Eurosurveillance MONTHLY

Surveillance report

Occurrence of Salmonella Enteritidis phage type 29 in Austria : an opportunity to assess the relevance of chicken meat as source of human salmonella infections

volume 9
issue 10
date October 2004
page 9-10

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Assuming that the various phage types of *Salmonella* Enteritidis (*S.* Enteritidis) are largely equally virulent, the importance of certain foods as sources of infection for human salmonellosis can be deduced from differences in the distribution of phage types in human and non-human samples. In 2002, *S.* Enteritidis phage type 29 (PT29) was first isolated from non-human test samples in Austria. S. Enteritidis PT29 accounted for 44 (27.7%) of 159 *S.* Enteritidis strains, derived from veterinary samples of chicken (e.g. meat, giblets) or chicken habitations (e.g. swabs from the coop and excrement). At the food retail level (chicken meat, chicken liver), five (13.1%) of 38 *S.* Enteritidis isolates were PT29. The proportion of *S.* Enteritidis PT29 in human samples was much lower. Only 0.4% (30 human primary isolates) of all *S.* Enteritidis isolates in the year 2002, and 0.33% (23 human primary isolates) of all human *S.* Enteritidis strains in 2003 were PT29. In our opinion, the discrepancy between the high prevalence of *S.* Enteritidis PT29 in broilers and chicken meat and the low number of PT29 cases in humans indicates that chicken meat of Austrian origin is currently only a minor source of human *S.* Enteritidis infections.

Introduction

In 1989 and 1990, human infections with *Salmonella* enterica subsp. enterica ser. Enteritidis (*S*. Enteritidis) increased markedly in Austria. A similar trend was observed in many European countries [1]. After a peak in 1992, the incidence of salmonella illness decreased. Since 2000, the numbers of infections have remained at a high level. In 2003, for example, there were 8271 laboratory confirmed (cultured) human salmonella infections. Of these, 7252 (87%) were serotype *S*. Enteritidis. The most important *S*. Enteritidis phage types (PT) were PT4 (45%), PT8 (32.1%) and PT21 (9.2%). Insufficiently cooked egg products and chicken meat are generally considered to be the main sources of human infection by *S*. Enteritidis, but which of these two is the main source had not previously been determined in Austria. Due to different eating habits around Europe and differences in the contamination rates of various foods, knowledge obtained from other countries cannot necessarily be applied to Austria. We hope that the following analysis of an outbreak of *S*. Enteritidis PT29 in Austria from 2000 to 2003 can assist in clarifying the relevance - in our opinion, very low - of chicken meat as a vehicle for human *S*. Enteritidis infections.

Materials and Methods

The national reference centre for Salmonella [Nationale Referenzentrale für Salmonellen] of the Österreichische Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety) receives the majority of all human and non-human salmonella strains isolated in Austria. The non-human bacterial strains are isolated from environmental samples, medical veterinary samples or food. The actual number of samples tested is not known and the representativeness of the isolates for all food and environmental contamination is uncertain. However, due to the widespread implementation of veterinary control programs in broiler chickens and egg production in Austria, and due to food control programs, which rely mainly on random sampling, the isolates derived from chicken are representative for the contamination of chicken. The salmonella isolates from the medical sector come mainly from stool samples of patients with diarrhoea. In addition to the strain, basic information such as date of sample, nature of sample, name, age and address of patient are available. Further information, such as travel history, is mostly incomplete and rarely obtained or transmitted. All salmonella

isolates received undergo serotyping (Kauffmann-White method). All S. Enteritidis isolates are phage typed [2]. Comprehensive phage typing of *S*. Enteritidis started in Austria in 1991.
We compared the proportions of S. Enteritidis PT29 among S. Enteritidis isolates of human (years 2002 and 2003), veterinarian and food origin (year 2002). Strains designated as poultry where the species was not stated were excluded from the analysis. A further subgroup of non-human strains, S. Enteritidis isolates from chicken as food from the year 2002, were evaluated. These isolates came from laboratories that specialise in analysing foodstuffs.

