Research Institute of Animal Health, Assiut.
Director: Prof .Dr .S.M. Nashed.

OCCURRENCE OF CAMPYLOBACTER JEJUNI AND LISTERIA MONOCYTOGENES IN HEN'S EGGS

(With 2 Tables)

By

SABAH MOUSTAFA

(Received at 25/10/1992)

تواجد الكامبيلوباكتر جيوجيناى والليستيريا مونوسيتوجين فى بيض الدجاج الطازج

صباح مصطفى

حيث أن البيض ذو قيمة غذائية عالية كما انه يدخل فى تصنيع العديد من المواد الغذائية الا انه يعتبر مصدرا للميكروبات الممرضه لذا فانه يشكل خطورة على صحة المستهلك.

للتعرف على مدى تواجد ميكروبى الكامبيلوباكتر جيوجيناى والليستيريا مونوسيتوجين فى بيض الدجاج الطازج تم جمع ٢٠٠ عينة عشوائية من اسواق ومحلات البقالة بمدينة أسيوط ممثلة فى ٥٠ مجموعة كل واحدة تحتوى على ٤ بيضات.

تم تجهيز البيض المراد فحصه لمحاولة عزل هذه الميكروبات من على السطح الخارجى للقشرة، من القشرة مع اغشيتها وكذلك من المحتويات الداخلية للبيضة.

تبين من هذه الدراسة ان ميكروب الكامبيلوباكتر جيوجيناى يتواجد بنسبة ١٢٪ فى بيض الدجاج الطازج الذى تم فحصه ولقد تم عزل الميكروب بنسبة ٢٪، ٦٪، ٤٪ من على سطح القشرة الخارجى، من القشرة مع اغشيتها ومن المحتويات الداخلية للبيضة كما انه لم يستدل على وجود ميكروب الليستيريا مونوسيتوجين.

تم مناقشة الاهمية الصحية للميكروبات التى عزلها وما يجب اتباعه لدرء خطرها.

CAMPYLOBACTER JEJUNI, LISTERIA MONOCYTOGENES HEN'S EGGS

SUMMARY

200 of fresh hen's eggs were collected at random from Assiut markets and different groceries. Both the shell and the contents of the eggs were examined bacteriologically for the presence of Campylobacter jejuni and Listeria monocytogenes. Cultures from eggs were performed from swabbing on their surfaces, from homogenates of shells and membranes, and from the egg contents. *C. jejuni* were detected in 12% of the analyzed samples. The organism was present in 2% of shell surface swabs, 6% of shell and shell membranes homogenates and in 4% of the egg white and yolk. *L. monocytogenes* failed to be detected in the examined egg samples. The significance of *C. jejuni* and *L. monocytogenes* as foodborne pathogens was discussed.

INTRODUCTION

Shell eggs have many uses in the home, in restaurants, and in institutions, either fried, hard cooked etc. or as ingredients in other foods. Egg products generally are utilized in the food industry in mixes, bakery foods, noodles, mayonnaise and salad dressings, candies, ice cream etc. There are also several other uses, such as its use in pet foods. The widespread use of eggs as ingredients of other foods makes them prime suspects in foodborne disease outbreaks. Thus potential hazard requires careful microbiological control in production and usage *(BERGQUIST et al., 1984)*.

The shell and the contents of the eggs at time of oviposition are either sterile or harbour very few microorganisms *(FORSYTHE et al., 1953; BROOKS & TAYLOR, 1955 and STADELMAN & COTTERILL, 1977)*. Contamination of the shell occurs afterwards from nesting material, floor litter, and avian faecal matter. The contaminating flora are mainly gram-positive cocci but gram-negative rods that cause spoilage of shell eggs are also present in small number *(BOARD, 1973)*.

