Recent trends in the epidemiology of non-typhoidal *Salmonella* in Israel, 1999–2009

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SUMMARY

The aim of the present study was to assess the recent trends in the epidemiology of non-typhoid *Salmonella* in Israel using a sentinel laboratory-based surveillance network. Between 1999 and 2009, 8758 *Salmonella* stool isolates were reported by five sentinel laboratories. There was a significant decrease in the incidence rate of *Salmonella* isolates from 70·5/100 000 in 1999 to 21·6/100 000 in 2005 followed by a slight increase to 30·3/100 000 in 2009. Of all *Salmonella*, 64·3% were isolated from children in the 0–4 years age group. Up to 2008, *S.* Enteritidis was the most prevalent serotype and in 2009 *S.* Infantis emerged as the most common *Salmonella* serotype. The decrease in the incidence of *S.* Enteritidis and *S.* Typhimurium and increase in *S.* Infantis among humans were associated with a similar trend among breeding flocks, which followed significant preventive interventions conducted against *S.* Enteritidis and *S.* Typhimurium infections in poultry. Tight surveillance and education of food handlers and consumers should be enhanced to reduce the foodborne transmission of *Salmonella* in Israel.

Key words: Antibiotic resistance, diarrhoea, epidemiology, foodborne infections, *Salmonella*.

INTRODUCTION

Non-typhoid *Salmonella* are an important cause of enteric diseases and are associated with severe morbidity and excess mortality worldwide [1].

Salmonellosis is a foodborne disease, commonly manifested by acute enterocolitis, with sudden onset abdominal pain, diarrhoea, nausea, sometimes vomiting and fever [2]. Symptoms usually appear within 1–3 days following exposure and persist for 3–5 days. Serious bloodstream infections can occur, particularly in the very young or in the elderly, who are also at the highest risk for morbidity and mortality, depending on the non-typhoid serotypes [3, 4]. The extent of morbidity and mortality associated with

non-typhoidal salmonellosis revealed the need for enhanced surveillance to follow sporadic (about 60–80 % of all cases [2]) and outbreak source cases. Surveillance networks such as FoodNet and EnterNet have been established in the USA and Europe aiming at early detection of enteric disease outbreaks, detection of changes in the epidemiology of the different agents causing these outbreaks and identifyng new genes encoding resistance to antibiotics.

The FoodNet surveillance run by the CDC's Emerging Infections Program reported a decrease in the incidence rate of *Salmonella* laboratory-confirmed infections when comparing 1996–1998 and 2007 data [5]. A decrease was also demonstrated in Europe by Denny *et al.* [6] and in England and Wales [7].

The incidence of salmonellosis in Israel increased from 31·6/100 000 in 1954 to 109·1/100 000 in 1996, decreased thereafter to 16·0/100 000 in 2005 and increased to 20·3/100 000 in 2009 [8].

Increasing antimicrobial resistance among *Salmonella* has been noted in several countries in the last decade [9, 10]. The incidence of multidrug-resistant *S*. Typhimurium and mostly multidrug-resistant *S*. Typhimurium DT104 rose markedly worldwide, including in Israel, during the 1990s and has thus become an increasing public health problem around the world [11–13].

A sentinel laboratory-based surveillance network was established in Israel in 1997 by the Israel Center for Disease Control (ICDC), which integrates information on *Salmonella* isolates from community and hospital laboratories and from the *Salmonella* National Reference Laboratory.

In this paper we present data generated by this network on agent and host-dependent characteristics of *Salmonella* enteric infections and antibiotic resistance patterns occurring in Israel in the last decade.

MATERIALS AND METHODS

The sentinel laboratory-based surveillance network for bacterial enteric diseases (salmonellosis, shigellosis, campylobacteriosis) was established in Israel in 1997. Five community laboratories and three hospital-based laboratories representing the different geographical regions of Israel participate in this network: Haemek Medical Center (HMC) and Haifa HMO (HH) in northern Israel, Sheba Medical Center (SMC), Maccabi Dan District Community Laboratory (MD) and Clalit HMO Petah-Tikva (CPT) in central Israel, Hadassah (Ein Karem and Mount Scopus) (EK and

MS) medical centres and Meuhedet Community Laboratory (MCL) in Jerusalem District and Soroka Medical Center (SMC), Beer-Sheba in southern Israel. Clalit HMO Petah-Tikva Community Laboratory was added to the network in 2004. The population served by the five community laboratories (HMC, HH, MD, MCL, SMC) is well defined and covers 30·7 % of the total Israeli population. The data presented in this paper refer to these five community laboratories for which the size and age distribution of the population they served was known and made possible the calculation of incidence rates. Demographic data on individuals with a positive stool sample for *Salmonella* and isolate characterization to the serogroup level were obtained from the sentinel laboratories and transferred to the ICDC, where they were integrated with the data on the further characterization of the isolates at the *Salmonella* National Reference Laboratory of the Ministry of Health.

