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MOLECULAR EPIDEMIOLOGY OF *SALMONELLA ENTERICA* SEROVAR SAINTPAUL ISOLATED FROM ANIMALS, FOOD, AND HUMANS IN 12 EUROPEAN COUNTRIES

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Abstract

The molecular epidemiological relationship among isolates of *Salmonella enterica* serovar (*S.*) Saintpaul, which was identified in animals, food, and humans in several EU countries, was investigated. Pulsed-field gel electrophoresis (PFGE) revealed a high degree of genetic diversity (82 *Xba*I PFGE profiles with 42.6% similarity) among 159 *S.* Saintpaul isolates from animals (n=91), food of animal origin (n=29), and humans (n=36) in 12 European countries during 2005 to 2009. Most frequent profiles (n=12) comprised almost 50% of the tested isolates. Profiles obtained in isolates from a single source within a particular geographical region or particular period of time were indistinguishable or closely related. Turkeys were confirmed as the major reservoir for *S.* Saintpaul. Indistinguishable PFGE profiles were identified in up to 19 isolates from turkey breeding and fattening flocks and food over the study period. Other animals, including food and pets, may also contribute to *S.* Saintpaul spread. International trade of animals and food, as well as travelling contributes to the spread of a specific clone to different geographical areas. Although control programmes in breeding turkey flocks, together with improved biosecurity, may interrupt the major transmission routes, it was concluded that *S.* Saintpaul will continue to represent a potential threat to human health.

Key words: *Salmonella* Saintpaul, epidemiology, molecular characteristics, pulsed-field gel electrophoresis, Europe.

Salmonellosis remains the second most common food-borne zoonosis in the European Union (EU). Since 2004, its incidence has been decreasing, but in 2010 it still accounted for 21.5 cases per 100,000 population (13). *Salmonella enterica* serovar (*S.*) Enteritidis and *S.* Typhimurium are the most frequent serovars, usually associated with food-borne disease. The incidence rates of *S.* Saintpaul are not available at international or national level, but the serovar was ranked ninth in the serovars reported in 2009 (14). It has also been associated with human salmonellosis in other parts of the world. In Japan, *S.* Saintpaul appeared in humans in 1997 and it was ranked ninth in 1999 and fourth in 2002 in a list of *Salmonella* serovars from human sources (18). Large and widely dispersed outbreaks caused by *S.* Saintpaul occurred in Australia (in 1999 and 2006), New Zealand (1967–1968), and the United States (2008) (17, 24, 26, 29).

In 2006 to 2007, a one-year baseline survey on *Salmonella* prevalence in turkeys in the EU ranked *S.* Saintpaul as the fourth most common serovar isolated from tested flocks and the leading serovar in 42.5% of infected breeding flocks. It was detected in 12 countries and although some of them are close neighbours (Austria, Czech Republic, Hungary, Poland, and Slovakia), no common source of infection was identified. Although no information on the trade of live birds or hatching eggs was available, the potential role of breeding turkey flocks in the transmission of *S.* Saintpaul was hypothesised (12). Several national risk factor analyses and epidemiological studies on these baseline results have been published (4, 6, 15). *S.* Saintpaul was also reported in beef in the EU in 2008 (10) and it was recently the most common serovar found in turkey meat (13, 14).

Taking into account the relative lack of information on the occurrence of *S.* Saintpaul and

reports indicating the possible public health impact of this serovar, the molecular epidemiological relationship among *S.* Saintpaul isolates, primarily from turkeys, and their spread into the food chain in several countries were investigated. Other infection sources, international trade, and impact of travel were also considered.

Material and Methods

In February 2009, an e-mail invitation was sent to the network of national *Salmonella* reference laboratories in 27 EU countries, as well as Former Yugoslav Republic of Macedonia, Norway, and Turkey, to participate in a survey on the epidemiology of *S.* Saintpaul. The laboratories were asked to provide *Xba*I pulsed-field gel electrophoresis (PFGE) profiles of available *S.* Saintpaul isolates cultured between 2005 and 2009. If PFGE profiling had not been done, up to 10 isolates were to be sent for PFGE profiling. Due to baseline study results (11), most isolates were expected to be of turkey origin. However, to reveal any other sources that might contribute to *S.* Saintpaul spread, isolates from animal feed, food of animal origin, or from any other sources were included in the study. Information on isolation methods or national surveillance schemes was not recorded.