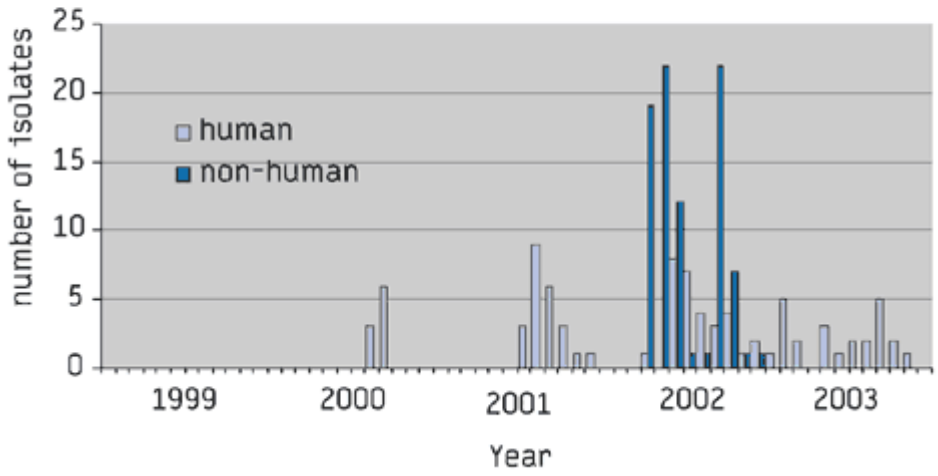
From a total of 172 isolates, 103 isolates of *S*. Enteritidis PT29 (56 human and 47 non-human isolates) were available for further subtyping by pulsed field gel electrophoresis (PFGE) using the XbaI restriction enzyme. Seventy strains were lost due to storage problems. The protocol was that specified by the European Salm-gene project [3]
All 24 patients infected with *S*. Enteritidis PT29 in 2003 were sent a questionnaire (as routinely used by the national reference centre for Salmonella in Austria), and 50% were returned (12/24). The results of the same questionnaire, sent to 598 patients with non-PT29 *S*. Enteritidis infection for other epidemiological purposes, were used as a control. The return rate in the control group was 67.1% (401/598).

Results

The temporal distribution of the isolations of *Salmonella* Enteritidis PT29 of human (n=86) and non-human (n=86) origin documented in Austria from 1999 to 2003 is shown in Figure 1.

FIGURE 1

Total number of *S.* Enteritidis PT 29 isolates of human and non-human origin, Austria, 1999-2003



Human S. Enteritidis PT29 isolates

On 27 August 2000, a human stool isolate of *S*. Enteritidis was typed as PT29 for the first time in Austria. In the same year, 9 human primary isolates were identified as *S*. Enteritidis PT29. At least 4 patients became ill during or within 7 days after a holiday in Croatia; no further information was available on these travel-associated cases. There were 23 human S. Enteritidis PT29 strains in 2001. In 2002, 30 human primary isolates from S. Enteritidis PT29 were detected in Austria. In the same year, 7459 S. Enteritidis primary isolates from human sources were registered. The proportion of S. Enteritidis PT29 was only 0.4% of the total number of human S. Enteritidis isolates. For 2003, the ratio was 24 *S*. Enteritidis PT29 strains out of 7252 human *S*. Enteritidis isolates (0.33%).

