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Prevalence of *Listeria monocytogenes* and *Salmonella* spp. in Ready-to-Eat Foods in Kefalonia, Greece

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Abstract

The presence of *Listeria monocytogenes* and *Salmonella* spp. was examined in ready-to eat meals, salads, desserts and ice cream in food establishments on the island of Kefalonia, Greece. Samples were collected or delivered from restaurants, canteens, cafes or ice cream parlors. Testing of the presence/absence of the pathogens was performed in 25 g samples of each product. Of the 1329 samples, 191 (14.4%) were positive for *L. monocytogenes* and 14 (1.05%) were positive for *Salmonella* spp. The elevated prevalence of *L. monocytogenes*, especially during the summer months, indicates high risk to consumers of ready-to-eat foods and shows the need for functioning regulatory actions.

Keywords: *L. monocytogenes*, *Salmonella* spp.; Ready-to-Eat Food; Greece; Survey

Abbreviations:

RTE: Ready-to-Eat; ISO: International Organization for Standardization; BPW: Buffered Peptone Water; T_{Tn}: Tetrathionate; RV: Rappaport-Vassiliadis; XLD: Xylose Lysine Desoxycholate; API: Analytical Profile Index; K-W: Kruskal-Wallis; CDC: Center for Disease Control

Introduction

Foodborne illnesses represent an extensive, yet largely avertable, problem on a global scale. Due to the fact that more and more people regularly eat their meals in public places, while food production and foodstuff commerce are imposing the use of the “cold chain”, *Listeria monocytogenes* is becoming increasingly relevant as emerging pathogen and so are the methods of its restraint, especially in food produced for people that have a high risk of infection. Furthermore, the Center for Disease Control (CDC) estimates that *Salmonella* spp. is responsible for more than 1.2 million illnesses each year, in the United States [1].

Although the two species are not related, both are of great interest. *Salmonella* spp. is the leading cause of food infection in the United States and causes the second largest number of human food-borne illnesses in Europe. Listeriosis caused by *Listeria monocytogenes* is low in incidence but its mortality rate is high placing the microorganism to the top pathogens contributing to food-borne illness resulting in death.

According to a 2010 FoodNet report, rates of infection in the U.S.A. were at least 25% lower for *Listeria* than they were a decade ago; *Salmonella* was only 10% lower [2]. In Europe, a 10.5% increase was reported for the year 2012 when compared to 2011 for *L. monocytogenes*. Salmonellosis on the other hand continued to decrease in 2012, with a 4.7% decrease in confirmed cases compared to 2011 [3]. Although progress has been made in decreasing

contamination of some foods and reducing illness caused by some pathogens, more information is needed to understand sources of infection and changes in incidence, and to help determine where to target prevention efforts.

The bacterial genus *Listeria* currently comprises 10 species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes*. *Listeria* species are omnipresent organisms that are broadly dispersed in the environment, particularly in plant matter and soil. *Listeria monocytogenes* is a Gram positive, aerobic to facultative anaerobic, psychrotrophic, non-spore forming bacterium. It is found in foods, water, soil, vegetables, animals and humans [3,4]. The principal method of transmission, to both animals and humans, is through ingestion of contaminated food. The bacterium can be found in uncooked nourishments and in processed foods which become contaminated on a post-process level. The temperature range for *L. monocytogenes* is -1 to 45°C, with the optimal temperature being 30-37°C. As the bacterium can grow at temperatures below 0°C, it has the potential to grow in food during refrigerated storage. The fact that this resilient bacterium can multiply at such low temperatures, makes the presence of *Listeria* in “ready- to-eat” (RTE) foods a fretful trepidation, when one takes into consideration the extended shelf-life of said products. Although the incidence of listeriosis is quite low, the mortality rate of 15.9% puts this organism among the most significant reasons of death from foodborne illnesses in developed countries [5].

Listeria monocytogenes infection manifestations include mild flu-like symptoms (nausea, diarrhea and fever) but there is also the case of invasive listeriosis, where more dire implications are involved (neonatal listeriosis, septicemia, meningitis, meningoencephalitis and less frequently endocarditis). Healthy young persons seldom acquire invasive listeriosis; most listeriosis cases occur in susceptible host populations such as pregnant women, organ transplant recipients, cancer and AIDS patients, individuals with other immunocompromising ailments as well as adults over 65 [6].