Campylobacter jejuni has been recognized as an important cause of human enteritis with isolation rates from diarrheia patients often equaling or exceeding the rates for Salmonella spp. or Shigella spp. Animals are the main reservoir for C. jejuni and an important means of transmission via foods of animal origin, Prominent among these foods are various poultry

products *(BLASER, 1982; PRESCOTT & MUNORE, 1982 and SKIRROW, 1982).*

The chicken is regularly cited as the likely source of C. jejuni infections in man. The presence of Campylobacters have been demonstrated in the intestines of chickens and their products *(FLETCHER & plastridge, 1964; SMIBERT, 1965 and SMITH & MULDOON, 1974).*

Listeria monocytogenes is a potential foodborne pathogen which can cause abortion in pregnant women and meningitis or meningio-encephalitis in imunocompromised men and women *(GRAY & KILLINGER, 1966; RALOVICH, 1984; and MARTH, 1988).* L. monocytogenes has been isolated not only from a large number of mammalian species, but also from at least 17 different avian species *(GRAY, 1958).* Listeria infection in fowls has been most commonly manifested by septicaemia, and the bacterium can be isolated from most of the viscera, particularly liver or spleen and occasionaly brain. Although the presence of necrotic lesions in the oviduct of some hens with listeria infecteion suggests the possibility that eggs may contain L. monocytogenes, this has never been confirmed *(GRAY and KILLINGER, 1966).*

This paper reports on the presence of C. jejuni and L. monocytogenes in fresh hen's eggs.

MATERIAL and METHODS

200 of fresh hen's eggs (50 groups) were collected at random from Assiut markets and different groceries. Every four eggs (one group) were packaged in sterile separate bag and dispatched to the laboratory.

Eggs were tested by swabbing on their surfaces, from homogenates of shells and membranes, and from egg contents. The prepared samples from the four eggs of each group were thoroughly mixed and analyzed for the presence of C. jejuni and L. monocytogenes.

Both selective broth enrichment and direct plating were used for isolating Campylobacters from the prepared sample groups of surface swabs, homogenates of shell and membranes, and the interior contents. Homogenous samples were placed in separate cotton-plugged flasks of Brucella-FBP broth supplement *(GEORGE et al., 1978).* Inoculated flasks were incubated under static microaerobic conditions at 42°C for 48 hours. Loopfuls of enrichment culture were streaked onto Brucella-FBP agar plates which inverted and placed in an anaerobic jar with a microaerobic atmosphere. The anaerobic jars were incubated at 42°C for 48 hours. Colonies resembling those of Campylobacter (smooth, convex, translucent, colourless to cream colured,

pin-point to 2 to 4 mm in diameter, or often showing confluent growth) were selected and transferred to semisolid Brucella medium tubes containing neutral red indicator. Inoculated tubes were incubated aerobically at 42°C and the culture is used for biochemical and growth tests according to the recommended methods outlined by *PARK* et al. *(1984)* and cited in the Compendium of Methods for the Microbiological examination of Foods.

Moreover, all the prepared samples were directly streaked onto brucella-FBP agar plates. All inoculated plates were incubated at 42°C for 48 hours in microaerobic gas mixture of 10% Co2, 5% O2 and 85% N2 using Campylobacter Gas Generating Kits (Oxoid). Suspected colonies were subjected to biochemical characteristics according to the procedures described by *PARK* et al. *(1984)*.

Isolation of L. monocytogenes from the prepared samples was done by suspending them in Tryptose phosphate broth supplemented by 40 mcg/ml naldixic acid, 30 U/ml polymxin B and 10 mcg/ml trypaflavine. Inoculated broth was kept at 28°C for 5 days then one loopful was transferred to *RALOVICH* et al. medium (Tryptose agar with 5% normal horse serum) modified by *HOFER (1982)*. Inoculated plates were incubated at 37°C for 48 hours and colonies showed a darkened central area were kept on Tryptose agar slants for confirmation. According to the procedures adopted by *GRAY and KILLINGER (1966) and RALOVICH (1984)* isolates giving a catalase reaction and that were motile at 21°C were examined further with API 20 S strips (Analytab Products). Serological slide agglutination tests were done on isolates thought to be L. monocytogenes using commercially prepared Listeria O Antiserum Poly (Difco).

Further, direct isolation from the examined samples was carried out on Tryptose agar plates and colonies resembling L. monocytogenes were confirmed as described above.

RESULTS

Results showing the recovery of C. jejuni and L. monocytogenes are presented in Tables (1 & 2). Of the 50 groups (200 eggs) examined 6 (12%) were found to contain C. jejuni. The recovery rate of the organism from the shell surfaces, homogenates of shell and shell membranes, and the egg contents was 2%, 6% and 4% respecively. On the other hand, L. monocytogenes could not be isolated from the exterior or the interior of the examined hen's eggs.

