Vet.Med.J.,Giza. Vol.50, No.4. (2002) : 571-579.

INCIDENCE OF LISTERIA AND YERSINIA SPECIES AMONG SLAUGHTERED POULTRY AND RABBIT WITH SPECIAL REFERENCE TO ITS ZOONOTIC IMPORTANCE

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Received: 7.8.2002.

Accepted: 25.8.2002.

SUMMARY

This work was undertaken to determine the degree of contamination by psychrotrophic pathogens (Listeria and Yersinia species) among slaughtered poultry (chicken, duck, geese, squabs) and rabbit. A total of 120 slaughtered poultry carcasses with their gizzard and liver and 30 rabbit carcasses with their liver only were randomly collected from selected retail poultry shops in Giza city. *L. monocytogenes* was obtained by 3.3% from chicken carcass, 6.6% in rabbit carcass and 3.3% from rabbitís liver. *L. innocua* and *L. grayi* were isolated with different percentages. *Y. enterocolitica* was isolated with percentages 33.3%, 50% and 53.3% in chicken carcass, gizzard and liver respectively. In ducks, it was 43.3, 53.3% and 53.3% for whole carcass, gizzard and liver respectively; while in geese it was 23.3%, 33.3% and 43.3%. In squabs it was 6.6% for carcass only but it failed to be detected in rabbit. *Y. intermedia* and *Y. frederiksenii* could be isolated. The health risks of the isolated Listeria and Yersinia species were discussed.

INTRODUCTION

Poultry (chicken, duck, geese, squabs) and rabbit are also popular for Egyptian people as well as red meat. Moreover, rabbit meat has a highly palatable and digestable quality for the consumers. The presence of *L.monocytogenes* in a wide variety of foods has become of great concern during the last two decades. The outbreaks of human listeriosis were confirmed due to the consumption of contaminated foods (Azadian et al. 1989 and Barnes et al. 1989). Sporadic cases have been traced back to a variety of foods, such as milk,

raw vegetables, seafoods, chicken, meats, enviroment, etc. (Petran et al.1988, Farber et al.1989 and Marrakchi et al. 1993). Chun choi- Young et al. (2000) isolated *L.monocytogenes* with an incidence of 10% from chicken. At the same time Baek et al.(2000) found that frozen foods, beef, pork and chicken were contaminated with L.monocytogenes with an overall percentage of 63.4%. *L.monocytogenes* has been strongly implicated particularly in the contamination of foods stored at low temperature (Peel et al.1988). Recent outbreaks of listeriosis were associated with consumption of precooked refrigerated chicken and turkey franks stored at 4°C (Kerr et al. 1990). Khalafalla (1993) isolated *L.monocytogenes* from 10 % of the processed rabbit carcasses examined. Whereas Zhi et al. (1993) and Khalafalla and Waffia (1995) isolated *L.monocytogenes* from 43.41% and 2.5% from ducks samples respectively.As well as 5% from squabs. Frozen and chilled chicken did show detectable levels of listeria reflecting the greater potential for contamination during poultry processing (Fenlon et al, 1996) . There is lack of information concerning the minimal infectious dose of *L.monocytogenes*, although it is generally thought to be relatively high (>100 viable cells). *Y.enterocolitica* usually does not cause large outbreaks compared with other pathogens, this organism can grow at refrigerated temperatures because of its psychrotrophic nature (Jiang et al. 2000). The incidence of human disease attributed to *Y.enterocolitica* is less than the other major microbial foodborne disease agents. Certain biological characteristics of Yersinia and human demographics and behaviors suggest that it is an emerging microbial threat (Funk et al.1998). Many researchers have isolated *Y.enterocolitica* from chicken meat samples collected from retail markets (Leistner et al., 1957; DE-Boer et al., 1982; Khalafalla, 1990 and Floccari et al., 2000). Also could be isolated from ducks (Turnkson et al., 1988 and Khalafalla, 1990). However, the presence of Listeria and Yersinia species in poultry and rabbit carcases should be considered, since the temperature abuse from the processing to the consumer and eventual lack of adequate cooking could favour the development of these pathogens at higher levels and lead to foodborne illness (Floccari et al.2000). In Egypt, Listeria and Yersinia organisms are not routinely searched for, so its epidemiological importance remains unknown. Therefore, this study was done to assess the presence of Listeria and Yersinia species in slaughtered poultry and rabbit after preparation purchased from commercial retail poultry shops in Giza governorate. The health risks due to these two pathogens for consumers were also assessed.