The genus *Salmonella* constitutes of two species: *S. enterica* and *S. bongori*. Salmonellosis in humans is usually described by severe fever, abdominal pain, nausea and sometimes vomiting. Symptoms are often

minor and most infections are self-limiting, lasting a few days. Nevertheless, in some patients the infection may be more serious and associated with dehydration or septicemia. Mortality is typically low with less than 1% of reported cases being lethal. The usual reservoir of *Salmonella* is the intestinal tract of animals from where a variety of foodstuffs of both animal and plant origin may become contaminated with fecal organisms either directly or indirectly. Contamination through food often occurs when organisms are introduced into food preparation areas and are allowed to multiply in food, e.g. due to insufficient storage temperatures, inadequate cooking or cross-contamination of RTE food. Even infected food handlers may act as a cause of contamination for foodstuffs.

The occurrence of both food-borne pathogens has been studied by several groups in several countries. With respect to *L. monocytogenes*, in China, it has been found that, in RTE foods, the contamination level of *Listeria monocytogenes* was 6.3-6.9% [7,8], in Sweden, data from two surveys, performed in 2010, showed that the prevalence of *L. monocytogenes* in some RTE food categories at $\geq 10^2$ cfu/g was low when meat-product or cheese were tested. This, however, was not the case for fish products and especially gravad (marinated) fish where 12% of the samples containing $\geq 10^2$ cfu/g were found [9]. In Turkey [10], in a study of several different salads (samples collected from food presented for sale in the refrigerator) *L. monocytogenes* was found in 6% of the samples. In Italy, *L. monocytogenes* was found in only 1.01% of the RTE samples [11]. In Spain, it was isolated from vegetables at 4.18% prevalence [12], whereas in Greece it was found at 6.8% [13] in RTE food in University canteens. In Ethiopia, its prevalence was 5.4% [14], in products of animal origin. In Portugal a 4.1% incidence was found for pastry products [15]. In Poland, in cakes and delicatessen (excluding meat products) incidence was 0.4% and 0.7% respectively [16].

The most prevalent reports on *Salmonella* incidence in food include a study in Greece where it was isolated from 17.9% (13) of the samples in RTE food in University canteens. In China, there are reports of 2-3.5% of RTE samples positive for *Salmonella* [17,18] as well as of 39% positive samples in the Hong Kong area [19]. In Spain, it was detected in 0.7% of lettuce samples tested [20]. In Nigeria, *S. typhimurium* was detected in 11.1% of the samples and *S. typhi* in 4.8% [21]. In Turkey, it was isolated from 8% RTE salads [10] and from

2.4% Tulum cheese [22]. In South Africa, it was detected in 16% of the samples obtained from retail delicatessens [23] and finally, in Egypt, it was detected at 2% incidence in dairy products [24].

The present study reports the prevalence of the two food pathogens (*Salmonella* spp. and *Listeria monocytogenes*) in food samples able to support their growth acquired over a period of two years in RTE foods on the island of Kefalonia, Greece. Care was taken so that the food samples represent all categories of RTE foods which restaurants, canteens and cafes sell as well as both ways that a consumer can obtain the RTE foods i.e. bought from the retail site or delivered. The study examines the prevalence of the two pathogens in a specific highly touristic area of Greece, the island of Kefalonia. It is the third report coming from Greece which differs from the two previous in that it examines food from several different manufacturing establishments as opposed to only food prepared in University restaurants and canteens. Furthermore, Kefalonia is an island on the west sea (Ionian Sea) of Greece, lightly populated, whereas the previous reports took place in the northern part of Greece (in the second highest populated city of Greece). Moreover, it is the first report on seasonal variation of incidence of *Salmonella* spp. and *Listeria monocytogenes* in ready-to-eat food in Greece.

Materials and Methods

Food samples

A total of 1329 food samples were purchased over a period of two years (August 2010 and July 2013) from restaurants, cafes and canteens on the island of Kefalonia, Greece. All food samples were purchased and transferred to the laboratory in sterile and sealed plastic containers or were delivered to the lab through the restaurant's delivery system. Samples bought and transferred to the lab were stored at refrigerated temperature for transportation whereas those delivered were transferred to the lab into the containers used by the restaurant's delivery system. All samples were immediately transported to the laboratory and stored at appropriate refrigeration temperatures. Conditions of the RTE foods at retail level are presented in Table 1. Microbiological analysis was carried out within 1-2 hours of purchase. All samples were tested twice.

