OCCURRENCE OF CLOSTRIDIUM PERFRINGENS IN SOME MEAT PRODUCTS IN SHARKIA AND KALYOBIA GOVERNORATES

Fatein, S. H.*; El-Taher, E. M. and Hemmat M. Ibrahim***

** Faculty of Veterinary Medicine, Moshtohour, Zagazig University*

*** Food Hygiene and Control Department, Animal Health Research Institute*

ABSTRACT

This study was conducted on 90 random samples of meat products (30 each of minced meat, beefburger and sausage. The examined samples were collected from different supermarkets at Sharkia and Kalyobia Governorates, and then subjected to bacteriological investigation for isolation and identification of Clostridium perfringens. The incidence of Clostridium perfringens in examined minced meat, beefburger and sausage was 26.67%; 16.67% and 40% respectively, from which 13.33%, 10% and 23.33% were Lecithinase positive strains. The isolated strains were typed into types A, B, D and C in 8.89%, 3.33%; 2.22% and 1.11 %, respectively. All isolated Clostridium perfringens type A strains resist heating at 100¡C for 60 minutes, while 87.5% resist for 120 minutes, meanwhile 62.5% resist for 150 minute and finally 37.5% resist for 180 minutes.

The importance of Clostridium perfringens and its effect on the public health as well as the scientific base which must be adopted in meat products industry was also discussed .

INTRODUCTION

Meat products proved to be available allover the world especially ready-to-eat types liable to harbour different types of microorganisms, among these microorganisms toxigenic anaerobes as Clostridium perfringens which is ubiquitous in nature usually detected in dust, soil food, intestinal contents of man, animals and work surfaces **(Bari et al., 1992 and Hayes, 1992).**

Clostridium perfringens spore may contaminate meat at abattoir level, during transmission as well as marketing level, while meat product contamination may occur during processing at cattering establishments and during home preparation **(Poxton et al., 1989)**. Clostridium perfring-

ens strains may cause a number of human diseases ranging from necrotic enteritis to wound infections and life threaten gas gangrene **(Norris and Pettipher, 1987)** and they are divided into five toxicological types on the basis of their toxins production **(Smith, 1979 and McDonel, 1980).**

Clostridium perfringens type A is responsible for food poisoning diseases as previously recorded by **(Tortora and Zebral, 1988, Hewitt et al., 1989 and Hayes, 1992).**

Clostridium perfringens type A strain produce enterotoxin which have markedly heat resistant spores are most frequently encountered with food poisoning. The ingesting of meat contaminated with Clostridium perfringens strains is considered to be unfit for human consumption and lead to food poisoning as previously mentioned by **Tortora and Zebral (1988); Hewitt et al. (1988); Poxton et al. (1989) and Hayes (1992).**

Clostridium perfringens strains were isolated from meat and meat products samples which had been investigated by **El-Naenaeey (1989); Itman et al. (1989); Guzman et al. (1990) and Hassan (1994).** Furthermore Clostridium perfringens strains were isolated from ready-to-eat meat products (minced meat, beefburger, sausage, luncheon etc) with different incidences as previously recorded by **El-Hosseiny (1987; El-Naenaeey 91989); Guzman et al., 1990); Edris et al. (1992); Hassan (1994); El-Lawendy (1996) and Hassan (1999).**

Clostridium perfringens might find their way into meat and meat products either directly from the slaughtered animals or by subsequent contamination from the hands of cookers, butchers and other meat handlers in slaughterhouses, meat markets and retail shops as previously mentioned by **Gotz (1976) and Milev (1976).**

Clostridium perfringens strains were survived heating at 100°C (Boiling or steaming) for variable times as recorded by; **Wijewanta (1972) and Hussein (1977).** Furthermore Clostridium perfringens type A strains were the most frequent heat resistant strains as recorded by **El-Bardisy (1984), El-Naenaeey (1989), Hassan (1994); El-Lawendy (1996) and Hassan (1999).**

Therefore the main purpose of the current study is to investigate the prevalence of Clostridium perfringens in some meat products marketed in Sharkia and Kalyobia Governorate, typing of the isolates and the effect of heat on the Clostridium perfringens isolates and throw light on the required sanitary measures which minimize the possible hazards during consumption of such meat products which had been contaminated with these toxigenic strains.

MATERIALS AND METHODS

A total of 50 samples of different meat products represented by minced meat, beefburger and

sausage (30 of each) were collected randomly from supermarkets in Sharkia and Kalyobia Governorates. Samples were transferred to the laboratory in ice box and kept in retail packages under complete aseptic conditions without undue delay for bacteriological examination

Clostridium perfringens count was assayed according to the technique adopted by ICMSF (1978) using cooked meat broth tubes incubated anaerobically for 24 hours at 37°C followed by subculturing onto Neomycin Blood Agar plates which were incubated also anaerobically at 37°C for 48 hour. Suspected colonies of Clostridium Perfringens were tested for Lecithinase activity by using Egg Yolk Agar plates (Nagler's reaction). Furthermore isolated strains were typed by intradermal inoculation test in Gina pigs. However, the heat resistant test of Clostridium perfringens spores was carried out at 100°C for 60, 120, 150 and 180 minutes according to **Hussein (1977)**.

