



Assessment of meat microbial contamination in Alkadro slaughterhouse, Khartoum State, Sudan.

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Abstract

This study was conducted to evaluate the bacterial contamination of meat in Alkadro slaughterhouse. One hundred and twenty swab samples were taken from different stages in carcasses line, as well as inspection of knives, hocks, tables, worker hands and air. The samples were subjected to viable bacterial count and the meat was tested for the pH values. Causes of condemnation of organs were also recorded. The percentages of causes of condemnations from the total number of condemned organs: in beef, were *fasciola spp* in livers were (46.03%), aspiration pneumonia in lungs (3%) and abscess in head (7.5%). In mutton, abscess in livers were (64.4%), hydatid cysts in livers (2.9%), pneumonia in (9.7%), Hearts due to the adhesions of pericardium (2.9%) and abscess (0.4%). For viable bacterial count, air samples showed a maximum level in evisceration site (80.8 CFU/plate), inspection site (63.25 CFU/plate), skinning site (51.80 CFU/plate) and in washing site (40.40 CFU/plate). Tables, workers' hands, knives, hooks and carcasses, showed that the maximum level counts in hooks (4.8×10^3 CFU/cm²) and the minimum level count were in hands (2.7×10^3 CFU/cm²). Carcasses after skinning showed the minimum level count 1.34×10^2 CFU/cm². pH of meat ranged between (5.7 – 6.4). This study revealed that there is high level of contamination in slaughterhouse. Hence to reduce of meat contamination should be start with the implementation of good hygienic practices and good manufacturing practices and implementing HACCP system. In addition to health education and training are needed to raise the awareness for meat handlers in the slaughterhouses.

Key words: Slaughterhouse, Meat, Contamination, Bacteria.

المستخلص

أجريت هذه الدراسة بغرض تقييم التلوث البكتيري للحوم في مسلخ الكدرو. تم أخذ مائة وعشرين مسحة من مراحل مختلفة في خط الذبيح وكذلك فحص السكاكين والشناكل والطاولات و أيدي العاملين والهواء. أخضعت العينات لتحديد حملتها من البكتيريا الهوائية وتم اختبار قيمة الرقم الهيدروجيني للحوم. و رصدت أيضا أسباب إعدام الأعضاء. كانت النسب المئوية لأسباب الإعدامات من العدد الكلي للأعضاء المعدمة: في لحوم البقر كان المتورقة في الكبد (46.03%) وكان الإلتهاب الرئوي (3%) وخراج الرأس (7.5%). في الضأن كانت خراج في الكبد (64.4%)، الكيسات المذنبة في الكبد (2.9%)، والالتهاب الرئوي (9.7%)، القلب سبب إلتصاق التامور (2.9%)، والخراجات (0.4%). حمولة البكتيريا الهوائية، أظهرت عينات الهواء أقصى مستوى حمولة عد بكتيري في موقع نزع الأحشاء (80.8 وحدة)، موقع الفحص (63.25 وحدة)، موقع السلخ (51.80 وحدة) وموقع الغسيل (40.40 وحدة). عينات الطاولات، أيدي العمال، السكاكين، الشناكل والذبائح أظهرت أقصى مستوى حمولة عد بكتيري في الشناكل 4.8×10^3 وحدة /سم مربع وأدنى مستوى حمولة عد بكتيري في أيدي العاملين 2.7×10^3 وحدة / سم مربع، عكست ذبائح بعد السلخ أقل متوسط حمولة عد بكتيري 1.34×10^2 وحدة / سم. وأظهرت نتيجة

الأس الهيدروجيني للحم مدى يتراوح ما بين (5.7-6.4). كشفت هذه الدراسة عن ارتفاع مستوى التلوث في المسلخ. وبالتالي، لتقليل تلوث اللحم يجب البدء بتطبيق الممارسات الصحية الجيدة وممارسات التصنيع الجيدة وتنفيذ نظام تحليل المخاطر ونقطة التحكم الحرجة. و بالإضافة إلى التعقيم والتدريب الصحي لزيادة الوعي لدى المنعاملون في اللحم بالمسالخ .

الكلمات المفتاحية: مسلخ، لحم، تلوث، بكتريا.