The typing was carried out at the National Veterinary Research Institute (NVRI, Poland), Finnish Food Safety Authority (EVIRA), National Institute of Public Health and the Environment (RIVM, the Netherlands), and Scottish *Salmonella*, *Shigella*, and *Clostridium difficile* Reference Laboratory. *Xba*I digestion of *S.* Saintpaul chromosomal DNA was applied according to the standardised protocol recommended by PulseNet – an international network for molecular typing of food-borne pathogens and disease surveillance (28). The results were analysed with BioNumerics (Applied Maths, Belgium) using PulseNet-recommended parameters: unweighted pair group method with arithmetic mean (UPGMA), Dice coefficient, 0.5% optimisation, and 1.5% position tolerance. Bands with a molecular weight of less than 33.3 kb were excluded from the analysis. To verify genetic similarity, a subset of 45 strains of major *Xba*I profiles was tested with *Bcu*I (*Spe*I) digest and at the same running and analysis conditions.

Three of invited laboratories (Estonia, Greece, and Macedonia) reported no *S.* Saintpaul isolates, whereas 11 laboratories from 10 countries participated in the study. Three of the participating laboratories provided PFGE profiles and seven sent isolates for testing. RIVM, besides Dutch isolates, offered single isolates from Czech Republic and Slovenia. Six laboratories provided 10 or more isolates. Altogether, PFGE profiles of 159 isolates cultured between January 2005 and May 2009 in 12 countries were available for analysis. A summary description of the isolates was given in Table 1. The analysed isolates originated mostly from animals (n=91), food of animal origin (n=29), humans (n=36), environment (n=1), feed (n=1), and an unknown source (n=1). Animal isolates were obtained from turkeys (n=59), chickens (n=11, broilers or laying hens), ducks (n=11), geese (n=4), pigs (n=3), and pets (n=3, cat, dog, and turtle).

A total of 10 isolates were from breeding flocks of turkeys (n=7), ducks (n=1), and geese (n=2). The isolates originating from food of animal origin were from turkey meat (n=20), broiler (n=3), or unspecified poultry meat (n=2), and single isolates from pork, beef, kebab meat, and unspecified food. Eighteen isolates were found in imported items, either animals or food, from at least six countries (Brazil, Denmark, France, Germany, Poland, and Scotland). Fifteen isolates were obtained from people from the Netherlands and the United Kingdom who had travelled to several countries in Africa, Asia, or South America prior to infection.

Results

PFGE analysis of 159 isolates revealed 82 different profiles. Of these, 57 were observed in single isolates, each of 13 profiles was seen in two isolates and the remaining 12 profiles were present in three to 19 isolates (Fig. 1). The overall profile similarity was 42.6%. Probability-based Simpson's index of diversity (D=0.970) confirmed high genetic variability of the tested isolates. The 12 most frequent profiles comprised almost 50% of the tested isolates and shared the following common features. They were isolated mostly in neighbouring countries (*i.e.* Poland and Slovakia). Two most common profiles were seen in a few isolates from geographically distant countries (*i.e.* Poland, Slovenia, and Ireland). Isolates with indistinguishable profiles came from several sources such as animals and food (*i.e.* pigs, turkey meat, and beef), at least two animal species (*i.e.* duck and turkey), and different production types (*i.e.* duck breeders, laying hens, and fattening turkeys) were involved.