RESULTS & DISCUSSION

The ubiquity of potentially life threatening pathogens in our environment, the ability of them to survive or proliferate in reduced oxygen atmosphere and under refrigeration as well as its presence in low number is necessary for disease production, which indicates the seriousness and potential hazards **(Ganowiak, 1986)**.

Table (1) illustrated that the sausage samples were more highly contaminated with Clostridium perfringens ($6.82 \times 10^3 \pm 1.05 \times 10^3$ /gm) by minced meat samples ($2.64 \times 10^3 \pm 0.46/10^3$/gm) while the beefburger samples were least contaminated ($9.27 \times 10^2 \pm 2.12 \times 10^2$/gm). At the same time Clostridium perfringens was more frequently isolated from sausage samples (40%); followed by minced meat samples (26.67%) and beefburger samples (16.67%). Moreover, among the isolated Clostridium perfringens strains from minced meat, beefburger and sausage samples 13.33%, 10% and 23.33% were Lecithinase positive, respectively. In this work, the incidence of Clostridium perfringens in sausage samples was lower than results which previously reported by **El-Naenaeey (1989); Guzman et al. (1990) and El-Lawendy (1996)**, where Clostridium perfringens was isolated from the examined sausage samples in 62.7%, 78.9%, 80.8% and 62%, respectively. On the other hand lower incidence was recorded by **Youssef (1984)** 25%; **El-Kelish et al. (1987)** 20% and **Hassan (1999)** 25%. In the current study Clostridium perfringens was isolated from 40% of minced meat samples. This results seems to be agree with those recorded by El-Naenaeey (1999) 40% and **Hassan (1999)** and oppositely higher findings were recorded by **Youssef (1984)** 77.1%.

Regarding to beefburger samples, Clostridium perfringens was isolated in 16.67%, this result agree well with those reported by **Hassan (1999)** 15%, while higher incidence was recorded by

Edris et al. (1992) 90% and **El-Lawendy (1996)** 60.32%. The variation may be attributed to different factors such as initial bacterial count of meat, hygienic status of meat plants; excessive handling during processing and addition of contaminated spices.

Regarding the results recorded in table (2) typing of 14 strains of Lecithinase positive Clostridium perfringens indicated that recovery of 8 strains type A, 3 type B, 2 type D and 1 type C from the examined minced meat product samples. Accurately, type A was detected from 16.67% sausage samples, 6.67% from minced meat samples and 3.33% from beefburger samples, while type B was recorded in 6.67% and 3.33% each of beefburger and minced meat samples respectively.

On the other hand type D was detected in minced meat and sausage samples (3.33% of each-0, whereas type C was only recovered from 3.33% of the examined sausage samples. This findings nearly similar to those recorded by **El-Naenaeey (1989); Hassan (1994); El-Lawendy (1996); Miwa et al. (1998) and Hassan (1999).**

Concerning data in table (3) Clostridium perfringens type A strains isolated from heat-treated sausage samples were resistant to cooking temperature (for 180 minutes at 100°C rather than beefburger samples till 120 minutes). Moreover isolates from minced meat was resistant for 150 minutes. Nearly similar results were obtained by **El-Naenaeey (1989) and Hassan (1999).** In this target **Smith (1979)** recorded that Clostridium perfringens type A strains can survive cooking and has the ability to produce heat resistant spores. Whereas **Robers and Derrick (1978)** reported that spores of classical strains which responsible for human illness other than food poisoning were more sensitive to heat than spores of food poisoning one. Hence there are apparent relationship between spore heat resistance and the response of spore to heat inactivation. **Baver et al. (1981)** declared that spores of Clostridium perfringens surviving cooking or resulting from post-processing contamination must multiply until sufficient numbers were developed to cause illness, and this may attributed to genetic differences among Clostridium perfringens strains regarding to the heat resistance of their spores **(Doyle, 1989).**

Clostridium perfringens food poisoning does not occur by accident but is usually caused by ignorance, which leads to mistakes in food handling. Meat hygiene covers all aspects of processing, preparation, storing, cooking and serving of meat to make safe to eat. The adaptation of recent quality assurance programs such as Hazard Analysis Critical Control System (HACCPS) and good manufacturing Practices (GMPs) are required to ensure food safety.