Food	# of samples	Conditions at retail site
Meals-1		
Sandwiches	103	Presented for sale in the fridge
Cheese and spinach pie	89	Kept in electric food warmer at restaurant
Cooked meat with vegetables meal	84	Kept in electric food warmer at restaurant
Total	276	
Meals-2		
Sandwiches	97	Delivered through restaurant's delivery system
Cheese and spinach pie	52	Delivered through restaurant's delivery system
Cooked meat with vegetables meal	71	Delivered through restaurant's delivery system
Total	220	

Salads-1		
Vegetables with olive oil	23	Presented for sale in the fridge
Vegetables with meat and dressing	30	Presented for sale in the fridge
Total	53	
Salads-2		
Vegetables with olive oil	27	Delivered through restaurant's delivery system
Vegetables with meat and dressing	35	Delivered through restaurant's delivery system
Total	62	
Desserts		
Oven baked pastries	256	Presented for sale in the fridge
Desserts with dairy cream	335	Presented for sale in the fridge
Total	591	
Ice cream	127	Presented for sale in the freezer
Grand Total	1329	

Table 1: Food categories and conditions at retail level of RTE foods.

Microbiological analysis

Salmonella spp. was detected according to ISO 6579:2002/Cor. 1:2004 [25]. Portions of 25 g from each sample were aseptically transferred in stomacher bags and homogenized with 225 ml of buffered peptone water (BPW) (Difco) for 120 s using a Stomacher Bag mixer (Interscience). Quantities of 1 and 0.1 ml were inoculated into Tetrathionate (TTn) broth with Novobiocin (Oxoid) and Rappaport-Vassiliadis (RV) broth (Difco), respectively. The enrichment broths were incubated for 24 ± 2 h at 37°C ± 1°C (for TTn broth) and 42°C (for RV broth), respectively. The positive cultures were streaked onto Xylose Lysine Desoxycholate (XLD) agar (Difco) and *Salmonella* chromogenic agar (Oxoid) and incubated for 24 ± 2 h at 37°C ± 1°C. Five presumptive colonies were picked from each plate, inoculated into Triple Sugar agar (Difco) and Lysine Iron agar slopes (Difco) and incubated at 37°C ± 1°C for 24 ± 2 h. Isolates with typical *Salmonella* reactions were streaked onto Trypticase Soy agar plates (Difco) and confirmed by the API-20E Enteric Identification System (bioMerieux, Inc.). The reference strains *S. typhimurium* ATCC 25241 and *S. enteritidis* ATCC 49223 were used as positive control strains.

L. monocytogenes was detected according to the ISO 11290-1:1996 [26]. Samples (25 g) were weighted into sterile stomacher bags and homogenized with 225 ml of Fraser broth (Bio-Rad) for 120 s using a Stomacher Bag mixer (Interscience). Following the incubation of samples at 37°C ± 1°C for 48 ± 2 h, positive broths were streaked onto *Listeria* Palcam agar (Oxoid) and incubated at 37°C ± 1°C for 24 ± 2 h. Characteristic colonies were streaked onto Trypticase Soy agar plates (Difco) and incubated at 37°C ± 1°C for 24 ± 2 h. All isolates were Gram stained, tested for motility and catalase activity and identified by the API *Listeria* system (bioMerieux, Inc.). As positive controls, the *L. monocytogenes* ATCC 19115 and ATCC 7644 strains were used.

Results were expressed as absence or presence of *Salmonella* spp. or *L. monocytogenes* per 25 g.

Statistical analysis

Statistical analysis was carried out by using the SPSS 20.0 software. The aim of the statistical analysis has been to detect differences between the mean percentages of infection incurred by *L. monocytogenes* or *Salmonella* spp. among the four seasons. Comparisons were made between the mean values of the infection percentages measured during each season over a two year period (2011-2013). A mean value for a specific season was evaluated from 6 individual measurements conducted in the corresponding months during the two year period. Due to the small sample size (6 measurements for each season), non-parametric (or distribution-free) statistical tests were employed. More specifically, the Kruskal-Wallis (K-W) test was initially applied among the mean values of all four seasons by employing the 95% significance level. In case the K-W test provided evidence for the existence of statistically significant differences (p<0.05), pairwise comparisons between all four mean values were conducted by using Mann-Whitney tests in order to identify statistically significant differences between two specific mean values.

