10^5$	$9.4x10^4$ $\pm1.2x10^3$	$5.9x10^3$	$9.1x10^3$	$4.5x10^3$ $\pm1.1x10^3$	$2.1x10^2$	$7.2x10^3$	$3.5\ x10^3$ $\pm0.6x10^2$
M_2.	18	$2.1x10^3$	$8.5x10^5$	$8.3x10^4$ $\pm1.1x10^3$	$5.0x10^3$	$6.5x10^3$	$5.6x10^3$ $\pm0.7x10^3$	$1.5x10^3$	$5.1x10^3$	$3.0\ x10^3$ $\pm0.7x10^2$
Ln_1	18	$8.1x10^3$	$1.6x10^5$	$9.2x10^4$ $\pm0.9x10^4$	$8.1x10^3$	$9.2x10^3$	$6.6x10^3$ $\pm0.5x10^3$	$1.2x10^2$	$9.2x10^3$	$7.4\ x10^3$ $\pm0.9x10^2$
Ln_2	18	$6.2x10^3$	$8.2x10^5$	$7.8x10^4$ $\pm0.8x10^3$	$5.1x10^3$	$9.1x10^3$	$3.1x10^3$ $\pm0.6x10^3$	$2.0x10^3$	$9.3x10^3$	$5.6\ x10^3$ $\pm0.7x10^2$
Total	144	$2.1x10^3$	$8.5x10^5$	$1.7x10^5$ $\pm0.8x10^4$	$2.0x10^3$	$9.2x10^3$	$2.5x10^3$ $\pm0.7x10^3$	$1.2x10^2$	$6.1x10^4$	$3.9x10^3$ $\pm0.9x10^2$

A.P.C=Aerobic Plate Count **P.C.= Psychrophilic Count**
E.C.=Enterobacteriacae Count

Table (4) Statistical analysis of bacterial counts contaminated the examined samples of the inspected pathological yellow colored sheep carcasses(n=72).

Sam ple	Total No.	A.P.C.			P.C.			E.C.		
		Min.	Max.	Mean ±Sd	Min.	Max.	Mean ±Sd	Min.	Max.	Mean ±Sd
P.F.	9	$8.5x10^4$	$8.2x10^5$	$4.1x10^5$ $\pm0.7x10^4$	$3.0x10^3$	$5.1x10^3$	$4.1x10^3$ $\pm0.7x10^3$	$3.5x10^3$	$3.1x10^4$	$1.7x10^4$ $\pm0.7x10^3$
L.T.	9	$2.1x10^4$	$7.9x10^5$	$4.6x10^5$ $\pm0.9x10^4$	$2.0x10^3$	$4.1x10^3$	$3.2x10^3$ $\pm1.6x10^3$	$8.0x10^3$	$8.2x10^4$	$4.5\ x10^4$ $\pm0.9x10^3$
H.Ln	9	$5.5x10^4$	$6.1x10^5$	$3.4x10^5$ $\pm1.1x10^4$	$2.2x10^3$	$9.0x10^3$	$5.6x10^3$ $\pm0.9x10^3$	$7.0x10^3$	$7.2x10^3$	$7.1x10^4$ $\pm0.6x10^3$
G.B.	9	$4.6x10^4$	$5.6x10^5$	$3.0x10^5$ $\pm0.8x10^4$	$5.2x10^3$	$9.1x10^3$	$7.1x10^3$ $\pm0.9x10^2$	$5.0x10^3$	$5.1x10^4$	$2.7x10^4$ $\pm0.8x10^3$
M_1	9	$5.0x10^4$	$6.8x10^5$	$3.3x10^5$ $\pm0.9x10^4$	$5.3x10^2$	$4.0x10^3$	$7.3x10^3$ $\pm1.2x10^2$	$8.5x10^2$	$7.9x10^4$	$3.9x10^4$ $\pm0.5x10^3$

M_2	9	$5.0x10^4$	$6.0x10^5$	$3.2x10^5$ $\pm0.9x10^4$	$8.6x10^2$	$8.9x10^3$	$4.8x10^3$ $\pm1.1x10^2$	$8.0x10^3$	$8.1x10^3$	$8.0x10^4$ $\pm0.4x10^3$
Ln_1	9	$8.9x10^3$	$9.2x10^4$	$5.0x10^4$ $\pm0.9x10^3$	$5.5x10^2$	$5.9x10^3$	$3.2x10^3$ $\pm0.9x10^2$	$8.1x10^3$	$9.3x10^4$	$5.1x10^4$ $\pm0.6x10^3$
Ln_2	9	$9.1x10^3$	$9.2x10^4$	$5.1x10^4$ $\pm0.9x10^3$	$4.5x10^3$	$5.1x10^3$	$7.3x10^3$ $\pm0.8x10^3$	$8.2x10^3$	$8.6x10^4$	$4.7x10^4$ $\pm0.3x10^3$
Total	72	$8.9x10^3$	$8.2x10^5$	$2.6x10^5$ $\pm1.1x10^3$	$5.3x10^2$	$9.1x10^3$	$5.3x10^3$ $\pm0.6x10^2$	$8.5x10^2$	$9.3x10^4$	$4.9\ x10^4$ $\pm0.3x10^4$

A.P.C=Aerobic Plate Count **P.C.= Psychrophilic Count**
E.C.=Enterobacteriacae Count

Conclusion

Bacteriological results of the examined physiological and Pathological yellow colored sheep carcasses indicated higher counts of A.P.C., P.C., E.C. in the samples collected from pathological than those collected from physiological yellow colored carcasses.

At the same time , E.coli, salmonella and coagulase (+)ve Staph. aureus could be isolated from some samples of physiological yellow colored carcasses and of higher incidence from those of pathological ones.

To reduce total aerobic, coli- form, and Escherichia coli counts on sheep carcasses, the microbiological contamination of the hindquarters and the general microbiological condition of the carcasses should be improved strict hygienic measures for preventing or removing contamination during dressing of the carcasses.