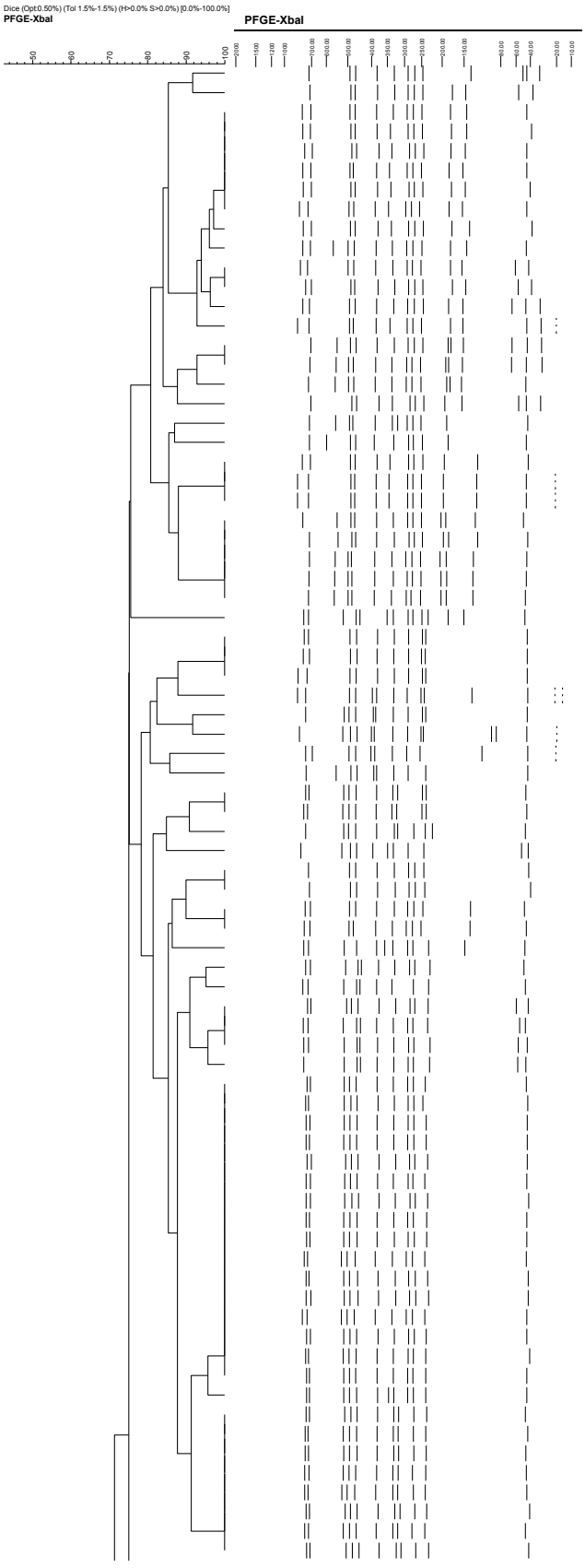
The average length of time, during which the most frequent profiles (n=12) were observed, was three years, while the most common profile (observed in 19 isolates) was found throughout the study period (2005–2009). In contrast, profiles seen in two isolates (n=13) were mostly from a single source type (n=10), at a certain point in time (the same year, n=7) and in a single country (n=10). Where two countries were involved, they were not neighbouring and travel (human), or the importation (animals or food) was indicated (n=7).

The turkey isolates (n=59) showed 25 profiles, six of which were represented by single, unrelated strains. The others were clustered in seven related profile groups (Clusters A–G). Four of these profile groups contained isolates from Hungary (Cluster B), United Kingdom (Cluster A), and Poland (Clusters C and F). Profile similarity within Clusters A and C approached 100%, but within Clusters B and F, it was approximately 85%. In the other three profile groups, the isolates originated from geographically close countries (Clusters E and G in Austria, Poland, and Slovakia and Cluster D in Poland and Austria) and the profile similarity within the clusters reached 91%–92%.