Results

A list of all the food samples tested and the prevalence of *L. monocytogenes* for all food categories tested is given in Table 2.

RTE food	<i>L. monocytogenes</i>	<i>Salmonella</i> spp.
Meals-1		
Sandwiches	20/103 ^a (19.4%) ^b	5/103 (4.9%)
Cheese and spinach pie	16/89 (18.0%)	1/89 (1.1%)
Cooked meat with vegetables meal	2/84 (2.4%)	0/84 (0.0%)

Total	38/276 (13.8%)	6/276 (2.2%)
Meals-2		
Sandwiches	16/97 (16.5%)	4/97 (4.1%)
Cheese and spinach pie	10/52 (19.2%)	0/52 (0.0%)
Cooked meat with vegetables meal	0/71 (0.0%)	0/71 (0.0%)
Total	26/220 (11.8%)	4/220 (1.8%)
Salads-1		
Vegetables with olive oil	3/23 (13.0%)	0/23 (0.0%)
Vegetables with meat and dressing	4/30 (13.3%)	1/30 (3.3%)
Total	7/53 (13.2%)	1/53 (1.9%)
Salads-2		
Vegetables with olive oil	3/27 (11.1%)	0/27 (0.0%)
Vegetables with meat and dressing	6/35 (17.1%)	0/35 (0.0%)
Total	9/62 (14.5%)	0/62 (0.0%)
Desserts		
Oven baked pastries	2/256 (0.8%)	0/256 (0.0%)
Desserts with dairy cream	76/335 (22.7%)	3/335 (0.9%)
Total	78/591 (13.2%)	3/591 (0.5%)
Ice cream	33/127 (26.0%)	0/127 (0.0%)
Grand Total	191/1329 (14.4%)	14/1329 (1.05%)
^a Number of positive samples/number of samples analyzed		
^b Percentage of positive samples		

Table 2: Prevalence levels of *Listeria monocytogenes* and *Salmonella* spp. in RTE foods in Kefalonia, Greece.

Meals bought on site

Dishes were selected based on what restaurants and canteens usually offer to consumers. It is customary, in Kefalonia, Greece, to have as main meal a dish comprising of meat (beef, pork, goat, sheep or chicken) cooked along with vegetables or a piece of pie which is usually made from fyllo pastrie filled with feta cheese or feta cheese and spinach. Out of the 276 such RTE foods examined, 38 (13.8%) were contaminated with *L. monocytogenes* and 6 (2.2%) were tested positive for *Salmonella* spp., while 2 samples (sandwiches) were contaminated with both pathogens. Of the three RTE food categories examined, the prevalence of *L. monocytogenes* was the highest in sandwiches and pies (cheese and spinach) with 20/103 and 16/89 (or 19.4% and 18.0%) of the samples being positive for the microorganism. In the case of meals composed of cooked meat with vegetables, *L. monocytogenes* was found in 2/84 (2.4%) of the samples. In the same RTE foods, *Salmonella* spp. was found in 6 of the 276 (2.2%) samples tested. Specifically, in the case of sandwiches and pies, *Salmonella* spp. was found respectively in 5/103 and 1/89 (4.9% and 1.1%) of the samples

examined. No cooked meat with vegetable meal was found to be positive for *Salmonella* spp.

Meals delivered through restaurant's delivery system

Meals were provided by restaurants only, as canteens do not have a delivery service in Kefalonia, Greece. Out of 220 samples of meals delivered through the restaurant's delivery system 26 (11.8%) were tested positive for *L. monocytogenes* and 4 (1.8%) were tested positive for *Salmonella* spp. No sample tested positive for both pathogens. *L. monocytogenes* was detected in 16/97 (16.5%) and 10/52 (19.2%) of the samples tested in sandwiches and pies (cheese and spinach) whereas no *L. monocytogenes* was found in meals composed of cooked meat with vegetables. *Salmonella* spp. was detected only in sandwiches in 4/97 (4.1%) of the samples examined.

Salads bought on site

The traditional Greek salad consists of a variety of vegetables with olive oil and either lemon juice or vinegar as dressing. However, during the last twenty years, salads with other dressings have appeared in restaurants. Frequently, these salads are considered whole meals when pieces of chicken or bacon are incorporated into them. During the examination of the salads that are sold in the restaurants and canteens, care was taken as to include both categories. Fifty three salads were examined and *L. monocytogenes* was found in 7 (13.2%) whereas *Salmonella* spp. was found in 1 (1.8%). No sample tested positive for both microorganisms. When considering the two categories of salads tested, *L. monocytogenes* was detected in both at 3/23 (13%) and 4/30 (13.3%) in vegetables with olive oil as dressing and vegetables with meat and dressing, respectively. *Salmonella* was detected only in one sample of vegetables with meat and dressing salad.