The *Salmonella* National Reference Laboratory performed serotyping, phage-typing and antibiotic resistance testing. In the mid-2009, the performance of phage-typing was discontinued. This paper includes phage-type data until 2008.

Salmonella of the same serogroup and serotype isolated from the same individual within a month were counted only once. This paper refers to non-typhoidal *Salmonella* isolates.

Susceptibility of *Salmonella* isolates of the serotypes *S*. Enteritidis, *S*. Typhimurium, *S*. Virchow and *S*. Hadar to ampicillin (AMP), sulfamethoxazole–trimethoprim (SXT), ceftriaxone (CRO), chloramphenicol (C), ciprofloxacin (CIP) and tetracycline (TET) was determined at the *Salmonella* National Reference Laboratory of the Ministry of Health, by the Kirby–Bauer disk diffusion method [14]. The tests were performed on Muller–Hinton medium using Oxoid antimicrobial susceptibility disks (Oxoid, UK). Multidrug resistance was defined as resistance to three or more antimicrobial agents. These tests were performed for the years 2002–2007. The data on antibiotic resistance are presented for the total eight sentinel laboratories.

Data analysis

Data analysis was performed using SAS software (release 9.1.3; SAS Institute Inc., USA). Information on the population size served by each sentinel laboratory was available by age group and sex. Incidence rates were calculated by year, month, age group and
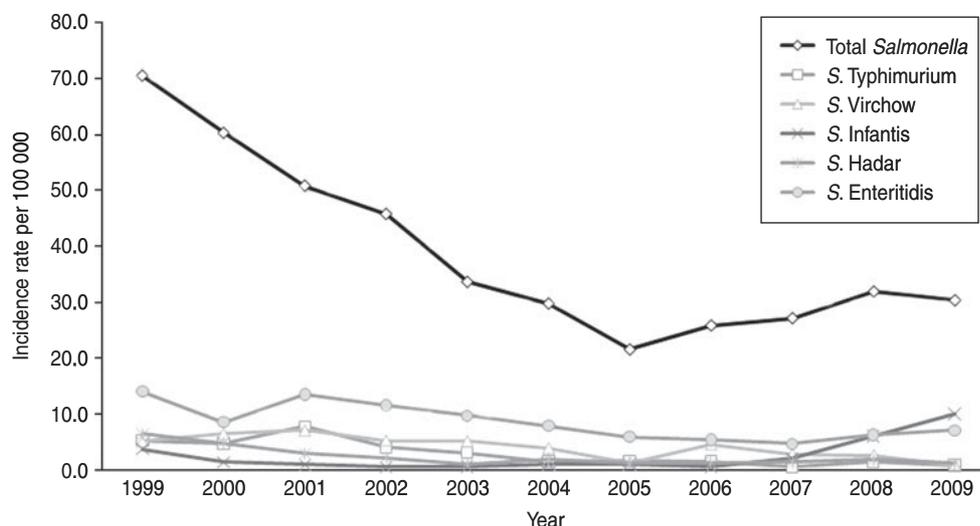
Fig. 1. Incidence rate (per 100 000) of total *Salmonella* isolates and five most commonly identified *Salmonella* serotypes in five sentinel community laboratories, 1999–2009.

for the five most prevalent serotypes by dividing the number of individuals positive for *Salmonella* by the population covered by that laboratory.

We investigated time trends in the incidence rates in Israel from 1999 to 2009, using linear regression models.

RESULTS

Between 1 January 1999 and 31 December 2009, data on 8758 isolates of *Salmonella* from stool specimens were reported to the ICDC by the five community laboratories according to the following breakdown: HH 2417 isolates (27·6%), SMC 1791 (20·4%), MCL 2046 (23·4%), MD 1783 (20·4%) and HMC 721 (8·2%).