DISCUSSION

Like other foods, the composition of the egg influences the types of organisms. Since the parts of the egg differ considerably in composition susceptibility, to spoilage or growth of pathogens differ considerably in each part *(BERGQUIST et al., 1984).* The egg contents may become contaminated by improper washing and storage methods. The physical and/or chemical barrier produced by the egg shell, shell membranes, and the antimicrobial substances in the albumin favour the penetration and the multiplication of the Gram-negative bacteria *(HARTUNG & STADELMAN, 1962; BOARD, 1964 and FOSTER, 1971).*

C. jejuni has received considerable attention as a prominent causative agent in cases of human gastroenteritis since *SKIRROW (1977)* identified the organism as the cause of a new disease. Clinical findings suggest that the organism is responsible for many cases of enteritis as in Salmonella sp. *(BLASER et al., 1977).*

The present study demonstrates that 6 out of 50 groups of hen's eggs (12%) are reservoirs of C. jejuni and constitutes potential sources of enteric infection. The incidence of C. jejuni in the shell surfaces, shell and shell membranes homogenates, and egg contents was 2%, 6% and 4% respectively. In this respect, C. jejuni was detected in 96% of cloacal samples from alive birds, 84% of 25 processed birds ready for sale, 55% of 200 caged laying hens and 25% of 200 freshly laid eggs. Cultures from eggs were performed from swabbing on their surfaces and from homogenates of shells and membranes *(FIGUERO et al., 1983). ACCUF et al. (1982)* failed to isolate C. jejuni from fertile turkey eggs or from newly hatched turkey poults obtained on two occasions from two commercial hatcheries. The organism was present in 16 to 76% faecal swabs of 15-to 19 day old turkey from two commercial facilities, and was isolated from litter and drinking water. Besides, *CRUICKSHANK et al. (1983)* reported that C. jejuni is not an essential constituent of the bowel flora in chickens and the incidence of excreting flocks varies from place to another. Natural egg transmission was not demonstrated.

L. monocytogenes has been recognized as a human and animal pathogen for more than 50 years; however its presence has only recently surfaced *(GRAY, 1963 and DOYLE, 1984).* Although L. monocytogenes has been often isolated from fowls with other disorders, there are numerous reports of primary listeria infection *(GRAY and KILLINGER, 1966).*

L. monocytogenes failed to be detected during the course of our study as the present widespread use of poultry feeds containing antibiotics have prophylactic value against listeria infection in domestic birds. This substantiates what have been reported by *CSONTOS* et al. *(1955) and GRAY & KILLINGER (1966).*

In our environment appropriate handling and cooking of chicken and eggs appear as an important steps in reducing the risk of human infection.

REFERENCES

Accuf, G.R.; Vanderzant, C.; Grander, F.A. and Golan, F.A. (1982): Examination of turkey eggs, poults and brooder facilities for Campylobacter jejuni. J. Food Prot. 45, 14: 1279-1281.

Bergquist, D.; Kraft, A.; Cotterill, O. and Magwire, H. (1984): Products. In the Compendium of Methods for the Microbiological Examination of Foods. 2nd Ed. Speck, M.L. (ed.). Washington, D.C. American Public Health Association.

Blaser, M.J. (1982): Campylobacter jejuni and food. Food Technology 36, 3: 89-92.

Blaser, M.J.; Berkowitz, I.D.; LaForce, F.M.; Cravens, J.; Reller, L.B and Wang, W.L.L. (1977): Campylobacter enteritis: Clinical and epidemiological features. Ann. Intern. Med. 91: 179-185.

Board, R.G. (1964): The growth of Gram-negative bacteria in hen's eggs. J. Appl. Bacteriol. 27: 350-364.

Board, R.G. (1973): The microbiology of eggs. In Egg Science and Technology. Stadelman, J. and Cotterill, O.J. (eds). The AVI Publishing Company, Inc. Westport, C.N.

Brooks, j. and Toylor, D.I. (1955): Eggs and egg Products. Rep.Fd. Invest. ,Bd, 60, H.M.S.O. London, England.

Csontos,L. ; Derzsy,D. and Baranyl, I.T. (1955): Listeriosis in young geese. Acta Vet.Hung. 5:261-277.