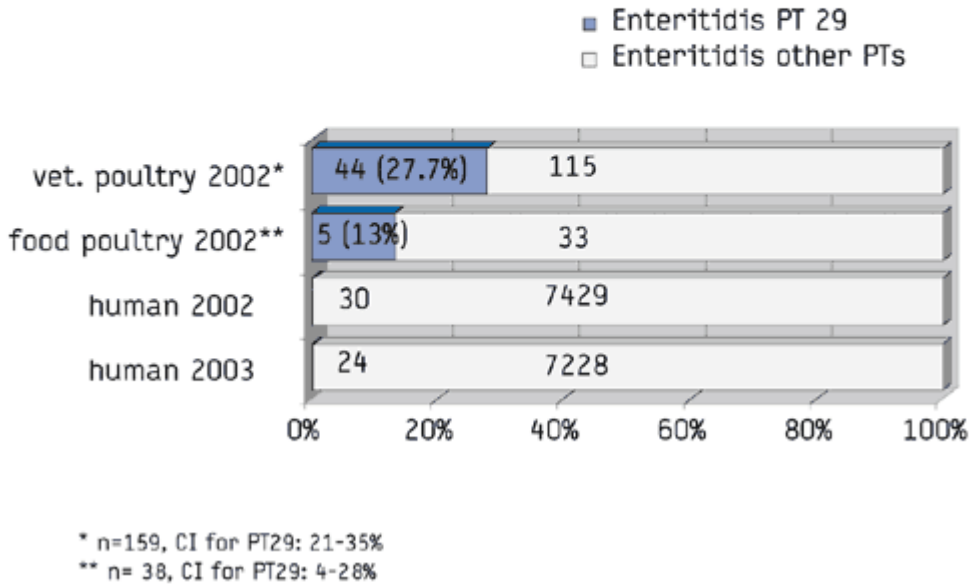
Non-human S. Enteritidis PT29 isolates

Non-human *S*. Enteritidis PT29 isolates were first identified in Austria in April 2002. That year, 86 non-human S. Enteritidis PT29 strains were isolated. *S*. Enteritidis PT29 has not been found in samples of non-human origin since January 2003.
Of the 86 isolates in 2002, 44 came from veterinary samples of chickens or chicken habitations (37 non-human S. Enteritidis PT29 strains lacked detailed information on origin; see below). In 2002, 159 *S*. Enteritidis isolates (all phage types) were isolated from veterinary samples from chickens: 27.7% of these (confidence interval (CI) 21% to 35%) from chickens or their

habitations were PT29. Five of the 86 non-human *S*. Enteritidis PT29 isolates were from food samples. These were labelled as chicken, chicken breast, chicken liver, chicken residue, and young broilers. The five food samples were obtained at different times. Testing of the samples took place in 3 laboratories in 2 federal states. In 2002, 38 S. Enteritidis isolates (all phage types) were isolated from foods: 13.1% of these (CI 4.4% to 28%) were PT29. Figure 2 presents a comparison of *S*. Enteritidis PT29 isolates with the total number of *S*. Enteritidis isolates from food samples.

FIGURE 2

Comparison of *S*. Enteritidis PT29 isolates to o total number of *S*. Enteritidis isolates from food samples and veterinary samples in 2002, and human isolates in 2002 and 2003



* n=159, CI for PT29: 21-35%
** n= 38, CI for PT29: 4-28%

Thirty seven non-human S. Enteritidis PT29 strains could not be assigned to a specific group (food or chicken) for the analysis, as the isolate origin was documented only as 'poultry', without specifying the origin. In general, non-human isolates originated from broiler chicken production. No isolate was obviously related to egg production.

The distribution of other phage types differs strongly from the distribution of *S*. Enteritidis PT29 in human and chicken. In Table 1, the relative frequency of the most common phage types of S. Enteritidis in humans and chickens are compared for

2002.

TABLE 1

Examples for the proportions of phagetypes among *S.* Enteritidis isolates of human and veterinarian origin, Austria, 2002

S. Enteritidis	Humans %	Humans (n)	Chickens %	Chickens (n)
PT 4	55.7%	(4151)	25.8%	(44)
PT 8	21.8%	(1626)	17.6%	(28)
PT 21	6%	(446)	8.8%	(14)
PT 6	4.1%	(307)	3.1%	(5)
PT 29	0.4%	(30)	27.7%	(44)
Total		7459		159

Fifty six of the 86 human *S*. Enteritidis PT29 isolates and 47 of the 86 non-human S. Enteritidis PT29 isolates were subtyped using PFGE. These *S*. Enteritidis PT29 isolates tested showed 3 distinct band patterns (dubbed E1, E2, and E3, Figure 3).