Introduction

Meat is considered as an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a highly favourable environment for the growth of pathogenic bacteria Lawrie and Ledward (2006). Meat products are perishable and unless processed, packaged, distributed and store appropriately can spoil in relatively short time (Sofos, 2005). Foodborne diseases and foodborne injury are at the best unpleasant; at worst, they can be fatal. But there are also other consequences. Outbreaks of foodborne illness can damage trade and tourism, and lead to loss of rings, unemployment and litigation (Iroha *et al.*, 2011; Hassan *et al.*, 2010). Food spoilage is wasteful, costly and can adversely affect trade and consumer confidence.

Meat inspection practice is one of the important activities of the veterinary services. Its aim to insure that meat is free from diseases, wholesome and fit for human consumption (Herenda *et al.*, 2000). According to Jenni (2012) meat hygiene as a system of principles designed to ensure meat and meat products are safe wholesome and processed in a hygienic manner and are fit for human consumption. The efficient meat hygiene practices begin in the farm and maintained throughout the chain i.e. in the animal collection centre, markets, during transportation of animal for slaughter, in abattoirs during transport of meat to butcheries and even at the consumer home. Meat hygiene is essentially a public health function, the primary role of which is to safeguard against infectious diseases by preventing their transmission to humans thereby providing safe wholesome meat and

meat products for human consumption (Gill, 2004). To insure that meat quality standards are maintained, slaughter of animals for human consumption should be done in slaughterhouse. A slaughterhouse has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals. Processing and effective preservation and storage of meat products for human consumption is also practiced in slaughterhouse (Jenni, 2012).

In general, the muscles of alive healthy animal are sterile while lymph nodes of some organs and especially the surfaces exposed to environment such as hides (pelts or fleeces the moth and the gastrointestinal track carry extensive contamination (Maja, 2007). Variety of source including air, water, soil, faces, feed, hides, intestine, and lymph, processing equipment, utensils and humans contribute to the microbial contamination of sterile muscles of health animals during resting prior to admission to slaughterhouse, feeding, Stunning, slaughtering method, skinning, evisceration and further processing and handling (Sofos, 2002a). Meat contaminating bacteria are the major direct cause of food-borne diseases and represent a potential source for the drug resistance of human pathogenic agents. The most commonly recognized food-borne infections are those caused by the bacteria like *Campylobacter spp*, *Salmonella spp*, *E. coli O157:H7*, *Listeria spp*, *streptococcus cereus* and by a group of viruses called Calici virus, also known as the Norwalk and Norwalk-like viruses (Olsen *et al.*, 2000). The viable count of bacteria (VC) expressed as organisms/cm² or as organisms/g on fresh meat or a meat product sets a limit to its shelf-life. Meat will

“spoil” with VC at $10^6/\text{cm}^2$ because of off-odor’s. Slime and discoloration appear at $10^8/\text{cm}^2$ (Hassan *et al.*, 2010). The aims of this study were:1/ To detect the cause of organ condemnation in Alkadro slaughterhouse. 2/ To assess the microbiological contamination of carcasses at different stages of meat preparation. 3/ To quantify the contribution of different processing stages on the final contamination of meat.4/To estimate the correlations between pH and the total number of viable bacteria in the carcasses

Materials and Methods

The study was carried out at Alkadro slaughterhouse in Khartoum State between

February to September, 2015. The numbers of animals slaughtered in period between May 2014 to April 2015 were 38.290 cattle and sheep Table (1). a total of 120 swab samples were taken. Meat samples were collected from different stages in critical control points of meat product processing which included: Tables surface, hocks, knives, and meat handler’s hand and carcasses surface the samples were taken by swabbing an area of 3 cm^2 for 15 second (Koller, 1984). Carcass – samples were taken from flank area covering an area of 10 cm^2 . The procedure was repeated after evisceration and inspection. Ten air samples were taken by opening of prepared Petri dishes for specific periods of time (30minutes) in the area to be assessed (Hill *et al.*, 1984).