Table 1
S. Saintpaul isolates analysed by pulsed-field gel electrophoresis (n=159)

Country	Type of origin	Source including country of origin, if not domestic, (number of isolates)	Year of isolation
Austria[a,b]	domestic	turkey (n=9)	2006–2009
		cat[c] (n=1)	
Belgium[b,d]	domestic	chicken (n=1)	2008
		feed (n=1)	
Czech Republic[e]	domestic	food (n=1)	2005
Finland[f]	domestic	turtle[c] (n=1)	2005–2008
	imported	food: Brazil (n=6), Germany (n=1), Poland (n=3)	
		goose: Denmark (n=1)	
Hungary[b,g]	domestic	turkey (n=7)	2007–2008
		pig (n=1)	
Ireland[b,h]	domestic	food (n=2)	2008–2009
		environment (n=1)	
	imported	food: unknown source (n=1)	
Netherlands[e]	domestic	chicken (n=1)	2005–2006
		duck (n=1)	
		food (n=1)	
	travel associated	human: Brazil (n=1), China (n=1), Egypt (n=1), Morocco (n=1), Suriname (n=1), Tanzania (n=1), Turkey (n=1)	
Norway[b,i]	imported	duck: France (n=3), Scotland (n=2), unknown (n=1)	2008
Poland[b,j]	domestic	chicken (n=6)	2005–2009
		duck (n=1)	
		goose (n=3)	
		pig (n=1)	
		turkey (n=30)	
		food (n=14)	
Slovakia[b,k]	domestic	chicken (n=1)	2005–2008
		turkey (n=9)	
Slovenia[e]	domestic	pig (n=1)	2006
United Kingdom	domestic	dog[c,l] (n=1)	2005–2009
		duck[b,m] (n=3)	
		chicken[b,m] (n=2)	
		turkey[b,m] (n=4)	
		unknown[b,m] (n=1)	
		human[l] (n=21)	
	travel associated	human[l]: Cuba (n=2), Dominican Republic (n=1), Egypt (n=2), Ghana (n=1), Nigeria (n=1), West Africa (n=1)	

[a] isolates provided by the Austrian Agency for Health and Food Safety, Austria.
[b] PFGE typing performed by the National Veterinary Research Institute, Poland.
[c] pet.
[d] isolates provided by the Veterinary and Agrochemical Research Centre, Belgium.
[e] isolates provided and PFGE typing performed by the National Institute of Public Health and the Environment, the Netherlands.
[f] isolates provided and PFGE typing performed by the Finnish Food Safety Authority Evira, Finland.
[g] isolates provided by the Central Agricultural Office, Hungary.
[h] isolates provided by the Central Veterinary Research Laboratory, Ireland.
[i] isolates provided by the Norwegian Institute of Public Health, Norway.
[j] isolates provided by the National Veterinary Research Institute, Poland.
[k] isolates provided by the State Veterinary and Food Institute, Slovakia.
[l] isolates provided and PFGE typing performed by the Scottish *Salmonella, Shigella* and *Clostridium difficile* Reference Laboratory, United Kingdom.
[m] isolates provided by the Veterinary Laboratories Agency (currently, the Animal Health and Veterinary Laboratories Agency), United Kingdom.