MATERIAL AND METHODS

Collection of Samples: A total of 120 slaughtered poultry samples; 30 each of chickens, ducks, geese, squabs; with their gizzard and liver. In addition 30 rabbit carcasses with their liver,were randomly collected from selected retail poultry shops in Giza city. The collected samples were

transferred in an icebox immediately to the laboratory.

Preparation of samples:

Carcass:

(a) Poultry (chicken, duck, geese and squabs) were collected from randomly selected retail poultry shops, slaughtered, put in the scalding tank, defeathered on the preparing table, eviscerated and washed in the same tank contain tape water which not frequently changed. The prepared carcasses were placed individually in a sterile double polyethylene bag (35 by 30 cm) with one liter of phosphate-buffered saline, pH 7.6 and manually vigorously massaged for 4 min. (Floccari et al. 2000). One corner of the bag was then wiped with alcohol and cut with a sterile scissor. The rinse was then placed in one liter capacity sterile flask and left for 15 minutes till clearance of the supernatant fluid. Then the upper layer of the fluid was discarded, while the lower part of the rinse (10 c.c.) was added to the enrichment for both listeria and yersinia separately, (Floccari et al. 2000).

(b) Rabbit carcass, were slaughtered, dressed, eviscerated and washed in the same tank. Whole carcass rinse was done as previously mentioned in poultry.

Gizzard and liver: Ten grams of liver and gizzard of each carcass, liver only in case of rabbit were separately homogenized with 90 ml sterile peptone water (APHA 1992) using universal laboratory aid made in Poland.

Isolation and Identification of Listeria species

Ten ml of the whole carcass rinse, gizzard and liver homogenate of each species were separately placed in sterile flask 250 ml containing 90 ml Buffered listeria enrichment broth (Oxoid, CM 897) with listeria selective enrichment supplement (Oxoid, SR 141) and incubated at 30°C for 24 hrs. A loopful from the broth was streaked on modified Oxford agar plate (Oxoid, CM 856) with listeria selective supplement (Oxoid, SR 140). Plates were examined for typical listeria colonies after 48 hrs incubation at 35°C. The suspected colonies were transferred to trypticase soya agar (Oxoid, CM 131) supplemented with 0.6% yeast extract (Oxoid) and incubated for overnight at 35°C. Biochemical tests, including Gram staining, catalase, oxidase, motility, B- hemolysis, carbohydrate utilization and the CAMP test, were performed according to Bhunia et al. (1994). Also an API- Listeria (BioMeriux, Marcyl, Etoile, France) Kit was used to confirm differences between Listeria species and *L.monocytogenes*.

Isolation and Identification of Yersinia species

Ten ml of the whole carcass rinse, gizzard and liver homogenate of each species of bird (chicken, duck, geese, squabs and rabbit) were separately added to phosphate - buffered saline supplemented with 1% sorbitol and 0.15% bile salt with pH

7.6 ± 0.2. The enrichment was incubated at 22°C for 48hrs (Avulsion et al., 1980 and APHA, 1992). Afterward, 100ul of inoculated broth was streaked onto Yersinia selective agar plate (Oxoid, CM 653) with Yersinia selective supplement (Oxoid, SR 109). Plates were incubated at 37°C for 24 hrs (Schumann and Waiters 1992). Three colonies showing *Yersinia characteristics* (three per selective agar plate) were then tested for catalase, Gram staining and motility at 25°C and 37°C, growth in triple sugar iron agar (Oxoid), urea broth, lysine decarboxylase, fermentation of Rhomnose and Simmonís citrate medium at 25°C.