Table (1): Number of animals slaughtered at AlKadaro Slaughter for local consumption during May 2014 – April 2015, Alkadro Slaughterhouse:

Month	Number of sheep	Number of cattle	Total
May 2014	1262	878	2140
2014June	938	882	1820
2014July	900	1005	1905
2014 August	903	144	1047
2014September	605	800	1405
2014 October	850	450	1300
2014November	1425	675	2100
December 2014	2825	713	3538
January 2015	4852	1536	6388
February 2015	4436	596	5032
March 2015	5151	714	5865
April 2015	4850	900	5750
Total	28.997	9293	38.290

The viable bacterial count was done. Each of the swab samples was taken, was emerged in 9 ml normal saline and shaken and serially diluted in 9ml normal saline i.e. 1/10, 1/100, 1/100, 1/10000 and $50\mu\text{l}$ from dilutions were spread over a Petri dish containing Plate Count Media and incubated at 37°C for 24

hours (Barrow and Feltham, 2003 and Monica, 2000).

pH value was carried out according to Nebraska, (2005). The pH meter was calibrated using pH 7 and pH 4 standardization buffers. Cut meat sample in small pieces were weighed Approximately 10

grams were put into a blender cup and 100 ml of distilled deionized water was added and blended for 30 seconds at a high speed, transferred sample to beaker, and the pH was

read as soon as possible. Stir bar was added to the beaker, turned on to stir plate and placed the pH electrode in the sample.

Results

Causes of condemnation in ALkadro slaughter house:

The main cause of condemnation in livers of beef carcasses was *Fasciolasp* (n=261)46.03%, lunges with aspiration pneumonia in was(n=32)3% and abscesses in heads were(n=43)7.5% (Table, 2). The main cause for condemnation in livers of mutton was abscesses n=603 (64.6%), hydatid cyst n=27(2.9%), pneumonia in lungs n=91(9.7%) and adhesion in hearts were n=28 (2.9%)Table (3).

Quantitative bacteriological findings:

Aerobic bacterial counts of tables' surfaces, worker's hands, knives, hooks and carcasses

are shown in Table (4). The result shows that the most mean count in hocks was 4.8×10^3 CFU/cm² and the lowest mean count was in worker's hands 2.7×10^3 CFU/cm². Carcasses after skinning showed the lowest mean count.

Viable count at different stages:

The result of viable count from air samples was as follows: highest mean in evisceration site was 80.8 CFU/plate, inspection site 63.25 CFU/plate, skinning site 51.80 CFU/plate and in washing site was 40.40 CFU/plate (Table 5). Correlation is significant at the 0.01 level (2-tailed). The correlations between pH and viable counts of bacteria were strong.

Table (2): Causes of beef organs condemnation, Alkadro slaughterhouse 2014 - 2015(Record ofAlkadro slaughterhouse).

Organs condemned	Causes of condemnation	NO. of condemned organs	Percentages of causes of condemnations from the total condemned organs (%)
Liver	Faciola	261	46.03%
	Abscess	47	8.3%
	Calcification	96	16.9%
	Fibroses	20	3.5%
	C.bovis	27	4.8%
	Congestion	11	2.1%
Lung	Pneumonia	32	5.8%
	Aspiration	17	3%
	Pneumonia	5	0.9%
	Abscess	8	1.4%
	Adhesion	8	1.4%
	Abscess	43	7.5%
Total		567	100%

Table (3): Causes of organs condemnation in mutton, Alkadro slaughterhouse 2014 - 2015(Record of Alkadro slaughterhouse).

Organs	Reasons of condemnation	N.O. of condemned organs	Percentages of causes of condemnations from the total condemned organs (%)
Liver	Hydatid cyst	27	2.9%
	Abscess	603	64.6%
	Calcification	128	13.7%
	Fibroses	16	1.7%
Lung	Pneumonia	91	9.9%
	Aspiration	37	3.9%
	Pneumonia		
Heart	Abscess	4	0.4%
	Adhesion	28	2.9%
Total		934	100%

Table (4): Mean (\pm SD) of quantitative bacteriological findings from different locations in Alkadro slaughterhouse:

Location	Number of Samples	Mean \pm SD	Maximum	Minimum
Tables	10	$4.2 \times 10^3 \pm 1.16$	1.2×10^5	2.2×10^1
Knives	10	$4.4 \times 10^3 \pm 0.41$	2.6×10^4	7.07×10^2
Hands	10	$2.7 \times 10^3 \pm 0.94$	3.9×10^4	1.9×10^2
Hocks	10	$4.8 \times 10^3 \pm 0.62$	3.9×10^4	1.9×10^2
Carcasses after skinning	10	$1.34 \times 10^2 \pm 0.49$	1.5×10^3	3.02×10^1
Carcasses after evisceration	10	$7.6 \times 10^2 \pm 0.42$	3.9×10^3	1.9×10^2
Carcasses after inspection	10	$1.8 \times 10^2 \pm 0.31$	5.01×10^2	3.9×10^1

Table (5): Mean (\pm SD) of viable count in different stage from air

Air location	Number of Sample	mean \pmSD	Maximum	Minimum
Skinning	10	51.80 ± 18.931	30	87
Evisceration	10	80.80 ± 7.396	72	92
Inspection	10	63.25 ± 11.449	50	81
Washing	10	40.40 ± 23.032	21	97
**		Highly significant ($P < 0.05$)		

Discussion:

Sanitation in the slaughterhouse is important to prevent contamination of meat. The type and extent of contamination depends on sanitation procedures, hygienic practices, product handling, processing, application of decontamination intervention, and condition of storage and distribution (CW Mwai, 2011). Data concerning the reasons of condemnation of meat following slaughtering were collected during May 2014 to April 2015. The major causes of condemnation of internal organs (liver; lung, head and heart) were as follows: livers (Faciolasis and abscesses), lungs (pneumonia and aspiration pneumonia), hearts (abscess and adhesion) and heads (abscess). Ali *et al.*, (2012) in Sudan, reported that the pathological conditions, causing liver condemnations. For instance, parasitic infections particularly food borne parasites such as fascioliasis, cysticercosis have been recognized. The whole carcasses were mainly condemned due to tuberculosis, cysticercosis, jaundice, pyemia and septicaemia, while abscesses were the main causes of partial condemnations of carcasses.

Gill (2004) has reported that wholesome meat which is hygienically produced, is pathogen free, retains its natural state and nutritive value, ensures to maintenance a degree of microbial contamination control and is unconditionally acceptable to the consumer. The study was conducted mainly to assess the evolution of the microbial contamination of mutton during its preparation process and to observe the use of hygienic practices which may reduce incidences of cross-contamination in the slaughterhouse and to check the bacterial load (count) that present in knives, tables, hooks, worker's hands, air and carcasses. Utensils, equipment's and cutting board

surfaces were identified as a major source of contamination in meat processing plant (Rahman, 2007).

In this study knives showed viable bacterial count of about 4.4×10^3 CFU/cm² (blade). This finding is in agreement with Hassan (2004) who reported that knives can carry 2.96×10^4 CFU/cm² (blade). Adzitey *et al.*, (2011) reported that the contamination could have been due partially to the contamination of carcasses and meat cuts by undisinfected tables and the handling of meat with unsterilized instruments such as knives. The study also shows that hocks had viable bacterial count of about 4.8×10^3 CFU/cm². This high account may be due to the rusting, lack of good hygienic operation and cleaning during slaughtering and the use of sanitizing agent.

Meat handler's hands showed viable bacterial count of about 2.7×10^3 CFU/cm². This was due to the weakness of awareness and guidance of the importance of personal hygiene. Guyon *et al.*, (2001) showed that preevisceration; de-fatting and associated workers materials are critical point for carcass. The low use of protective practices increased the risk of cross contamination because meat handlers were probable sources of contamination for microorganisms. This is in agreement with reports of the World Health Organization (WHO, 2004).