Cluster	Strain No.	Date	Country	Source
-	4910	2005	Finland (import: Brazil)	Food (turkey meat)
-	S.8022-06	2006	UK	Duck
-	10306507	2008	Norway (import: France)	Duck
-	4454-07	2007	UK	Unknown
-	S.4715-06	2006	UK	Duck
A	S.74-08	2008	UK	Turkey
A	S.8405-07	2007	UK	Turkey
A	S.9459-07	2007	UK	Turkey
A	S.7731-07	2007	UK	Turkey
-	10306506	2008	Norway (import: France)	Duck
-	0401/2009	2009	Poland	Goose (breeding flock)
-	L.544-06	2006	UK	Poultry
-	2903	2007	Finland (import: Denmark)	Goose
-	S/20071987	2007	UK, Scotland	Human
-	10200871	2008	Norway (import: France)	Duck
-	10209236	2008	Norway (import: Scotland)	Duck
-	1385/2008	2008	Poland	Goose
-	S.21-07	2007	UK	Duck
B	P-125	2007	Hungary	Turkey (fattening flock)
B	P-625	2007	Hungary	Turkey (fattening flock)
-	10209230	2008	Norway (import: Scotland)	Duck
-	S/20051609	2005	UK, Scotland	Human
-	S/20061380	2006	UK, Scotland	Human
-	09/00000735	2009	Austria	Cat
B	P-112	2007	Hungary	Turkey (fattening flock)
B	P-152	2007	Hungary	Turkey (fattening flock)
B	P-405	2007	Hungary	Turkey (fattening flock)
B	P-677	2007	Hungary	Turkey (fattening flock)
-	0683/2005	2005	Poland	Food (pork)
C	0108/2006	2006	Poland	Turkey (fattening flock)
C	BST-KIE-004B	2006	Poland	Turkey (fattening flock)
-	S/20062165	2006	UK, Scotland	Dog
-	S/20070822	2007	UK, Scotland	Human
-	S/20061658	2006	UK, Scotland	Human
-	S/20090004	2009	UK, Scotland	Human
-	S/20080563	2008	UK, Scotland	Human
-	S/20080833	2008	UK, Scotland	Human
-	1462/2008	2008	Poland	Food (turkey meat)
-	1463/2008	2008	Poland	Food (poultry meat)
-	7169	2007	Slovakia	Turkey (breeding flock)
-	0804310/2	2008	Belgium	Feed
-	3536	2006	Finland (import: Brazil)	Food (turkey meat)
-	4698	2006	Finland (import: Brazil)	Food (turkey meat)
-	1090500890	2005	Netherlands	Duck
-	11030285	2008	Norway (import: unknown)	Duck
-	BST-KIE-003E	2006	Poland	Turkey (fattening flock)
D	09/00000258	2009	Austria	Turkey
-	1011/2008	2008	Poland	Food (turkey meat)
D	07/00001043	2009	Austria	Turkey
D	1224/2008	2008	Poland	Turkey (fattening flock)
-	1821/2008	2008	Poland	Food (turkey meat)
-	S09-000458	2009	Ireland (import: unknown)	Food (turkey meat)
E	0103/2006	2006	Poland	Turkey
E	0565/2006	2006	Poland	Turkey (fattening flock)
E	06/00006827	2006	Austria	Turkey
E	06/00007163	2006	Austria	Turkey
E	06/00007263	2006	Austria	Turkey
E	07/00000086	2007	Austria	Turkey
E	07/00000348	2007	Austria	Turkey
E	07/00002193	2007	Austria	Turkey (fattening flock)
-	1090501192	2005	Czech Republic	Food (turkey meat)
-	1090503120	2005	Netherlands (travel: Turkey)	Human
-	1090801464	2005	Netherlands (travel: Morocco)	Human
-	1090804608	2008	Hungary	Slaughter pigs
E	1358/2008	2008	Poland	Turkey (fattening flock)
-	1833/2008	2008	Poland	Laying hen flock
-	1883/2008	2008	Poland	Food (turkey meat)
E	BST-SZ-090C	2007	Poland	Turkey (fattening flock)
E	BST-SZ-111A	2007	Poland	Turkey (fattening flock)
-	0424/2006	2006	Poland	Duck (breeding flock)
E	0558/2006	2006	Poland	Turkey (fattening flock)
-	1090500428	2005	Slovakia	Laying hen flock
E	1155/2005	2005	Poland	Turkey
E	8490	2008	Slovakia	Turkey (fattening flock)
E	BST-KAT-149D	2007	Poland	Turkey (fattening flock)
E	BST-SZ-004D	2006	Poland	Turkey (fattening flock)
E	BST-SZ-019A	2006	Poland	Turkey (fattening flock)