RESULTS AND DISCUSSION

It is of importance to emphasize that *L. monocytogenes* could be isolated only from whole chicken carcasses, whole rabbit carcasses and rabbit liver samples at the percentages of 3.3, 6.6 and 3.3, respectively. While *L.innocua* had been detected in the chicken, duck and geese sample but failed to be isolated from squabs and rabbit samples. Whereas *L.grayi* could be isolated only from the whole chicken carcasses (10%), chicken liver (3.3%), and whole rabbit carcasses (3.3%). Listeria species failed to be detected in squabs. High incidence of *L.monocytogenes* in raw meat of chicken, pork and beef was reported by Kerr et al. 1990 who pointed out that recent outbreaks of listeriosis were associated with consumption of precooked refrigerated chicken and turkey franks stored at 4°C. Moreover, Hudson et al. (1992) found *L. monocytogenes* in 12.5% of ready to eat chickens in New Zealand,while, Baek et al.(2000) found *L.monocytogenes* in 4.3, 19.1 and 30.2 % in beef , pork and chickens in domestic foods, in Korea respectively. Higher figure (10%) was reported in Korea also by Chun choi et al. (2001), while a high incidence in rabbit carcasses, (Khalafalla, 1993). Regardless that the isolation ratio of L.monocytogenes during this study was lower than those of the previously mentioned reports; more thorough precautions to avoid contamination by *L.monocytogenes* should be taken. At the same time the isolation of other species of Listeria which are not pathogenic to human being indicates that there is a potential risk of contamination with L. monocytogenes. In this respect, Soriano et al. (2001) discussed the importance of strict hygiene measures during handling practices in order to avoid contamination of the food product with listeria species. Genigeorgis et al.(1989) and Khalafalla, (1993) attributed the presence of *L. monocytogenes* in slaughtered poultry and rabbit to the contamination during processing from either the intestinal contents or environmental contamination followed by multiplication to hazardous concentrations during prolonged cold storage. Ryser and Marth (1991) discussed the public health hazards due to *L.monocytogenes* infections.They pointed out that in cases not involving pregnancy, about two thirds of patients suffer from bacteraemia only, and about one third suffer from meningitis. Meanwhile, a small percentages have local lesions, including septic arthritis, este-

omyelitis, pericarditis and endocarditis, without reported bacteraemia. The authors further added that the symptomes caused by *L.momocytogenes* infection in perinatal cases in people whose immunity has been impaired by age, conditions such as cancer, organ transplantation, corticosteroid use or AIDS (acquired immunity deficiency syndrome) are generally only a mild fever in the mother with or without slight gastroenteritis of flu-type symptoms, but the consequences for the foetus or newborn are often major or fatal. Intrauterine death occurs most often before the third trimester of pregnancy, while later in pregnancy the child may be stillborn or born prematurely and severely ill. Septicaemia is the most common in these cases, but sometimes accompanied by meningitis. The results in table (2) illustrated that Y.enterocolitica was isolated from 13.3%, 50% and 53.3% of whole carcasses rinse, gizzards and liver of chicken respectively,while at a level of 43.3%, 53.3% and 53.3% from whole carcasses, gizzards and liver of duck respectively. Whereas in case of carcasses, gizzards and liver of geese samples, the organism was isolated at a level of 23.3%, 33.3% and 43.3% respectively. In squabs, it was isolated from 6.6% of whole carcasses rinse, and failed to be detect in squabís gizzard and liver samples. It is of interest to recognize that *Y.enterocolitica* could not be detected in rabbit samples, Whereas, *Y. intermedia* could be isolated from all investigating samples but at different percentages. Moreover, *Y. frederiksenii* failed to be isolated from the liver samples of both duck, geese and squabs, while isolated from the rest of the samples at different levels. Low figure was obtained by Floccari et al. (2000) who isolated the organism from chicken carcasses by 10% ; according to the following percentages: *Y. enterocolitica* was 4.3%, *Y. intermedia* was 1.4% and *Y. frederiksenii* was 4.3%. In this respect, Ramirez (2000) reported that *Y.enterocolitica* was the most frequent species isolated, followed by *Y. frederiksenii, Y. kristensenii, Y. intermedia* and *Y. aldovae*. The presence of *Y.enterocolitica* and related species in chickens reinforces the increasing concern about the microbiological quality of poultry in Mexico. Processing plants are adopting quality assurance programs based on hazard analysis and critical control points concept to improve the overall quality of their product and reduce the incidence of microorganisms that may be the cause of food borne disease. The occurrence of Y. enterocolitica in processed poultry may be attributed to the contamination of poultry carcasses during processing particularly during scalding and defeathering. The data obtained during this study and tabulated in table (2), also indicated that the samples of both chicken and ducks were density contaminated with yersinia species, followed by the samples of geese, while the samples of squabs and rabbit were less density contaminated. This may be due to faulty evisceration , bad hygienic measures during preparation. This agrees with that reported by Ibrahim (1992). In this respect, Mousa (1989) recovered *Yersinia enterocolitica* from carcass surfaces in a high frequency which