Salads delivered through restaurant's delivery system

In this case, as in the case of meals delivered through restaurant's delivery system, salads were purchased from restaurants only and not canteens. No sample examined showed to be positive for *Salmonella*. Out of the 62 salads 9 (14.5%) were tested positive for *L. monocytogenes*. *L. monocytogenes* was detected at 3/27 (11.1%) and 6/35 (17.1%) in vegetables with olive oil as dressing and vegetables with meat and dressing, respectively.

Desserts

Desserts in Kefalonia, as well as in Greece in general, can be divided into two categories. Those that are prepared and oven baked, where there is no manipulation of the product after receiving the heat treatment and those that include dairy cream filling introduced to the product after the heat treatment. The later often include ingredients such as eggs or their parts which often do not receive enough heat treatment. All desserts were bought on site (restaurants, canteens and cafes) and transferred to the lab. A total of 591 products were examined and *L. monocytogenes* was found in 78 (13.2%) whereas *Salmonella* was detected in 3 (0.5%). The three positive for *Salmonella* spp. samples were also positive for *Listeria monocytogenes*. The vast majority of *L. monocytogenes* positive samples belonged to the category of desserts with dairy cream as the microorganism was detected in 76/335 (22.7%) samples while it was found in only 2/256 (0.8%) desserts which are oven baked. *Salmonella* spp. was detected only in desserts with dairy cream 3/335 (0.9%).

Ice cream

All ice cream samples were bought on site from cafes and ice cream parlors. Out of the 127 samples tested 33 (26.0%) were found positive for *L. monocytogenes* and none was found to be positive for *Salmonella* spp. After contacting the manufacturers, it was known to us that the usual ingredients of ice cream are pasteurized milk and/or dairy cream (in some cases dry milk is used as a substitute), egg and/or egg products, several flavorings, nuts and chocolate.

Seasonal variation

The monthly distribution of *L. monocytogenes* and *Salmonella* spp. on 1329 RTE food samples collected over 24 months in the years 2011-2013 in Kefalonia, Greece is shown in the following (Tables 3 and 4).

Year	Winter			Spring			Summer			Fall		
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
2011	6/55 ^a								9/55	11/55	6/55	4/55
2012	5/55	9/55	10/55	7/55	11/55	8/55	9/55	10/55	11/55	8/55	6/56	7/56
2013		4/56	1/56	9/56	9/56	9/56	10/56	12/56				
Total/month	11/110	13/111	11/111	16/111	20/111	17/111	19/111	22/111	20/110	19/110	12/111	11/111
Total/season	35/332			53/333			61/332			42/332		

^aNumber of positive samples/number of samples analyzed

Table 3: Monthly distributions of *L. monocytogenes* on 1329 RTE food samples collected over 24 months in the years 2011-2013 in Kefalonia, Greece.

	Winter			Spring			Summer			Fall		
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
2011	0/55 ^a								1/55	0/55	0/55	0/55
2012	0/55	0/55	1/55	0/55	1/55	2/55	2/55	2/55	1/55	1/55	0/56	1/56
2013		0/56	0/56	0/56	0/56	1/56	1/56	0/56				
Total/month	0/110	0/111	1/111	0/111	1/111	3/111	3/111	2/111	2/110	1/110	0/111	1/111
Total/season	1/332			4/333			7/332			2/332		

^aNumber of positive samples/number of samples analyzed

Table 4: Monthly distributions of *Salmonella* spp. on 1329 RTE food samples collected over 24 months in the years 2011-2013 in Kefalonia, Greece.

The results of the statistical analysis are given below, separately for each microorganism.

***L. monocytogenes*:** By applying the Kruskal-Wallis test among the mean percentages of incidence observed in all four seasons a p value of 0.027 (i.e. <0.05) was obtained, thus providing evidence for the existence of statistically significant differences. Subsequent application of pairwise Mann-Whitney tests showed that the mean percentages of contamination incurred by *L. monocytogenes* in the Summer was higher than the ones in Winter, Spring and Fall at 95% statistical significance. On the other hand, no statistically significant differences were detected between the mean percentages of contamination observed in Winter, Spring and Fall.