Cruickshank, J.G. ; Egglestone,S.I. ; Gawler, A.H.L. and Lanning, D.G. (1981): Campylobacter Jejuni and the broiler chicken process. In Campylobacter: Epidemiology, Pathogenesis and Biochemistry, Newell, D.G. (ed.) MTP Press Limited, International Medical Publishers, Lancaster Boston the Hague.

Doyle, M.P. (1984): Listeria monocytogenes-A pathogen of renewed interest. Annual Spring Meeting. Food Research Meeting. Food Research Institute. University of Wisconsin-Madison, U.S.A.

Figueroa, G.; Hidalago, H.; Troncoso, M.; Rosende, S. and Soto, V. (1983): Campylobacter jejuni in broilers, hens and eggs in a developing country. In Campylobacter II. Proceedings of the Second Internationa Workshop on Campylobacter Infections. Brussels, 6-9 September 1983. Pearson et al. (edss.). Public Health Laboratory Service. London.

Fletcher, R.D> and Plastridge, W.N. (1964): Difference in physiology of Vibrio spp. from chicken and man. Avian Diseases 8: 72-75.

Forsythe, R.H.; Ayers, J.C. and Radlo, J.L. (1983): Factors affecting the microbiological populations of shell eggs. Food Technology 11: 56-60.

Foster, E.M. (1971): The control of Salmonella in processed foods. A classification system and sampling plan. J. Assoc. Offic. Agr. Chemists 54, 2: 259-266.

George, H.A.; Hoffman, P.S.; Smibert, R.M. and Krieg, N.R. (1978): Improved media for growth and aerotolerance of Campylobacter fetus. J. Clin. Microbiol. 8: 36-41.

Gray, M.L. (1958): Listeriosis in fowls- a review. Avian Diseases 2: 296-314.

Gray, M.L. (1963b): Epidemiological aspects of listeriosis. Am. J. Public Health 53: 551-563.

Gray, M.L. and Killinger, A.H. (1966): Listeria monocytogenes and listerc infections. Bacteriol. Rev. 30: 309-382.

Hartung, T.E. and Stadelman, W.T. (1962): Influence of metallic ions on the penetration of egg shell membranes by Pseudomonas flourescens. Poultry Sci. 41: 1590-1596.

Hofer, E. (1982): Bacteriological and epidemiological studies on the occurrence of Listeria monopcytogenes in helathy cattle. Zbl. Bakt. Hyg. A 250: 175-183.

Marth, E.H. (1988): Disease characteristics of Listeria monocytogenes. Food Technology 24, 4: 165-168.

Park, C.E>; Smibert, R.M.; Blaser, M.J.; Vanderzant, C.V. and Stern, N.J. (1984): Campylobacter. In the Compendium of Microbiologal Examination of Foods. 2nd Ed. Speck, M.L. (ed). Washington, D.C. American Public Health Association.

Prescott, J.F. and Munore, D.L. (1982): Campylobacter jejuni enteritis in man and domestic animals. J. Am. Vet. Med. Assoc. 181: 1524-1530.

Ralovich, B. (1984): Listeria research-Present situation and perspective. Akademial Kiado, Budapest, Hungary.

Skirrow, M.B. (1977): Campylobacter enteritis: a new disease. Br. Med. J. 2: 9-11.

Skirrow, M.B. (1982): Campylobacter enteritis. The first five years. J. Hyg. 89: 175-184.

Smibert, R.M. (1956): Vibrio fetus var intestinalis isolated from the intestinal contents of birds. Am. J. Vet. Res. 30: 1437-1442.

Smith, M.V. and Muldoon, P.J. (1974): Campylobacter fetus ssp. jejuni (Vibrio fetus) from commercially processed pultry. Appl. Microbiol. 27: 995-996.

Stademan, W.J. and cotterill, O.J. (1977): Egg Science and Technology. AVI Publishing Co., Westport, C.N.

Table 1

Incidence of C. jejuni and L. monocytogenes in hen's eggs

Organisms	No. of samples exmined	No. positive	Percent positive
C. jejuni.	50	6	12
L. monocytogenes	50	0	0

Table 2

Recovery rates of C. jejuni from the examined hen's eggs

Sample description	No. positive/No. analyzed	% positive
Shell surface	1/50	2
Homogenates of shell and membranes.	3/50	6
Egg contents.	2/50	4
Total	6/50	12