Table (1): Incidence of Listeria species among slaughtered poultry and rabbit

type of sample	*L. monocytogenes*		*L. innocua*		*L. grayi*	
	Positive samples	%	Positive samples	%	Positive samples	%
Whole chicken carcass	1	3.3	5	16.6	3	10
gizzard	0	0	3	10	0	0
Liver	0	0	4	13.3	1	3.3
Whole duck carcass	0	0	3	10	1	3.3
gizzard	0	0	2	6.6	0	0
Liver	0	0	2	6.6	0	0
Whole geese carcass	0	0	1	3.3	0	0
gizzard	0	0	1	3.3	0	0
Liver	0	0	0	0	0	0
Whole squabs carcass	0	0	0	0	0	0
gizzard	0	0	0	0	0	0
Liver	0	0	0	0	0	0
Whole rabbit carcass	2	6.6	0	0	1	3.3
Liver	1	3.3	0	0	0	0
total	4	0.9	21	5	6	1.4

N.B. Number of examined samples were thirty.

Table (2): Incidence of Yersinia species among slaughtered poultry and rabbit of Yersinia

type of sample	*Y. entercolitica*		*Y. intermedia*		*Y. frederiksenii*	
	Positive samples	%	Positive samples	%	Positive samples	%
Whole chicken carcass	10	33.3	12	40	5	16.6
gizzard	15	50	10	33.3	4	13.3
Liver	16	53.3	10	233.3	3	10
Whole duck carcass	13	34.3	9	30	2	6.6
gizzard	16	53.3	10	33.3	1	3.3
Liver	16	53.3	10	33.3	0	0
Whole geese carcass	7	23.3	7	23.3	6	20
gizzard	10	33.3	9	30	6	20
Liver	13	43.3	7	23.3	0	0
Whole squabs carcass	2	6.6	5	16.6	4	13.3
gizzard	0	0	3	10	2	6.6
Liver	0	0	10	33.3	0	0
Whole rabbit carcass	0	0	2	6.6	3	10
Liver	0	0	4	13.3	3	10
total	93	22.2	108	25.7	39	9.3

N.B. Number of examined samples were thirty.

reflected the possibility of cross contamination from the already infected intestinal contents to the carcass surface during evisceration and primary processing, and unhygienic condition prevailing at retail shops. On the other hand, Hassan, (1997) stated that the surface of chicken carcases were contaminated with *Y.enterocolitica* from viscera and polluted water, then distributed to meat during its preparation and handling. *Y.enterocolitica* is a potential cause of food borne disease in humans. Infants, children, adolescents, the elderly, and patients with immunodeficiency or impaired iron metabolism are particularly susceptible (Schumann 1979). *Y.enterocolitica* causes infections to gastrointestinal tract, with a tendency toward systemic infections in liver, kidney, spleen and lung . Inflammatory acute enteritis, occasionally bloody with fever and diarrhea is the most frequent resulting condition especially in children. The appendicitis- like syndrome, mesenteric lymphadenitis and terminal ileitis can also be included in the diagnosis. In cases of immune suppressed individuals, septicaemia can also develop (Bottone, 1997). Even though, *Y. enterocolitica* is the most frequently related to human infection, the other species may also cause gastroenteritis (Brenner et al. 1980 and Ursing et al. 1980). Finally we recommended that good hygienic practices in retail poultry shops is required for elimination of listeria and yersinia speciec from slaughtered poultry and rabbit and their carcasses and offals should be washed in tanks containing pure potable water to avoid cross contamination from one species to another.