***Salmonella* spp.:** By applying the Kruskal-Wallis test among the mean percentages of infection observed in all four seasons a p value of 0.096 (i.e. >0.05) was obtained, thus providing evidence for the absence of statistically significant differences between them.

Discussion

To date, limited information has been available on the prevalence of *L. monocytogenes* and *Salmonella* spp. in RTE food in Greece, and to our knowledge, no such study has been reported for the island of Kefalonia yet. Our results show the prevalence of *L. monocytogenes* and *Salmonella* spp. in the most common RTE foods sold in the food establishments of Kefalonia in the two year period between August 2011 and July 2013. A total of 1329 samples were examined. *L. monocytogenes* was found in 191 whereas *Salmonella* was found in 14 of the samples.

With respect to *L. monocytogenes*, it was found that 13.8% and 11.8% of the samples of meals (presented for sale in the fridge and delivered through restaurants delivery system, respectively) examined were contaminated with the microorganism. However, its incidence in cooked meat and vegetable meals was low (2.4% and 0% in meals presented for sale in the fridge and delivered through restaurants

delivery system, respectively), whereas it was high in the other two categories of meals examined. In the case of sandwiches, contamination was observed in 19.4% and 16.5% of the sandwiches examined (presented for sale in the fridge and delivered through restaurants delivery system, respectively). Two reports from Greece have recorded 7.7% and 7.3% prevalence of the microorganism in sandwiches examined in Northern Greece [13,27]. The ingredients of specific products examined in our study were bread, cheese, meat product, tomato, salad greens and dressing. *L. monocytogenes* contamination has been previously related partially to cheese, ham, vegetables, mayonnaise and mayonnaise salad constituting the sandwiches [27]. As the use of organic vegetables is a preferred practice in the island, the much higher contamination observed may be attributed partly to that practice. Occurrence of *L. monocytogenes* in cheese and spinach pie was also very high (18% and 19.2% in the products presented for sale in the fridge and delivered through restaurants delivery system, respectively). In the case of these products, they are usually produced by specific companies, frozen and transported in this condition to the restaurants, canteens or any other retail store. The places that serve these foods, bake them usually while in the frozen state and serve them. The major problem in this kind of handling is that it does not ensure that the combination of time and temperature used to cook the product is enough to kill all pathogens present in the frozen food.

The incidence of *L. monocytogenes* in the samples of salads examined was high (13.2% and 14.5% in the salads presented for sale in the fridge and delivered through restaurants delivery system, respectively). Ingredients in all salads examined, in this study, were salad greens, tomato, cucumbers. Furthermore, some of the salads included some kind of meat (chicken or bacon). The dressing used in salads with vegetables only constituted from olive oil and vinegar or lemon juice whereas the salads with meat had a mayonnaise base dressing. Although some of the *L. monocytogenes* load can be attributed to the presence of meat in the salad, equally important if not more, is the load found on raw vegetables. As has already been mentioned, organic vegetables are preferred in the island and usually carry a heavy bacterial load. Results on incidence of the microorganism in salads from reports from other countries vary. A report from Turkey has recorded a 13.4% contamination with *L. monocytogenes* in Caesar salad (a salad composed of boiled or fried chicken, fried bread, lettuce, parsley, tomato, cucumber and boiled corn) [10], results that agree with ours. Jamali et al. [28] found that, in Malaysia, the high prevalence of *L. monocytogenes* was distributed in salads and vegetables. In South China, 26.7% of the cold vegetables dishes in sauce were found to be contaminated [7]. No *L. monocytogenes* was detected in RTE salads in Italy [29].

Out of all food categories examined, the presence of *L. monocytogenes* was the highest in desserts with dairy cream and ice cream (22.7% and 26%, respectively), both of which are predominantly made with whipped cream or with pastry cream both of which involve much handling throughout the final formulation process. Another factor is the omnipresence of uncooked eggs throughout kitchen working stations since the pasteurized liquid/powdered egg option is not favored. This fact, escalated by the occasional addition of raw eggs in the final preparatory stages, leads to cross-contamination of RTE desserts and ice cream. Kotzekidou [13] reported a high prevalence rate of 20% in desserts with dairy cream and a 17.7% in desserts [27] in studies in Northern Greece.