Age and gender distribution

Data on patient's age was achievable for 8649 *Salmonella* isolates. A total of 5558 (64·3%) isolates were from children in the 0–4 years age group. The proportions of isolates from the 5–14, 15–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years age groups were 11·7% (1011 isolates), 5·4% (468), 4·7% (403), 3·1% (271), 3·6% (311), 2·7% (236) and 4·5% (391), respectively. Of children aged < 5 years, 1683 (30·3%) were isolated in the first year of life, 2123 (38·2%) in the second year of life, 975 (17·5%) in the third and 777 (14·0%) in the fourth and fifth years of life. Of the 8620 isolates for whom data on patient's gender were available, 53·2% (4587 isolates) were from male patients.

Salmonella serogroups, serotypes and phage types

The five most commonly identified serogroups were: *Salmonella* C1 (26·2%), *Salmonella* D (26·1%), *Salmonella* B (17·8%), *Salmonella* C2 (12·5%) and *Salmonella* E (2·0%). Of the total isolates, 11·8% were identified just by biochemical features and reported as *Salmonella* spp. without specification of serogroup.

The ten most commonly identified serotypes over the period 1999–2009 were: *S.* Enteritidis (26·5%), *S.* Virchow (12·7%), *S.* Typhimurium (8·8%), *S.* Infantis (8·4%), *S.* Hadar (7·3%), *S.* Bredeney (3·3%), *S.* Montevideo (3·0%), *S.* Java (2·2%), *S.* 9,12:l,v:- (2·1%) and *S.* Newport (1·9%). *S.* Enteritidis was the most commonly isolated serotype in all age groups.

Routine phage-typing was performed for *S.* Enteritidis and *S.* Typhimurium using the Israeli phage-typing scheme. The three most prevalent phage types among *S.* Enteritidis were B3 (47·4%), F3 (21·5%) and C8 (18·5%). The three most prevalent phage types among *S.* Typhimurium were 2(4+) (44·6%), Group 2 (22·3%) and R9 (9·0%).

Time trends

There was a significant decrease in the incidence rate of *Salmonella* isolates from 70·5/100 000 in 1999 to 21·6/100 000 in 2005 (P value < 0·01) followed by an increase to 30·3/100 000 until 2009 (P value = 0·03 for trend) (Fig. 1). This time trend was evident in all five community laboratories (data not shown).

Table 1. *Proportions of antibiotic resistance patterns of* Salmonella *serotypes, 2002–2007*

	AMP R (%)	n	SXT R (%)	n	CRO R (%)	n	C R (%)	n	CIP R (%)	n	TET R (%)	n
S. Typhimurium	99 (45·2)	219	10 (4·6)	219	0 (0·0)	219	83 (37·9)	219	0 (0·0)	219	116 (53·0)	219
S. Enteritidis	23 (3·7)	617	4 (0·6)	617	2 (0·3)	617	4 (0·6)	617	0 (0·0)	617	11 (1·8)	617
S. Virchow	41 (9·4)	435	165 (37·9)	435	0 (0·0)	435	140 (32·2)	435	0 (0·0)	435	171 (39·3)	435
S. Hadar	36 (16·4)	219	11 (5·0)	219	0 (0·0)	219	1 (0·5)	219	1 (0·5)	219	194 (88·6)	219

R, Resistant; AMP, ampicillin; SXT, sulfamethoxazole–trimethoprim; CRO, ceftriaxone; C, chloramphenicol; CIP, ciprofloxacin; TET, tetracycline.

Table 2. *Antibiotic resistance patterns of* Salmonella *serotypes, 2002–2007*

	Pattern	AMP	SXT	CRO	C	CIP	TET	n (%)
S. Typhimurium	1	R	S	S	R	S	R	79 (36·1)
(n = 219 isolates tested)	2	S	S	S	S	S	R	27 (12·3)
	3	R	S	S	S	S	S	11 (5·0)
S. Virchow	1	S	R	S	R	S	R	112 (25·8)
(n = 435 isolates tested)	2	R	R	S	R	S	R	25 (5·8)
	3	S	R	S	S	S	R	21 (4·8)
	4	R	S	S	S	S	S	8 (1·8)
S. Enteritidis	1	R	S	S	S	S	S	16 (2·6)
(n = 617 isolates tested)	2	S	S	S	S	S	R	5 (0·8)
S. Hadar	1	S	S	S	S	S	R	152 (69·4)
(n = 219 isolates tested)	2	R	S	S	S	S	R	33 (15·1)

AMP, Ampicillin; SXT, sulfamethoxazole–trimethoprim; CRO, ceftriaxone; C, chloramphenicol; CIP, ciprofloxacin; TET, tetracycline.