Table (1) : Incidence of Clostridium perfringens isolated from the examined meat product samples (n=30).

Meat product	Positive samples		Lecithinase positive		Max.	Min.	Mean ± S. E.
	No.	%	No.	%			
Minced meat	8	26.67	4	13.33	6.40×10^3	8.70×10^2	$2.64 \times 10^3 \pm 0.46 \times 10^3$
Beefburger	5	16.67	3	10.00	1.04×10^3	5.90×10^2	$9.27 \times 10^2 \pm 2.12 \times 10^2$
Sausage	12	40.0	7	23.33	2.10×10^4	1.26×10^3	$6.82 \times 10^3 \pm 1.05 \times 10^3$

Table (2) : Typing of Lecithinase positive strains of Clostridium perfringens in the examined meat products samples (n=30).

Toxin / Meat product	A		B		C		D		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Minced meat	2	6.67	1	3.33	-	-	1	3.33	4	13.33
Beefburger	1	3.33	2	6.67	-	-	-	-	3	10.00
Sausage	5	16.67	-	-	1	3.33	1	3.33	7	23.33
Total	8	8.89	3	3.33	1	1.11	2	2.22	14	15.56

Table (3) : Heat resistance of Clostridium perfringens type A strains isolated from the examined meat product samples.

Product	Type of strains	Resistance Time							
		60 minutes		120 minutes		150 minutes		180 minute	
		No.	%	No.	%	No.	%	No.	%
Minced meat	2	2	100	1	50	1	50	-	-
Beefburger	1	1	100	1	50	-	-	-	-
Sausage	5	5	100	5	100	4	80	3	60
Total	8	8	100	7	87.5	5	62.5	3	37.5

REFERENCES

Bari, A.; Amar, M. Y.; Siddiqui, S. A. and Asif, M. (1992) : Anaerobic and aerobic enterotoxigenic bacteria in ready-to-eat foods in Pakistan. Proceeding of 3rd World Congress Foodborne Infection and Intoxication, Berlin, Germany.

Baver, F. T.; carpenter, J. A. and Reagan, J. D. (1981) : Prevalence of Clostridium perfringens in pork during processing. J. Food Protec. 44 (4): 279-283.

Doyle, M. D. (1989) : Foodborne Bacterial pathogens. Pp. 193-231. Marcel Dekker, Inc. New York and Basal.

Edris, A. M.; El-Bardisy, M. and Mousa, M. M. (1992) : Studies on microbial status on some marketed meat products with special reference to C. perfringens. Egypt. J. Appl. Sci., 7 (12): 19-30.

El-Bardisy, M. M. M. (1984) : Bacteriological studies C. perfringens on in meat. M.V.Sc. Thesis (Microbiology) Fac. Vet. Med. Cairo University.

El-Hosseiny, M. M. (1987) : Bacteriological studies on meat and meat products. Ph.D. Thesis (Microbiology). Fac. Vet. Med. Cairo University.

El-Kelish, H.; Morrshdy, A.; El-Atabany, A. and Hafez, A. (1987) : C. perfringens in fresh sausage marketed in Zagazig city. Zagazig Vet. J. (1) :159-165.

El-Lawendy, H. M. T. (1996) : Occurrence of C. perfringens in locally produced meat products. M.V.Sc. Thesis (Meat Hygiene) Fac. Vet. Med., Moshtohour Benha Branch, Zagazig University.

El-Naenaeey, E. Y. M. (1989) : Occurrence of C. perfringens type A. Ph. D. Thesis (Microbiology) Fac. Vet. Zagazig University.

Ganowiak, Z. (1986) : Objectives of investigations of foodborne disease outbreaks. Proceedings of the 3rd Congress, Foodborne Infection and Intoxication Berlin, Germany.

Gotz, J. (1976) : The presence of C. perfringens in bovine, broilers and possible contamination during slaughtering. Fleischwirtschaft., 56 (2): 231-235.

Guzman, A. M. S. de; Micalizzi, B.; Pagano, C. E. T. and Gimenez, D. F. (1990) : Incidence of C. perfringens in fresh sausage in Argentina. J. Food Protec. 53 92): 173-175.

Hassan, M. A. (1994) : Incidence and serotyping of C. perfringens among animals slaughtered at Cairo and Giza abattoirs. M. V.Sc. Thesis (Meat Hygiene) Fac. Vet. Med., Moshtohour, Benha Branch, zagazig University.

Hassan, M. A. (1999) : Follow up of some pathogens in meat products and their resistance to certain preservatives. Beni-Suef vet. Med. J. 9 (3): 417-429.

Hayes, P. R. (1992) : Food Microbiology and Hygiene. 2nd Ed Elesevir Applied Science London New York.

