Prevalence of Some Foodborne Pathogens Isolated From Rabbit Meat

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Abstract

This study was conducted to establish the microbiological contamination of rabbit carcasses, and assess the spread of foodborne pathogens in fresh and frozen rabbit meat. A total of 50 New Zealand white rabbits, 25 freshly slaughtered rabbits from markets, farms and butcheries, as well as 25 frozen rabbit carcases from local retail supermarkets in Gharbia governorate, were collected for bacteriological examination. Neither *Salmonella* nor *Clostridium perfringens* were detected in frozen rabbit meat, while only 4% and 8% of fresh rabbit samples were contaminated with *Salmonella typhimurium* and *Clostridium perfringens* respectively. *Yersinia enterocolitica* was one of the most prevalent foodborne pathogenic bacteria investigated; it was detected on 4 samples (16%) of fresh rabbit carcasses and 2 samples (8%) of frozen rabbit meat. The prevalence of *Listeria monocytogenes* on fresh rabbit carcasses were 16%, while it was isolated from 4% of frozen rabbit meat samples. *Aeromonas hydrophila* were detected in 16% of freshly slaughtered rabbits, but not of frozen samples, with a mean count of 2.3 ± 1.2 log CFU/g.; indicating that fresh rabbit meat can act as a possible vehicle for the dissemination of *Aeromonas* gastroenteritis. *E. coli* was the most investigated foodborne pathogenic bacteria; it was detected on 6 samples (24%) of fresh rabbit carcasses, with a mean count of 2.4 ± 1.3 log CFU/g; and 3 samples (12%) of frozen rabbit meat, with a mean count of 2.1 ± 1.2 log CFU/g. The isolated strains were O26:k60, O55:k59, O86:k61, O114:k90, O119:k69, O126:K71, O128:K67 and O142:k86 while *E. coli* O157 was not detected. The high microbial E. coli contamination found in carcasses suggests a major public health risk, probably due to enteropathogenic phenotypes O114, and O119 strains. The prevalence of *Staphylococcus aureus* on both fresh rabbit carcasses and frozen rabbit meat samples was 8%, with a mean count of 3.1 ± 1.1 and 2.2 ± 0.8 log CFU/g, respectively. The present study concluded that

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rabbit carcasses and meat can contribute to microbiological risk of food borne pathogens. Consequently, strict maintenance of good practices of slaughter hygiene, strengthened by maintaining the cold chain during transport, distribution and carcass commercialization is of central importance to ensure both public health protection and meat quality.

Introduction

Rabbits are an important source of meat, especially in Mediterranean countries; rabbit meat is a common item in the diet. According to the Food and Agriculture Organization (FAO) of the United Nations, world rabbit meat production is estimated to be over 1.4 million tons annually. In 2006, the major producer was China (500,000 tons), followed by Venezuela, Italy, Egypt, Spain, and France.

Rabbit meat is appreciated for its nutritional and dietary properties (**14**): its lipids are highly unsaturated (60% of total FA), high in protein content (20–21%), low in cholesterol and sodium and rich in potassium, phosphorus and magnesium (**43** and **3**).

In view of food-borne diseases, the impact of latent zoonoses has increased in recent years (**17**). The healthy animal thereby represents a reservoir for food-borne pathogens, because fecal carriage is correlated with the probability of carcass contamination, these pathogens may enter the food chain during slaughter. Beside bacteria originating from the animals (hide, feet and intestine), pathogens may enter the food chain by cross-contamination from various environmental sources during slaughter and processing.

Even though rabbit carcasses have basically the potential to carry pathogenic bacteria and rabbit meat is marketed and consumed worldwide, data on the microbiology of rabbit meat are very scarce and limited to a few papers (**31**; **5**; **45**; **37**; **47**; **13**; **34**; **35**; **36** and **56**). However, rabbit carcasses are obtained, processed and stored in the same manner as meat from other animals, and according to European Union legislation (**15** and **16**), who stated that rabbit carcasses and cuts meet the same conditions that apply to

poultry and that they be similarly stored and checked for health and hygienic status.

Among the microbial hazards that are of importance in meat are pathogens from living animals such as salmonella, *Yersinia enterocolitica*, and *Escherichia coli*; pathogens from the environment that can enter the meat chain wherever environmental contamination may occur as *Listeria monocytogenes*, and some species of motile aeromonads; and pathogens that are carried by product handlers, as *Staphylococcus aures*, although this bacterium is capable of colonizing certain items of processing equipment and is also associated with some diseases of commercial rabbits, such as subcutaneous abscess mastitis, and pododermatitis **(48)**.