REFERENCES

American Public Health Association, APHA (1992): Compendium of Methods for the Microbiological Examination of Foods; 3rd edition, Edwards Brothers, Washington.

Avulsion, C.C.; Nehlman,I.J. and Sanders,A.C.(1980) : Alkaline method for rapid recovery of Y.enterocolitica and Y.pseudotuberculosis from foods. Appl. Environ. Microbiol. 93: 135-140.

Azadiann,B.S.; Finnerty, G.T. and Pearson, A.D. (1989): Cheese borne Listeria meningitis in immunocompetent patients. Lancet. 322-323.

Baek, S.Y.; Lim, S.Y.; Lee, D.H.; Min, K.H. and Kim, C. M. (2000): Incidence and characterization of Listeria monocytogenes from domestic and imported foods in Korea. J.Food Prot.63 :186-189.

Barnes,R.; Archer, P.; Strack,J. and Istre, G.R. (1989): Listeriosis associated with the consumpition of turkey franks. Morbid. Mortal. Weekly Rep. 38: 267-268.

Bhunia, A.K.; Steele,P.J.;Westbrook,D.J.; Bly,L.A.; Maloney,T.P. and Johnson, M.G. (1994): A six ñhours in vitro virulence assay for Listeria monocytogenes using myeloma and hybridoma cells from murine and human sources. Microbiol. Pathogen. 16: 99-110.

Bottone, E.J. (1997): Yersinia enterocolitica : the Charisma continues. Clin. Microbiol. Rev. 10 : 257- 276.

Brenner, D.J.; Bercovier, U.J.; Alonson, J.M.; Steigerwalt, A. G.; Fanning,R.G.; Carter,G.P. and Mollaret, H.H. (1980): Yersinia intermedia: a new species of Enterobacteriaceae composed of rhamnose positive, melibiose-

positive , raffinose- positive strains (formerly called atypical Y.enterocolitica or Y.enterocolitica like). Curr. Microbiol. 4 :207- 212.

Chun choi,Y.; Young cho,S.; Kill park,B.;Hwa chung,D. and Hwan oh,D.(2001): Incidence and characterization of Listeria Spp. from foods available in Korea. J.Food Prot.64: 554-558.

DE- Boer,E.; Hartog,B.J.and Oosterom, J.(1982): Occurrence of Yersinia enterocolitica in poultry products. J. Food Prot.45: 322-324.

Farber. J.M.; Sanders,G.W. and Johnson,M.A. (1989): A survey of various foods for the presence of Listeria species. J. Food Prot.52: 456-458.

Fenlon, D.R; Wilson, J. and Donachie, W. (1996): The incidence and level of Listeria monocytogenes contamination of food sources at primary production and initial processing. J. Appl. Bacteriol.6: 641-650.

Floccari, M.E. ; Carranza, M.M. and Parada, J.L. (2000): Yersinia enterocolitica Biogroup 1A, serotype O:5 in chicken carcasses. J.Food Prot.63, 1591-1593.

Floccari, M.E. and Peso, O.A. (1984):Yersinia enterocolitica and related species in sewage samples from Buenos Aires city : Use of Schiemannís medium for its isolation). Rev. Argent. Microbiol. 16: 57-66.

Funk,J.A.; Fred,T.H.; Isaacson,R.E. and Flosser,C.P. (1998): Prevalence of pathogenic Yersinia enterocolitica in groups of swine at slaughter. J.Food Prot. 61: 677-682.

Hassan, F. M. (1997): Studies on meat quality assurance and safety system in university hostel., M.V.Sc. Thesis, Fac. Vet. Med.Benisuef, Cairo Univ.Egypt.

Hudson,J.A.; Mott,S.J.; Delay, K.M. and Edridge, A.L. (1992): Incidence and coincidence of Listeria spp.,motile aeromonads and Yersinia enterocolitica on ready to eat flesh foods. Int. J.Food Microbiol.16: 99-108.