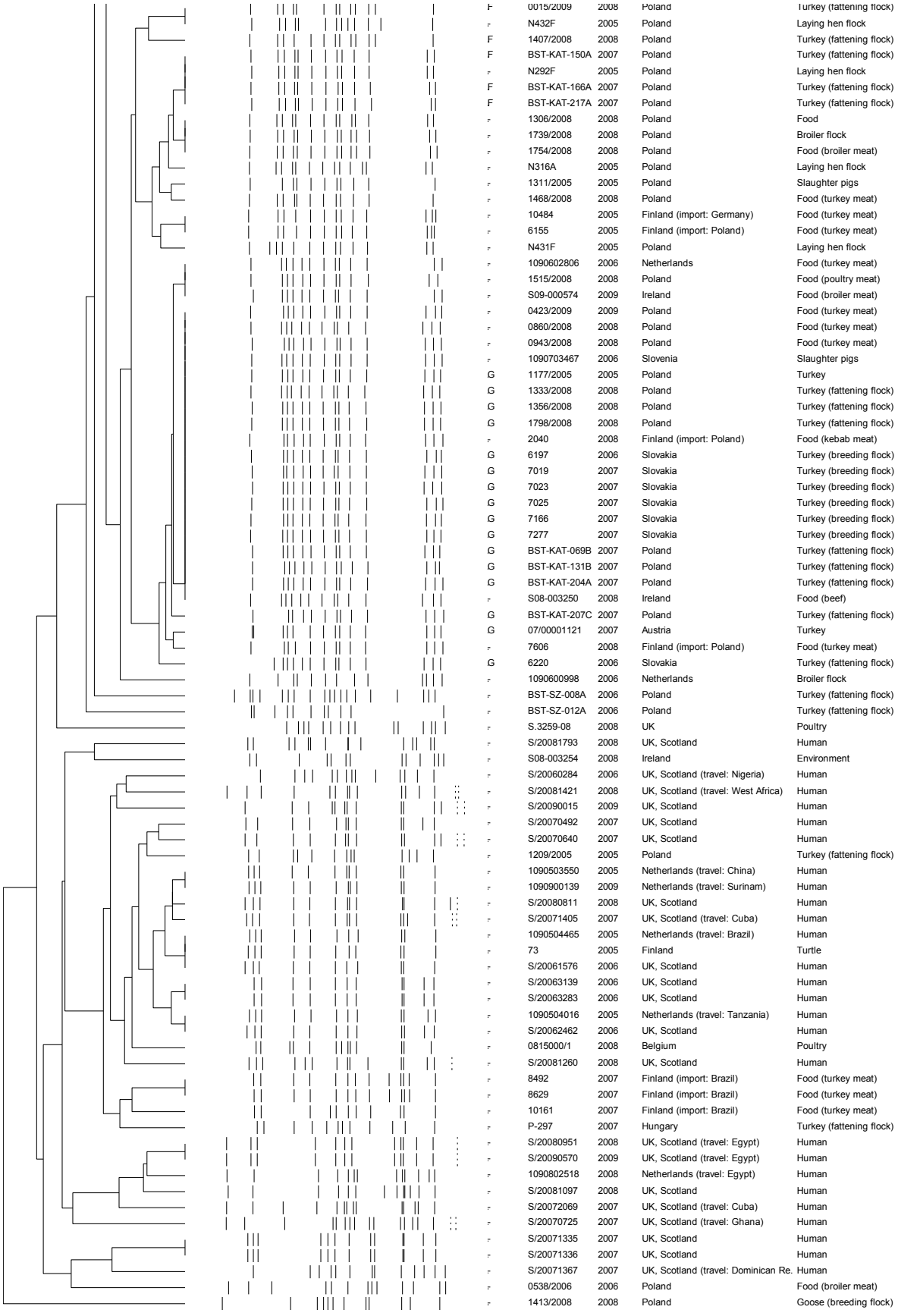


Fig. 1. Pulsed-field gel electrophoresis *Xba*I profile analysis of tested *S.* Saintpaul isolates (n=159); Letters A–G indicate related profile groups (Clusters A–G) of turkey isolates (n=59).

At least half of the isolates in each cluster showed an indistinguishable profile. Nine of the 13 indistinguishable turkey isolates in Cluster G originated from breeding flocks in Slovakia and the remaining four were isolated from fattening flocks in Poland. There were also six profiles observed in single isolates from fattening turkeys in Slovakia, Poland, and Hungary, which were unrelated to any of the described clusters. Indistinguishable profiles that had been seen in turkey isolates were also found in isolates from food (in Clusters D, E, and G), Dutch citizens, who travelled to Morocco and Turkey (Cluster E), a dog from Scotland (Cluster C), a cat from Austria (Cluster B), laying hens (Clusters E and F), and ducks (Cluster E).