There is no doubt that foodborne pathogenic bacteria are the cause of illness and death for many people each year, at great economic cost and human suffering **(7)**. Several studies have been conducted on the microbiological quality of red meat, poultry and their products **(1; 10; 27; 28; 58** and **59)** but there is some lack of information on the microbiological quality of rabbit meat which may be contaminated with organisms of various kinds including potentially pathogenic bacteria, like many raw foods of animal origin. On the other hand, health regulations applicable to rabbit meat production systems are not specific, due to the absence of regulations regarding quality and product safety, in which microbiological conditions should be specified. This situation could compromise the quality and food safety of rabbit meat.

Therefore, the aim of this study was to obtain data on the microbiological contamination of rabbit carcasses, and assess the spread of foodborne pathogens in fresh and frozen rabbit meat.

Materials and Methods

Sample collection

A total of 50 New Zealand white rabbits, 25 freshly slaughtered rabbits from markets, farms and butcheries, as well as 25 frozen rabbit carcasses from local retail supermarkets in Gharbia governorate, were collected. Each sample was packed in plastic bag and transferred immediately to the laboratory in an ice box for bacteriological examination.

Sample preparation for microbiological analysis

Defrosting of frozen meat was started in a refrigerator before the sample preparation. The sample were prepared and examined according to the technique recommended by **(29)** as follows: 25 gm meats were removed aseptically from the hind leg and transferred to a sterile polyethylene bag. Then, 225 ml of 1% sterile peptone water were aseptically added to the content of the bag and then homogenized at 200 rpm for 1-2 minutes to prepare the initial 1/10 dilution which was used for the preparation of other serial dilutions.

Isolation and identification of pathogenic bacteria

- *Salmonella* spp. were isolated and identified according to **(26)**.
- *Clostridium perfringens* were isolated and identified according to **(29)**
- *Yersinia enterocolitica* were isolated and identified according to **(57)**
- *Listeria monocytogenes* were isolated and identified according to **(25)**
- Aeromonas Hydrofla were isolated and identified according to **(22)**.
- *E. coli* were isolated and identified according to **(18)**.
- *Staphylococcus aureus* were isolated and identified according to **(1)** and confirmed by the coagulase test as described by **(12)**.

Results and Discussion

Table (1): Prevalence of bacterial food-borne pathogens on fresh rabbit meat samples (n= 25).

Samples	*Salmonella*		*Clostridium perfringens*		*Yersinia enterocolitica*		*Listeria monocytogenes*		Aeromonas hydrophila			*Escherichia coli*			*Staphylococcus aureus*		
	No*	%	No	%	No	%	No	%	No	%	count	No	%	count	No	%	count
Fresh rabbit carcasses	1	4%	2	8%	4	16%	4	16%	4	16%	2.3 ± 1.2	6	24%	2.4 ± 1.3	2	8%	3.1 ± 1.1

No* number of positive samples

Table (2): Prevalence of bacterial food-borne pathogens on frozen rabbit meat samples (n= 25).

Samples	*Salmonella*		*Clostridium perfringens*		*Yersinia enterocolitica*		*Listeria monocytogenes*		Aeromonas hydrophila		*Escherichia coli*			*Staphylococcus aureus*		
	No*	%	No	%	No	%	No	%	No	%	No	%	count	No	%	count
Frozen rabbit meat	0	0%	0	0%	2	8%	1	4%	0	0%	3	12%	2.1 ± 1.2	2	8%	2.2 ± 0.8

No* number of positive samples

Microbiological data, especially of food-borne pathogens from rabbits are rare. Only recent studies from Spain have addressed this topic comprehensively, although they were based on only chilled rabbit carcasses and meat samples **(45** and **46)**. Most other studies were dedicated to rabbits suffering from disease, specific pathogens, or factors influencing nutritional and sensory traits of rabbit meat.