Hewitt, J. H.; Begg, H.; Hewish, J.; Rawaf, S.; Stringer, M. M. and Theodore, G. B. (1989) : Large outbreaks of C. perfringens food poisoning associated with the consumption of boiled salmon. J. of Hygiene, 97 91): 71-80.

Hussein, A. Z. (1977) : The physical conditions of cattle before slaughtering and its relationship to probable isolation of C. perfringens from carcasses. Kande at nayk, Varonsih Agriculture Institute, USSR.

International Commission on Microbiological specification for Foods "ICMSF" (1978) : Microorganisms in Foods. Their Significance and Methods of Enumeration 2nd ed. Univ. of Toronto Press, Toronto, Buffalo, London.

Itman, R. H.; Ikbol Farrag; Arab, F. M. A. and Makhareta, M. A. M. (1989) : A preliminary investigation on some anaerobic bacteria in the liver of buffaloes and camels. Vet. J. Giza, 37 (3): 489-500.

McDonel, J. L. (1980) : Clostridium perfringens toxins (Type A, B, C, D, E). Pharmacology and Therapeutics, 10: 617-655.

Milev, M. (1976) : Normal status of Bacillus cereus and C. perfringens in the body of normal animals (cattle, sheep and swine). Vorogenizma Veterinarerneditisinki Nauki, 13 (4): 53-58.

Miwa, N.; Nishina, T.: Kubo, S.; Atsumi, M. and Honda, H. (1998) : Amount of enterotoxigenic Clostridium perfringens in meat detected by nested PCR. J. Food Microbiol., 43 (3): 195-200.

Norris, J. R. and Pettipher, G. L. (1987) : Essays in Agriculture and Food Microbiology. John Wiley and Sons Chichester, New York, Brisbane, Toronto.

Poxton, L.; Brown, R.; Fraser, A. G. and Colle, J. G. (1989) : Enteropathogenic Clostridia and Clostridium botulinum. Practical Medical Microbiology. 13th Edition Churchill Livingstone London and New York.

Roberts, T. A. and Derrick, C. (1978) : Cited after Doyle (1989). Smith, L. D. S. (1979) : Virulence factors of Clostridium perfringens.Review of Infectious Diseases, 1: 254-262.

Tortora, J. D. de O. and Zebral, A. A. (1988) : A human food poisoning outbreak in Riode Ja-

neiro caused by sausage contaminated with enterotoxigenic Clostridium perfringens type A. Rvista de Microbiologia, 19 (2): 133-118 CBA.

Wijewanta, E. A. (1972) : Isolation of heat resistant Clostridium perfringens from healthy cattle. Cornell Veterinarian, 62 (1): 26-31.

Youssef, H. (1984) : Incidence of Clostridium perfringens in meat products in Assuit city. Assiut Vet. Med. J. 12 923); 145-147.

الملخص العربى

مدى تواجد الكلوستريديم برفرنجينز فى بعض منتجات اللحوم بمحافظتى الشرقية والقليوبية

د/ فـاتـن سـيد حسانين د/ السيد جودة محمد الطاهر*

د/ همت مصطفى إبراهيم

كليـــة الطـب البيطـرى بمشـتهر - جامعـــة الزقـــازيق

* قسم الرقابـة الصحية على الأغذيـة - معهـد بحـوث صحـة الحيـوان

أجريت الدراسة على عدد تسعون عينة عشوائية من منتجات اللحوم (٣٠ عينة من كل من اللحم المفروم والبيف بيرجر والسجق) جمعت تلك العينات من الأسواق المختلفة بمحافظتى الشرقية والقليوبية وتم فحصها بكتيريولوچيا لعزل وتصنيف ميكروب الكلوستريديم برفرنجيتر.

كانت نسبة ميكروب الكلوستريديم برفرنجينز ٢٦٫٦٧ - ١٦٫٦٧ و ٤٠٪ لكل من اللحم المفروم والبيف بيرجر والسجق على التوالى منها ١٣٫٣٣٪ و ١٠٪ و ٢٣٫٣٣٪ كانت إيجابية لإنزيم الليسينيز.

صنفـــت العـــتـــرات المـعـــزولة إلى أ، ب، ج، د بنســـب ٨٩٫٨٪، ٣٣٫٣٪، ٢٢٫٢٪، ١١٫١٪ على التوالى.

كانت كل عترات النوع أ مقاوم للحرارة عند ١٠٠م لمدة ساعة و٨٧٫٥٪ منها مقاومة للحرارة لمدة ساعتين بينما ٦٢٫٥٪ مقاومة لمدة ساعتين ونصف و٣٧٫٥٪ مقاومة لمدة ثلاث ساعات.

كما نوقشت أهمية تلك الميكروبات وتأثيره على الصحة العامة وكذلك التوصيات الواجب إتباعها فى مصانع منتجات اللحوم.