The decrease in the incidence rate of *Salmonella* isolates from 1999 to 2005 and the increase from 2005 and onwards were consistent in all age groups and in both males and females (data not shown). The highest incidence rates throughout the years were observed in the 0–4 years age group, which also experienced the most significant decrease from 437·7/100 000 in 1999 to 121·0/100 000 in 2005 followed by and increase to 176·4/100 000 in 2009. Other age groups showed the same trends with about tenfold lower incidence rates.

There was a clear pattern of seasonality in the number of *Salmonella* isolates over the years with an increase in April–May, a peak in August and a decrease thereafter. A shifting in the most prevalent serotype was observed in 2009. The incidence of *S*. Enteritidis, the most prevalent serotype during 1999–2008, decreased from 14·0/100 000 in 1999 to 4·7/100 000 in 2007 and increased to 7·1/100 000 in 2009. The incidence of *S*. Infantis increased markedly from 0·6/100 000 in 2006 to 10·0/100 000 in 2009, becoming the most prevalent *Salmonella* serotype in Israel in 2009 (Fig. 1).

Antibiotic resistance

Between 2002 and 2007, 1490 (59·8%) of 2491 isolates were tested for antibiotic resistance (617 *S*. Enteritidis, 435 *S*. Virchow, 219 *S*. Typhimurium, 219 *S*. Hadar). The proportions of antibiotic resistance to these antibiotics are presented in Table 1. *S*. Typhimurium was highly resistant to tetracycline, ampicillin and chloramphenicol. *S*. Virchow was resistant to tetracycline, sulfamethoxazole–trimethoprim and chloramphenicol. *S*. Hadar was resistant to tetracycline. Over 95% of the *S*. Enteritidis isolates were susceptible to all six antibacterial drugs. Of 1490 isolates tested for antibiotic resistance, 15·4% were multidrug resistant (resistant to three or more antimicrobial agents). Multidrug resistance was found in 37·9% of *S*. Typhimurium strains, 32·6% of *S*. Virchow strains, 0·5% of *S*. Enteritidis and in 0·5% of *S*. Hadar strains.

Common antibiotic resistance patterns are presented in Table 2. The most frequent pattern (36·1%) belonged to *S*. Typhimurium isolates which were

resistant to ampicillin, chloramphenicol and tetracycline (R-Type ACT). Another common resistance pattern was that of *S.* Virchow isolates which simultaneously showed resistance to sulfamethoxazole–trimethoprim, chloramphenicol and tetracycline (25·8%).

DISCUSSION

In this article we report on 11-year data generated by a sentinel, laboratory-based surveillance network of salmonellosis established in Israel in 1997. These data show that the incidence of salmonellosis in Israel decreased markedly from 70·5/100 000 in 1999 to 21·6/100 000 in 2005, and increased to 30·3/100 000 in 2009. The parallel incidence rates reported by the Department of Epidemiology of the Israel Ministry of Health based on the national passive surveillance system which was established at the beginning of the 1950s, were 63·0/100 000 in 1999, 16·0/100 000 in 2005 and 20·3/100 000 in 2009 [8]. Although both systems of surveillance identified a similar trend in the incidence of salmonellosis in Israel, the figures yielded by the national passive surveillance system were lower and probably reflect under-reporting. Moreover, by integrating data on the isolation of *Salmonella* at the sentinel laboratories with information related to patients (gender, age, address, etc.) and characterization of isolates at the National Reference Centers of the Ministry of Health, the sentinel laboratory-based surveillance could show that the decrease in the incidence of non-typhoidal salmonellosis encompassed both genders and especially children aged 0–4 years and could display the changes in the relative importance of the leading *Salmonella* serotypes throughout 11 years of surveillance.