In the present study the carcasses surface after skinning, after evisceration and after inspection showed viable bacterial count of about

1.34×10^2 CFU/cm², 7.6×10^2 CFU/cm² and 1.8×10^2 CFU/cm², respectively. The results are in agreement with those stated by Abdalla *et al.*, (2009) who pointed out that the sources of meat contamination include the hands, arms of meat handlers, equipment and contact surfaces. These results are in agreement with those stated by Mohamed

(2002) and Hassan (2004). The number of bacteria detected in samples collected from Elssabauga and Alkadro slaughterhouses were 15×10^4 and 3.693×10^4 CFU/cm², respectively indicated that hygienic and sanitary condition were lacking, but the result disagree with those stated by Ahmad *et al.*, (2013); Harhoura *et al.*, (2012) and Paul and Sylvia (2014) who reported that a viable count in carcass surface were 2.6×10^5 CFU/g, 6.8×10^4 CFU/cm² and 1.64×10^9 CFU/g, respectively which were higher than Alkadro slaughterhouse indicating poor hygiene.

In this study the highest contamination was found on surfaces after evisceration which might be due to intestine rupture during evisceration leading to contamination of the area as well as dirty clothes or dirty hands of the workers (these results are in agreement with those stated by Mwai, C.W. (2011). The presence of a high number of viable bacterial counts expressed as organisms/cm² on fresh meat, a limit to its shelf-life. Meat will spoil with viable bacterial count at 10^6 /cm² because of off-odors. Slime and discoloration appears at 10^8 /cm² as described by the Agriculture and Consumer Protection Department (Hassan *et al.*, 2010). The degree of contamination of the skins of animals has a direct impact on the contamination of carcasses. The main factors determining the levels of viable bacterial count are contamination during slaughtering and processing, further contamination during storage, temperature, pH and relative humidity.

In this study air samples were taken from skinning, evisceration, inspection and washing sites. The viable count ranges were 30 – 87CFU, 72 – 92CFU, 50 – 81CFU and 21 – 97CFU, respectively. The result is in agreement with that stated by Hassan (2004) and Wafa and Elsanousi (2018) who pointed that the high count may be due to the high occupants of slaughter hall with different

workers and their continuing talking and movement, also the lack of good ventilation and exchange techniques. This study revealed that the main causes of condemnation in livers beef were *Fasciola spp* and in livers of mutton were abscesses. The level of contamination on carcasses was high especially after evisceration. The slaughterhouse building itself contributed to the low hygienic quality of meat. It was found that the equipment, workers, sanitation and air are the most important causes of microbial contamination and that will effect on health of the consumers. Low pH leads to decrease in growth of aerobic bacteria. The best solution to decrease meat contamination starting with the implementation of good hygienic practices and good manufacturing practices will be a step towards implementing HACCP system itself in the future. In addition to the more efforts in health education and training are needed to raise the awareness for meat handlers in the slaughterhouses.

References

- Abdalla, M. A.; Suliman S. E.; Ahmed D. E. and Bakhiet A. O. (2009). Estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan), *African Journal of Microbiology research*, 3(12) 882-886.
- Ahmad, M. U. D.; Sarwar, A.; Najeeb, M. I.; Nawaz, M.; Anjum, A. A.; Ali, M. A. and Mansur; N. (2013). Assessment of microbial load of raw meat abattoirs and retail outlets, *The Journal of Animal and Plant Sciences*, 23(3):745-748.
- Adzitey, F.; Teye, G. A., Kutah, W. N. and Adday, S. (2011). Microbial of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana, *Livestock research for rural development*, 23(5).