Identifying bacterial pathogens in natural hosts is important because they constitute potential reservoirs for zoonotic transmission **(23)**. The reported prevalence of pathogens such as *Salmonella* and *E. coli* in raw meat varies considerably, depending on the meat species as well as on its processing and origin **(36)**. Contamination of carcasses and cuts by these types of bacteria depends on their prevalence and numbers on the hide, fleece, pelt, or feathers and in the gastrointestinal tract. Contamination is also significantly affected by the degree of cross-contamination occurring from these and environmental sources during slaughter and subsequent processing **(52)**.

Salmonella remains a major cause of food borne disease in human world wide **(17)**. A limited number of surveys suggested that less than 1% of healthy rabbits at the abattoir are positive for *Salmonella* on the basis of fecal samples **(6** and **9)**. This study detected *Salmonella typhimurium* on one sample (prevalence, 4%) of fresh rabbit carcasses (Table, 1). Comparable to our results, Salmonella were not detected or only detected at a low prevalence (< 2%), on rabbit carcasses or meat in most studies **(11; 46; 55; 4; 32** and **56)**. In Egypt, **(91)** and **(37)** reported a prevalence of 5 % for *Salmonella typhimurium* on fresh rabbit carcasses, at the retail level. Moreover, recent publication indicated that rabbit meat exhibited the highest contamination from Salmonella (2.1%), followed by pork (1.9%), lamb (1.7%), and beef (1.1%) **(36)**; *Salmonella typhimurium* was the most frequent serotype isolated from raw meats. On the other hand, the present study did not detect Salmonella on frozen rabbit meats (Table 2), which is in agreement with data recorded by **(37)** and **(35)**.

This study has confirmed that *Clostridium perfringens* were contaminants of fresh rabbit carcasses (Table 1) and not of frozen rabbit meat (Table 2). The prevalence of *C. perfringens* in fresh examined samples was 8%. (**53**) found that the incidences of presumptive *C. perfringens* in frozen rabbit meat (65%) were higher than those in frozen beef (25%). Meanwhile, (**4**) and (**5**) recorded that *Clostridium perfringens* was not detected on rabbit carcasses. However, it should be considered that strains of *C. perfringens* isolated from rabbit meat were involved in two cases of food poisoning outbreaks **(50).**

Yersinia enterocolitica was one of the most prevalent foodborne pathogenic bacteria investigated; it was detected on 4 samples (16%) of fresh rabbit carcasses (Table, 1) and 2 samples (8%) of frozen rabbit meat (Table, 2). These results were not in agreement with (**31**) and (**4**) who reported that *Yersinia enterocolitica* could not be isolated from any examined sample (freshly slaughtered rabbits and processed rabbit carcasses). However, **(46)** found that two rabbit carcasses (3.9%) were positive, by PCR, for *Y. enterocolitica* yst gene, although viable *Y. enterocolitica* cells were not recovered from these samples by the culture method. *Yersinia enterocolitica* and other *Yersinia* species are ubiquitous in the natural environment, and may be recovered from water, soil, animals, and food. The presence of pathogenic *Y. enterocolitica* in food products is a special concern since those organisms are capable of growth at refrigerator temperatures. Pathogenic *Y. enterocolitica* organisms are significant causes of human disease in many parts of the developed world **(38).**

The prevalence of *Listeria monocytogenes* on fresh rabbit carcasses in this study were 16% (Table 1). Numerous studies have been conducted to determine the prevalence of listeria spp. and *Listeria monocytogenes* in rabbit meat. High prevalence of *Listeria monocytogenes* (> 20%) was detected in Italy on rabbit carcasses from four main slaughterhouses, this high incidence was attributed to the general wear and tear to work surfaces

and equipment and the uneffectiveness of hygienic practices **(13).** On contrary, *Listeria monocytogenes* was not isolated from all examined rabbit carcasses **(4; 34** and **56).** Our data compare well with those from **(46)** who recorded that the rates of listeriae and *Listeria monocytogenes* were 13.7 and 5.9%, respectively, but the prevalence was higher on rabbit meat at retail than on carcasses. In Egypt, **(31)** did not find *listeria* spp. on freshly slaughtered rabbit carcasses but did find them at the retail level with the prevalence of *Listeria monocytogenes* being 10%. Moreover, **(2)** found that the initial mean log count of *L. monocytogenes* in the rabbit meat samples was 3.813. On the other hand, the present study indicated also that *Listeria monocytogenes* was isolated from one sample (prevalence 4%) of frozen rabbit meat (Table 2). These findings could be supported by **(4)** who stated that *Listeria monocytogenes* is able to survive freezing temperatures of -18°C for several weeks in various food substrates.