FIGURE 3

S. Enteritidis PT 29 isolates. PFGE subtypes using *Xba*I, Austria, 2000-2003

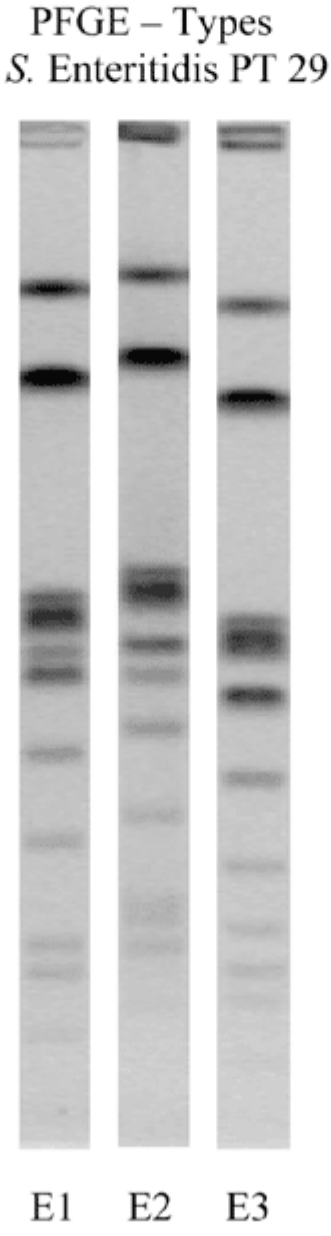


Table 2 summarises these PFGE subtyping results.

TABLE 2

Results of PFGE pattern analyses (E1, E2, E3) performed on 55 human and 47 non-human PT 29 isolates, Austria, 2000-2003

Year	2000		2001		2002		2003		Total	
PFGE pattern	A	B	A	B	A	B	A	B	A	B
E1	0	0	3	0	3	0	3	0	9	0
E2	0	0	4	0	13	46	12	0	29	46
E3	3	0	9	0	5	1	1	0	18	1

A: Human *S.* enteritidis PT29 isolates (n=56)
B: Non-human *S.* enteritidis PT 29 isolates (n=47)

While 5 of 12 (41.7%) *S.* Enteritidis PT29 patients reported consumption of chicken meat within 24 hours before onset of illness, 35 of 401 (8.7%) patients with non *S.* Enteritidis PT29 infections reported consumption of chicken meat. This corresponds to an odds ratio of 7.4 (95%, CI 2.3 - 24.8).

Discussion

In contrast to other phage types *S.* Enteritidis PT29 was found exclusively in the meat production line of the poultry industry. This restriction makes it possible to estimate the relevance of chicken meat as source of human infections. In our opinion, the discrepancy between the high occurrence rate of *S.* Enteritidis PT29 in broilers and chicken meat in Austria in 2002 and the low number of PT29 cases in humans may indicate that chicken meat of Austrian origin is a source of only minor importance for all human *S.* Enteritidis infections at the present time.
Case-control studies are frequently used to identify risk factors for infectious diseases. Many tests prove the consumption of inadequately heated egg products as the currently most important risk factor for causing human infections of *S.* Enteritidis [4-7]. Results concerning the influence of chicken meat are divided. Some studies [6,7] find a clear association between consuming chicken and illness, while other studies cannot prove any connection [5,6]. For methodological reasons, case-control studies can explain only some of the infections [8]. Salmonella can also be transferred to other foodstuffs, causing secondary contamination (e.g. transfer of pathogens from chicken meat to spices, lettuce, etc.). Infections that no longer

seem to be connected to consumption of chicken meat can therefore occur. The quantitative relevance of such infection is not known [9].