- Ali, D. K., Ismail, H. M. And Abdelgadir, A. E. (2012). Status of Milk and Meat Hygiene in Sudan. *J. Vet. Med. And Anim. Prod.* Vol. 3, No 1
- Barrow, G.I. and Feltham R. K. A. (2003). Cowan and Steel's Manual for the identification of medical bacteria, Cambridge University Press, London.
- CW Mwai. (2011). Risk of contamination of cattle carcasses with *Escherichia coli* O157 from slaughterhouse in Nairobi, Kenya (Doctoral dissertation, Faculty of veterinary medicine, University of Nairobi).
- Gill, E. O. (2004). Visible contamination on animals and carcasses and the microbiological condition of meat. *J. Food Prot.* 6 (2): 4] 3-19.
- Guyon, R.; Dorey, M. J. P. and Leclercq, A. (2001). Hazard Analysis of *Escherichia Coli* 0157: H7 contamination during Beef slaughtering in Calvados, France. *Journal of food Protection.* 64:9, 1341-1345; 42 refs.
- Harhoura, K. H.; Boukhors, K. T.; Dahmani, A.; Zenia, S. and Aissi, M., (2012). Survey of hygiene in ovine slaughterhouses of Algiers region by bacteriological analysis of carcasses. *African Journal of Microbiology Research* 6(22), 4722-4726.
- Hassan, A. N.; Farooqui, A.; Khan, A.; Ameera, Y.; Khan, A. Y. and Kazmi S. U. (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *J. Infect. Developing Countries.* 4(6): 382-388.
- Hassan, E. F. (2004). Sanitation and its impact on meat preparation at ALkadro export slaughter house, "Thesis for M.V.Sc. degree" University of Khartoum.
- Herenda, D.; Chambers, P. G.; Ettriqui, A.; Seneviratna, P. and Da Silva, T. J. P. (2000). Manual on meat inspection for developing countries. FAO animal production and health paper 119, ISBN 92-5-103304-8.
- Hill, R. A.; Wilson, D. M.; Burg, W. R. and Scotwell, O. C. (1984). Viable fungi in corn dust. *Appl. Environmental microbial* 47:84-87.
- Iroha, I. R.; Ugbo, E. C.; Ilang, D. C.; Oji, A. E. and Ayogu, T. E. (2011). Bacterial contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. *J. Public Heal. Epid.* 3 (2): 49-53.
- Jenni, N. (2012). Implementation of a quality management system in food production. M. Sc. Thesis, Sveriges Lantbruks University.
- Koller, W. C. (1984). Recovery of test bacteria from surfaces with a simple new swab-rinse-technique; a contribution to methods for evaluation of surface disinfectants. *Zentralbl. Bacterial, microbial. Hyg. I ABT. B.* 179.2: 112-124.
- Lawrie, R. A. and Ledward, D. A. (2006). Meat science. 7th Ed. Boca Raton Boston New York Washington, Cambridge England.
- Maja, M. (2007). Meat inspectors' manual abattoir hygiene, Veterinary Services National Department of Agriculture Republic of South Africa. Pretoria.
- Mohamed, B. A. (2009). Assessment of meat hygiene statues at Alssabaloga slaughterhouse in Khartoum state, M.Sc. Thesis, U. of K, Sudan.
- Mohamed, Y.A. (2002). Surface bacterial contamination of mutton Carcasses at the production and retail levels in Omdurman in Khartoum state, Sudan, M.Sc. Thesis, U. of K.
- Monica, C. (2000). District laboratory in tropical countries; part 2; United Kingdom Cambridge University Press.

Nebraska (2005). University of Nebraska-Lincoln, Institute of Agriculture and Natural Resources, Lincoln, NE 68588. 402- 472-7211.

Olsen, S. J.; Mac, K. L. C.; Goulding, J. S.; Bean, N. H. and Slutsker, L. (2000). Surveillance for foodborne-disease outbreaks-United States, 1993-1997. *MMWR CDC SurveillSumm*, 49(1), 1-62.

Paul, B. and Sylvia, A. B. (2014). Microbiological quality of meat at the abattoir and butchery levels in Kampala city, Uganda. *Internet Journal of Food Safety*, Vol.16, p.29-35.

Rahman, M. S. (2007). Handbook of food preservation, 2nd Ed. Taylor and Frances Group. Retail sale in Cote d'ivoire. *American Journal of Food*.

Sofos, J. N. (2002a). *Decontamination methods at slaughter-presented at pathogen reduction; A scientific dialogue a scientific symposium sponsored by the food safety and inspection service of the United States, Department at Agriculture, Washington.*

Sofos, J. N. (2005). Improving the safety of fresh meat, 1st Ed. Woodd head, Ltd and CRC press LLC.

Wafa, E. Ebrahim, and Elsanousi, S.M.(2018). Evaluation of Meat Safety Based on Prerequisite Programme of Hazard Analysis Critical Control Points in Alkadro Slaughterhouse, Khartoum State. *Sudan J. Vet. Res.*, Vol.33, p.47-53.

WHO, (2004). Developing and maintaining food safety control systems for Africa: current status and prospects for change. Proceedings of second FAO/WHO global forum of food safety regulators; Bangkok, Thailand Pp 12-14.