Aeromonas spp. are primarily aquatic organisms that are frequently found in food, drinking water, and aquatic environments **(21; 41; 44** and **38).** Although the significance of Aeromonas in foods remains undefined, epidemiological studies indicated that *A. hydrophila* may be an important cause of food-borne gastroenteritis **(40).** Of all the freshly slaughtered rabbit meat samples tested, 4 (16%) yielded detectable numbers of motile aeromonads (*A. hydrophila*), with a mean count of 2.3 $\pm$ 1.2 log CFU/g (Table 1). This prevalence of aeromonads was lower than those reported by **(46)** who found that the contamination rate of the rabbit meat samples for motile *Aeromonas* spp. (average count, 1.77 $\pm$ 0.62 log CFU/g) was 35.3%, most of the *Aeromonas* strains were *A. hydrophila* (19.6 %). **(24)** found that *A. hydrophila* was a reasonable predictor of human diarrhea. This species along with *A. cauiae* and the *A. veronii boivar sobria* accounts for more than 85% of the gastroenteritis-associated isolates **(33).** Overall, the intestinal carriage of this organism in food animals cannot explain the high prevalence of *Aeromonas* on meat, and the water used in washing has been considered the most likely source **(40).** However, because aeromonads can form biofilms, they can become endemic in slaughterhouses and other processing environments **(20).** The high prevalence of motile aeromonads in

carcasses and the isolation of potentially pathogenic *Aeromonas* strains may indicate that rabbit meat can act as a possible vehicle for the dissemination of *Aeromonas* gastroenteritis.

The present study indicated that *E. coli* was the most prevalent foodborne pathogenic bacteria investigated, it was detected in 6 samples (24%) of fresh rabbit carcasses, with a mean count of 2.4 ± 1.3 log CFU/g (Table, 1); and 3 samples (12%) of frozen rabbit meat, with a mean count of 2.1 ± 1.2 log CFU/g (Table, 2). Previous studies reported that *E. coli* was usually present at low levels both in rabbit carcasses and meats **(31; 37** and **13)**. The isolation frequency rate was as high as 62.4% **(56)**. On the other hand, the present study indicated also that the isolated strains from fresh rabbit carcasses were O26:k60, O86:k61, O114:k90, O119:k69, O126:K71 and O128:K67; and from frozen rabbit meat were O55:k59, O119:k69, and O142:k86 while *E. coli* O157 failed to be detected. The high microbial *E. coli* contamination found in carcasses suggests a major public health risk, probably due to *E. coli* O114, and O119 strains, which can be considered as enteropathogenic phenotypes, and should be evaluated using other in vitro and molecular procedures for confirmation of human health risk phenotypes. These observations were in compliance with **(19)** and **(34)** who indicated rabbits as a potential reservoir for enteropathogenic and enterohemorrhagic *E.coli* that may pose a zoonotic risk for humans. Moreover, wild rabbits living close to cattle excreting *E. coli* O157 were identified as vector in an outbreak of diarrhea and hemolytic–uremic syndrome involving visitors to a wildlife park **(49)**. However, *E. coli* O157 was not previously detected in rabbit carcasses and meats **(4** and **46)**.

This study revealed that the prevalence of *Staphylococcus aureus* on **both** fresh rabbit carcasses and frozen rabbit meat samples was 8%, with a **mean** count of 3.1 ± 1.1 and 2.2 ± 0.8 log CFU/g, respectively (Table **1&2)**. Recent studies indicated that *Staphylococcus aureus* was a prevalent foodborne pathogenic bacteria in rabbit meat samples and carcasses **(46; 47; 51; 34** and **35)**. High incidence was recorded by **(35)** (26%), **(56)** (36.0%), **and (46)** (52.9%); while the general average of contamination registered was <1.8 cfu/g **(5)**, 1.0 – 1.6 log CFU/g **(51)**, $4.7x10^2$ CFU/g **(35)** and 4×10^3 ±

4×10^{2} CFU/g **(31).** Contamination of meat by *Staphylococcus aureus* pathogens is considered an important issue in terms of food safety. Since food animals arriving at abattoirs are frequently carriers of *S. aureus*, the organism is commonly found on carcasses and cuts, and staphylococcal contamination may or may not result from lesions **(39). (47)** concluded that, *S. aureus* isolates from rabbit meat were probably of human origin or without host specificity. This bacterium may also be introduced by contact surfaces, equipments and utensils used in food processing. Therefore, contamination of raw meat with low numbers of *S. aureus* is common and not always related to human contamination.