A decrease in the incidence of non-typhoidal salmonellosis in the last decade was also seen in other countries. According to FoodNet, the incidence rate of *Salmonella* laboratory-confirmed infections decreased by 8%, comparing data from 1996–1998 and 2007 [5]. Data from Denny *et al.* showed a similar trend [6]. In England and Wales, the estimated cases of non-typhoidal *Salmonella* fell to less than half between 1992 and 2000 [7]. The incidence of *Salmonella* infections in Israel is still higher than that reported from other developed countries. In the USA, the reported incidence rate was 14·9/100 000 in 2007 [5], in comparison with 27·2/100 000 in the present study, for the same year. We cannot rule out the possibility that this difference could be at least in part due to differences in referral of specimens, testing for pathogens or other reasons.

Salmonella was consistently most common in children aged <5 years. Similar age distributions have been reported in the USA [5, 15–17] and Australia [18]. The reason for the high incidence rate in this age group has been described previously [19] and includes the high susceptibility of the intestinal microflora since the immune system is still developing and because this age group is more likely than others to expose infected items to their mouths.

We have shown a marked pattern of seasonality of salmonellosis, with incidence rates rising in the warm months and decreasing in the cold months, as expected. This seasonal pattern was also demonstrated in the USA [15, 16] and Canada [11].

S. Enteritidis was the most common serotype detected of all serotypes in the 11 years of surveillance. Other prevalent serotypes were *S.* Virchow, *S.* Typhimurium, *S.* Infantis, *S.* Hadar, *S.* Bredeney, *S.* Montevideo, *S.* Java, *S.* 9,12:l,v:- and *S.* Newport, by rank. According to the FoodNet Emerging Infections Program, in 2007, the most common serotypes in the USA were: *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, *S.* 1,4,[5],12:i:-, *S.* Javiana, *S.* Heidelberg and *S.* Montevideo [5]. The WHO Global Salm-Surv country databank, where 31 countries submitted data on 125 745 human *Salmonella* isolates worldwide, reported that during 2002, *S.* Enteritidis was also the most common serotype among humans, accounting for 65% of all isolates [20]. After *S.* Enteritidis, the nine most common serotypes in humans were *S.* Typhimurium, *S.* Newport, *S.* Heidelberg, *S.* Infantis, *S.* Hadar, *S.* Virchow, *S.* Javiana, *S.* Saintpaul and *S.* Montevideo, by rank [20].

S. Enteritidis is related to foodborne outbreaks and sporadic cases of human gastrointestinal diseases worldwide [21] are mostly associated with undercooked eggs [22–25]. In Germany, the decline in the incidence rate of *Salmonella* between 2001 and 2008 was related to a decrease in the incidence rate of *S.* Enteritidis [26].

The Public Health Laboratory Information System (PHLIS), a national *Salmonella* surveillance system, which collects data on reported laboratory-confirmed *Salmonella* isolates in the USA, reported that *S.* Typhimurium and *S.* Enteritidis both declined substantially (28% and 34%, respectively) between 1995 and 2005 [17]. The most marked decrease in our study was observed for *S.* Typhimurium, consistently

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throughout the surveillance period, with an incidence rate of 5·3/100 000 in 1999 to 0·8/100 000 in 2009. The FoodNet surveillance system in the USA also reported a decrease in the incidence of *S*. Typhimurium [5]. In Europe, a decline in *S*. Enteritidis was observed between 1998 and 2005 [27] and in *S*. Typhimurium between 1998 and 2003 [28].

The decrease in the incidence of *S*. Typhimurium is of particular importance due to the high rate of antibiotic resistance acquisition of this serotype.

Between 1999 and 2006, *S*. Infantis was ranked fifth among *Salmonella* serotypes in Israel. Since December 2007, the incidence of this serotype increased markedly and in 2009, *S*. Infantis became the most prevalent *Salmonella* serotype in Israel. Worldwide, *S*. Infantis was ranked fifth among *Salmonella* serotypes in 2002 [20] and in the USA, *S*. Infantis was ranked ninth between 1987 and 1997 [15].