Ibrahim,H.M.(1992): Sanitary improvement of meat meals in governmental hospitals in rural areas. Ph.D, Thesis, Fac. Vet. Med.,Moshtoher, Banha-Branch., Zagazig Univ.,Egypt.

Jiang, G.C.; Hyun Kang, D.and Fung, D.Y.(2000): Enrichment procedures and plating media for isolation of Yersinia enterocolitica. J.Food Prot.63: 1483-1486.

Khalafalla, F.A. and Waffia, H.A. (1995): Bacteriological study on ducks and squabs. J. Vet. Med. Ass.55, 629-634.

Khalafalla, F.A. (1993): Microbiological status of rabbit carcasses in Egypt. Z.lebensm Unters Forsch, 196: 233-235.

Khalafalla, F.A. (1990): Yersinia enterocolitica in processed poultry. Fleischwirtsch. 70(3), 305-306.

Kerr, K.G.; Rotowa, N.A.; Hawkey, P.M. and Lacey, R.W. (1990): Incidence of Listeria species in precooked, chilled chicken products as determined by culture and enzyme, linked immunoassays (Elisa). J.Food Prot.53: 606-607.

Lee,W.H. (1977): Two plating media modified with tween 80 for isolating Yersinia enterocolitica. Appl. Environ. Microbiol. 33: 215-216.

Marrakchi,A.; Hamama,A. and El Othmani, F. (1993): Occurrence of Listeria monocytogenes in milk and dairy products produced or imported into Moroco.J.Food Prot.56: 256-259.

Mousa,M.M.(1989): Monitoring of Yersinia enterocolitica in fresh slaughtered broiler chickens. J.Egypt Vet. Med. Assoc.49,109.

Petran, R.L.;Zottola, E.A. and Gravani,R.B. (1988): Incidence of Listeria monocytogenes in market samples of fresh and frozen vegetables. J.Food Sci. 53: 1238-1240.

Ralovich, B.S.(1984): Listeriosis Research- present situation and perspective. Akademiai Kiado, Budapest.

Ramirez. E.I; Salinas, C.V.; Rodas-suarez,O.R.and Pedroche, F.F.(2000): Isolation of Yersinia from raw meat (pork and chicken) and precooked meat (Porcine tongues and sausages) collected from commercial establishments in Mexico City. J.Food Prot.63: 542-544.

Ryser, E.T. and Marth, E.H. (1991): Listeria, listeriosis and food safety. New York: Marcel Dekker, Inc.3: 211-213.

Schumann, D.A. (1979): Synthesis of a selective agar medium for Yersinia enterocolitica. Can. J.Microbiol. 25: 1298-1304.

Schumann, D.A and Waiters, G.(1992): Yersinia, In Vanderzant C. and Splitstoesser D.F.(ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C. P.433-450.

Soriano, J.M.; Rico,H.; Molto, J.C. and Manes, J.(2001): Listeria species in raw and ready to eat foods from restaurants. J.Food Prot. 64: 551-553.

Turnkson, P.K.; Lindqvist, K.J. and Kapperud, G. (1988): Isolation of Campylobacter spp.and Yersinia enterocolitica from domestic animals and human patients in Kenya. Acta. Batho. Microbiol. Immunol. Scand. 96, 141-143.

Velazquez, L.; Escudero, M.E. and Stefanini de Guzman, M. (1995): Yersinia enterocolitica and related species isolated in San Luis, Argentina, 1989- 1993. Contrib.Microbiol. Immunol. 13: 59- 61.

Ursing,J.; Brenner, D.J.; Bercovier,H.; Fanning, G.R.; Sycigerwalt, J.and Mollart, H.H.(1980): Yersinia frederiksenii: a new species of Enterobacteriaceae composed of rhamnose-positive strain (formerly called atypical Yersinia or Yersinia enterocolitica like). Curr. Microbiol. 4: 213-217.

Zhi,Y.Q.; Tai, P.T.; Zin,Z.S; YU,H.Z.; Mei,Z.A.and Ze,W. (1993): Investigation of listeria spp.in livestock poultry and their processed products. The 11th Intertional Symposium of the World Association of Veterinary Food Hygienists, Proceeding, Bangkok, Thailand.