In conclusion, although further microbiological studies are needed to determine the prevalence and risk of food borne pathogens in rabbit carcasses and meat, our data and most of the limited information to date suggest that some zoonotic agents occurring in livestock are not frequently found in rabbit meat. However, the present study can also contribute to microbiological risk assessments from the handling of contaminated raw meat, the insufficient cooking of meat, and the cross contamination of ready-to-eat food. Consequently, strict maintenance of good practices of slaughter hygiene, strengthened by maintaining the cold chain during transport, distribution and carcass commercialization is of central importance to ensure both public health protection and meat quality.

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انتشار بعض الميكروبات الغذائية الشائعة المعزولة من لحوم الأرانب

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الملخص العربي

أجريت هذه الدراسة لمعرفة مدى التلوث البكتيري لذبائح الأرانب وتقيم مدى تواجد البكتريا الممرضة في كل من لحوم الأرانب الطازجة والمجمدة حيث تم فحص عدد ٥٠ أرانب نيوزلاندى ، جمعت من منافذ البيع المختلفة الغربية بواقع ٢٥ عينة مختلفة مذبحة حديثاً من الأسواق والمزارع والمحلات وكذلك ٢٥ عينة من لحوم الأرانب المجمدة من محلات السوبر ماركت المختلفة . وقد أو ضحت النتائج إنه لم يتم عزل ميكروبات السالمونيلا ولا كلوستريديوم برفرينجينز من لحوم الأرانب المجمدة بينما كانت ٤ % ، ٨ % من عينات لحوم الأرانب الطازجة ملوثة بهذه الميكروبات ، ولقد كان ميكروب يرسينيا إنتيروكولوتيكا من الميكروبات الأكثر شيوعا حيث تم عزلها من ١٦ % من لحوم الأرانب الطازجة و ٨ % من لحوم الأرانب المجمدة ، كما تم عزل ليستريا مونوسيتوجين من لحوم الأرانب الطازجة بنسبة ١٦ % بينما لم يتم عزلها إلا من ٤ % من عينات لحوم الأرانب المجمدة . وقد أشارت النتائج إلي عزل ميكروبات إيروموناس هيدروفلا من ١٦ % من لحوم الأرانب الطازجة فقط بمتوسط عدد كلي ٢,٣ ± ١,٢ لوج خلية بالجرام مدللة إمكانية انتشار الالتهاب المعدية المعوية الخاصة بهذا الميكروب عن طريق لحوم الأرانب الطازجة . وقد أثبت الدراسة إلي أن ميكروبات إي كولاى كانت الأكثر شيوعاً حيث تم عزلها من ٢٤ % من لحوم الأرانب الطازجة بمتوسط عدد كلي ٢,٤ ± ١,٣ لوج و ١٢ % من لحوم الأرانب المجمدة بمتوسط عدد كلي ٢,١ ± ١,١ لوج خلية بالجرام و كانت العترات المعزولة : O26 : K60, O55 : K59 , O 86 O26:K 71, O128:K67 and O142:K86 ، K51 , O114: K90, O11:K69,O157 وقد أشارت النتائج على أن التلوث العالي بميكروب إي كولاي في ذبائح الأرانب يشكل خطورة على صحة الإنسان ، خاصة بسبب العترات المعوية الممرضة O114:K90, O119:K69 ، بينما كانت نسبة التلوث بميكروب العنقودي الذهبي في كل من لحوم الأرانب الطازجة ، وقد خلصت الدراسة بمتوسط عدد كلي ٣,١ ± ١,١ ، ٢,٢ ± ٠,٨ لوج خلية بالجرام على التوالي ، وقد خلصت الدراسة إلي أن الأرانب من الممكن أن تشكل خطورة ميكروبية على صحة المستهلك ولذلك يجب الاهتمام الصحي بعمليات الذبح وسرعة تبريد اللحوم بعد الذبح وأثناء النقل والتوزيع والبيع لما له من أهمية قصوى للمحافظة على صحة الإنسان وجودة لحوم الأرانب.