The significant decrease in salmonellosis caused by *S*. Enteritidis and *S*. Typhimurium and the emergence of *S*. Infantis in Israel as the most prevalent serotype parallel similar changes in the incidence and distribution of *Salmonella* serotypes occurring at the level of the main natural reservoir of *Salmonella*, i.e. poultry. Actions taken to reduce *S*. Enteritidis and *S*. Typhimurium infections in poultry as tight surveillance of all breeding flocks and hatcheries, culling or treatment of all flocks infected with *S*. Enteritidis or *S*. Typhimurium, improvement of biosecurity and infrastructure, and systematic immunization against *S*. Typhimurium and *S*. Enteritidis started in 1994 and 1996, led most probably to the marked decrease in the incidence of these serotypes in poultry. In 1996, *S*. Enteritidis or *S*. Typhimurium were isolated from 33·6% of the breeding flocks and in 2009 only 0·4% were positive to one of these two pathogens (E. Berman, personal communication). Since 2008, *S*. Infantis has been the most frequent serotype among breeding flocks, accounting for 27·9% of all poultry between June 2008 and May 2009 (E. Berman, personal communication). The temporal association between the increase in incidence of *S*. Infantis in breeding flocks and in humans and the similar pulsed-field electrophoresis profiles of *S*. Infantis human and poultry isolates [29] reveal the important role of the animal reservoir and the food-borne transmission of *Salmonella* in Israel.

The incidence rate of *S*. Virchow infections was ranked second over the 11 years of surveillance in Israel. The high prevalence of *S*. Virchow among non-typhoid *Salmonella* serotypes is unique to Israel [30]. In the USA and Europe, *S*. Virchow accounts for <1% and <5% of total non-typhoid *Salmonella* isolates, respectively [30] and in 2002, *S*. Virchow was ranked only seventh among human *Salmonella* isolates worldwide [20]. Physicians should pay attention to isolation of *S*. Virchow since it tends to invade the bloodstream and to cause invasive infection, especially in infants aged <2 years [3]. We will follow this pattern to discover whether *S*. Enteritidis will regain its position of leading importance among the *Salmonella* serotypes isolated in Israel.

The data we provide on the distribution of the *S*. Enteritidis and *S*. Typhimurium phage types are based on the Israeli phage-typing scheme, which prevents international comparisons, except for the incidence of *S*. Typhimurium phage type 2(4+) (according to the local scheme) which was documented as being identical to DT104 (definitive phage-type 104) [13]. The proportion of this *S*. Typhimurium phage type declined from 56·0% in 1999 to 35·1% in 2008. The reduction of DT104 phage type is of public health importance due to the resistance of this specific phage type to multiple antibacterial drugs.

We found that *S*. Typimurium and *S*. Virchow were more resistant to antibiotics compared to *S*. Enteritidis and *S*. Hadar. *S*. Typimurium and *S*. Virchow were also highly multidrug resistant. High rates of multidrug-resistant *S*. Virchow were also reported in Denmark for the period 2003–2005 [31].

In spite of being the most prevalent serotype, *S*. Enteritidis did not acquire antibiotic resistance over the years, as documented by a comparison of the findings of a study conducted in southern Israel between 1994 and 1996, when over 95% of *S*. Enteritidis isolates exhibited susceptibility to various antibiotic drugs [13]. Reports from the USA also indicate a low resistance rate of *S*. Enteritidis [32]. During 2002–2007, the period for which antibiotic resistance patterns are presented in this paper, *S*. Infantis was not tested routinely for antibiotic resistance. However, data on *S*. Infantis resistance patterns in Israel including 71 randomly selected isolates of *S*. Infantis which were identified in Israel during 2007–2009 (21 human sources, 28 poultry sources, 22 food sources) and were tested for antibiotic resistance showed that the dominant clone of *S*. Infantis was multidrug resistant (96%), with a combined resistance pattern to nalidixic acid, nitrofurantoin and tetracycline, with or without additional resistance to trimethoprim–sulfamethoxazole [29].

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It should be borne in mind that the laboratory-based surveillance system underestimates the real burden of salmonellosis. Only a fraction of individuals with gastroenteritis consult a physician, and only a small part of them have a stool culture performed. A population survey performed by FoodNet reported a ratio of 1:39 between laboratory-confirmed cases and *Salmonella* infections in the community [33].

In conclusion, despite the significant decrease in incidence rates over the last 11 years, *Salmonella* infections are still common in Israel with specific emerging serotypes such as *S.* Infantis which have become more prevalent than *S.* Enteritidis and *S.* Typhimurium. There is still a clear need for improvement of hygiene and sanitary standards in breeding flocks and hatcheries. It is expected that these actions, coupled with enhanced education of food handlers and consumers with regard to prevention of foodborne disease transmission will further decrease the incidence of salmonellosis in Israel.

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DECLARATION OF INTEREST

None.

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