Phage typing of *S*. Enteritidis was developed to clarify epidemiological relationships after the worldwide increase in infections [2]. While *S*. Enteritidis PT4 is predominant in western Europe, PT8 and PT13a are mainly seen in North America [1]. Epidemiological studies show that large outbreaks can also be caused by rare phage types as long as transfer occurs through suitable vectors, e.g. eggs [10,11].
Most phage types of S. Enteritidis differ very little in their ability to cause human infection. Assuming the largely identical virulence of various phage types, conclusions can be drawn about the importance of chicken meat as a source of infection for human salmonellosis, based on the distribution of S. Enteritidis in human versus non-human sample material. The outbreak of *S*. Enteritidis PT29 (in humans and in chickens) which we are presenting here lasted for 4 years in Austria. In 2000 and 2001 only human infections occurred. Epidemiological investigation (data not shown) indicated that most of these infections were acquired in Croatia. Since April 2002 S. Enteritidis PT29 has also been isolated from chicken habitations in Austria. A large breeding business had bought breeding eggs from Croatia. *S*. Enteritidis PT29 established itself in several breeding businesses for broiler chickens over the following months (Dr Pless, Styrian veterinary administration, personal communication). Little information is available about the phage type distribution of S. Enteritidis in humans and non-humans in different European countries. *S*. Enteritidis PT29 is not listed in published tables, indicating that *S*. Enteritidis PT29 is a rare type of *S*. Enteritidis in Europe [12,13].

PFGE enabled the clonal origin of these chicken isolates to be determined. With only one exception (E3), all 47 non-human isolates tested were classified as PFGE type E2. Among the human isolates, type E3 was predominant in the first 2 years - 2000 and 2001; 12 of the 19 isolates (63.2%) tested belonged to this PFGE type. The PFGE type E2, dominant in Austrian chicken (veterinary and food samples), was found as an infective agent with humans as of 2001 and became dominant among human isolates only as of 2002 (25 of the human strains tested in 2002 and 2003, i.e. 69.4%).
In our opinion, two separate events are behind the S. Enteritidis

PT29 outbreak. In 2000 and 2001 there were mainly travel-associated infections (Croatia). Contamination of domestic chicken meat with S. Enteritidis PT29 first appeared in 2002. More than 10% of all S. Enteritidis contamination from domestically slaughtered poultry in 2002 was caused by PT29. This assumption is supported by the number of S. Enteritidis PT29 in food at retail level (13% of all S. Enteritidis found in edible chicken). The S. Enteritidis PT29 positive food samples were widely distributed in time and place. The rate of S. Enteritidis PT29 in the veterinary medical samples and in the food samples was, however, much higher than the remarkably small proportion of S. Enteritidis PT29 isolates from human samples. Only 0.40% of the human *S*. Enteritidis strains from 2002 and 0.33% of the S. Enteritidis strains of 2003 were typed as PT29.

Chicken meat is often frozen and stored for a long time, which means that human isolates of 2003 must also be taken into consideration to determine the relevance of chicken meat as source of infection for human illness. All the patients with human cases of S. Enteritidis PT29 in 2003 were approached and asked to complete a questionnaire. From the completed questionnaires, we deduced that *S*. Enteritidis PT29 was predominantly transmitted to humans by the consumption of chicken meat, although the possibility of other sources cannot be dismissed. Nevertheless, if other routes of infections had been of importance, our conclusions would still be valid.
From the data presented here, we conclude that Austrian chicken meat is probably only of minor importance as a source of human *S*. Enteritidis infections, regardless of phage type. This applies to chicken meat as direct source of infection as well as infections from secondary contamination. The incidence of human *S*. Enteritidis infections remains high in Austria. The main focus of preventive measures should be directed at reducing the danger of infection caused by the consumption of eggs [4-7]. The efforts of the European Commission, which requires chicken carcasses to be free of salmonella by 2010, are nonetheless welcome [14].

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