Different criteria or recommendations for *L. monocytogenes* have been established in RTE foods in different countries. In the USA and Italy, a zero tolerance policy has been adopted in RTE foods. In other EU countries (i.e. Germany, Netherlands, France and Greece), there is a tolerance of below 10^2 cfu/g of food at the time of consumption [30, 31]. As already mentioned *L. monocytogenes* can grow at refrigeration temperatures and most of these products use refrigeration as the sole method to control pathogen growth.

Salmonella spp. contamination was lower than that observed for *L. monocytogenes*. The highest incidence of *Salmonella* spp., was found in meals 6/276 and 4/220 (2.2% and 1.8%) of the meals presented for sale in the fridge and delivered through restaurants delivery system, respectively. Out of the ten positive samples for *Salmonella* nine belonged to the sandwich category. These data show a lower incidence than that found in previous studies in Northern Greece [13] and South Africa [23] where 17.5% and 20% of the sandwiches tested were found to be contaminated with *Salmonella* spp. The other sample positive for *Salmonella* was a cheese pie. Results from a previous Greek study show a 13.7% contamination level in oven baked pastries [13], results which are substantially higher than ours. In the salads category 1/115 (0.1%) was found positive for the pathogen. The specific salad consisted of raw vegetables, chicken and mayonnaise-base dressing. Incidence of *Salmonella* spp. in salads has been studied in Spain, South Africa, Turkey, Nigeria with 0.7%, 11%, 8.0% and 8.0% [10,20,21,23], respectively, being positive for the bacterium.

Desserts with dairy cream had the second highest incidence of *Salmonella*, with 3/335 (0.9%) of the samples tested being positive. This result is much lower than what was detected in a study in Northern Greece [13] where a 12.5% incidence of the pathogen was found in the specific food group. The overall *Salmonella* spp. positive samples were 14/1329 (1.05%). No *Salmonella* was detected in cooked meat with vegetables meals, vegetables with olive oil (salads) or ice cream, indicating proper procedures during preparation, handling and storage of these food items.

With regard to the dependence of the frequency of contamination on the time of the year, the results of our study indicate a statistically significant higher presence of *L. monocytogenes* during summer. On the other hand, our experimental data did not provide strong evidence for the existence of the same dependence for *Salmonella* spp. These findings are consistent with those published by EFSA [3] for *L. monocytogenes* reported cases for the European Union. A similar periodic incidence was reported for *Salmonella* spp. as well [3]. The observed very low contamination frequencies in combination with the small number of data (6 measurements) are the most probable causes for the failure of our statistical analysis to detect statistically significant differences between the different times of the year for *Salmonella* spp.

Results showed that RTE foods can be contaminated with *L. monocytogenes* and *Salmonella* spp. The high prevalence of *L. monocytogenes* in many of the RTE foods examined (sandwiches, cheese and spinach pie, salads, desserts with dairy cream and ice cream) shows that there is a need for improved in-house control to reduce risks of human listeriosis, as the most probable sources of contamination include contaminated raw material and post-processing contamination, even though the possibility that the cooking process is not sufficient in some cases, cannot be ruled out.

A major issue in the island of Kefalonia is the preference shown by the consumers for organically grown food. Indeed, consumer concern over the quality and safety of food has intensified in recent years

resulting in a rise in the demand of organically grown food. Hence, from a marketing point of view, RTE products appear to be more attractive to consumers when organic ingredients and few or no additives are used. Furthermore, the use of sanitizers in organic fresh produce is strictly regulated, resulting in very low concentrations of such compounds and thus raising sanitation questions [32]. Essential oils, which have been extensively studied, could constitute an effective and legal solution to this difficulty and become an alternative for synthetic preservatives [32-34].

Conclusions

In conclusion, this study revealed a low incidence of *Salmonella* in RTE foods sold in restaurants, canteens and cafes in the island of Kefalonia. However, the prevalence of *L. monocytogenes*, in the same food samples was high indicating high risk to consumers as RTE foods are consumed directly after purchase without any further treatment to reduce the microbial load. The island is in need not only of legal and appropriate manufacturing practices but also of intensified hygiene inspections pertaining to foodstuff. Additionally, operative control actions should be employed in order to develop a higher microbiological quality of RTE foods. Such actions could potentially be systematizing workshops, instructive seminars or conferences on food handling and hygiene practices for all involved parties of RTE food production.

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