There were other indistinguishable profiles seen in isolates of various origins. One of them was found in isolates from a person from Scotland, Dutch citizens, who had visited Brazil and a turtle from Finland. In addition, two human isolates from Scotland and an isolate originating from a duck exported to Norway also shared an indistinguishable PFGE pattern. Five PFGE profiles were noted in 10 human isolates (two isolates per profile) from travellers, who had been to China and Suriname, a Scottish domestic case and a Dutch citizen, who had travelled to Tanzania, a person in Scotland, who had travelled to Egypt, and four people in Scotland with no travel history. The remaining human isolates (n=19) showed PFGE profiles unrelated to any of the tested isolates.

The sole isolate obtained from animal feed showed a unique PFGE profile of ≤84.9% similarity to any of the other profiles seen in our study.

Thirty five (78%) out of 45 tested strains representing seven major *Xba*I profiles showed *Bcu*I (*Spe*I) profiles that did not improve discrimination of the method (data not shown). The strains showing different *Bcu*I (*Spe*I) and the same *Xba*I profiles were anyhow closely related and no more than two band difference was noted.

Discussion

The findings, although based on a single typing method, were able to define possible reservoirs and infection routes of *S.* Saintpaul. Turkey had already been confirmed as a plausible reservoir (5-7, 16, 20, 30). Indistinguishable isolates found in breeding and fattening turkey flocks in three countries (Slovakia, Poland, and Hungary) during the five-year study, suggest the presence of an established subclinical infection at the top of the production pyramid, which should be considered a primary source of infection for fattening farms (4, 12, 27, 28).

It could be expected that *S.* Saintpaul isolates within a country would be highly related. Surprisingly, the level of similarity in PFGE profiles in isolates from different countries was higher than that seen in the same country. One reason for this could be that an infected breeding flock might provide hatching eggs or poults for fattening farms in different countries. Similarly, turkey fattening farms in a country may be serviced by various breeding centres, resulting in the variety of profiles found and low within-country profile similarity.

Vertical transmission of *S.* Saintpaul in German and Dutch turkey flocks has been already reported (6). Strict biosecurity rules implemented in breeding farms may explain the lower prevalence of *Salmonella* infections there compared with that of fattening turkey flocks (3, 4, 11). The efforts to produce *Salmonella*-free hatching eggs might, however, be compromised by *S.* Saintpaul contamination of the hatchery environment, which could lead to the introduction of the pathogen into fattening flocks (3, 4). As in other studies (17), the finding of *S.* Saintpaul in goose and duck breeders indicates that animals unrelated to turkeys may be reservoirs of infection, no direct relation was found to fattening flocks of geese and ducks or to food of thereof (Fig. 1).

The PFGE profiles, both *Xba*I and *Bcu*I, observed over the five-year study period in isolates from a variety of sources might demonstrate a surprisingly high genetic stability of *S.* Saintpaul lineages. Genetic homogeneity within different serovars has been reported in turkeys and other animals and production types, with the pathogen being transmitted horizontally between flocks *via* contaminated farm utilities as a result of inappropriate flock management and biosecurity measures (4, 15, 27, 30). In the current study this mode of transmission is supported by indistinguishable PFGE profiles noted in isolates from turkey, geese, ducks, pigs, chickens (broilers and layers), as well as in food, occurring in several European countries for up to five years.

As in the case of other *Salmonella* serovars, the public health implications of *S.* Saintpaul infections are directly related to animal infections (1, 5, 6, 19). This was shown by finding an indistinguishable PFGE profile in isolates originating from animals and food of animal origin in Austria, Czech Republic, Hungary, and Poland, as well as from people from the Netherlands who had travelled to other countries.

Typing results of the isolates from Finland and Norway suggest that international trade of animals and food, at both European and transcontinental level, might be crucial for the spread of *S.* Saintpaul. This finding is in accordance with other observations (2, 24). In our study, indistinguishable PFGE profiles were found in Finland in isolates from food imported from Brazil, Germany, and Poland, and isolates identified in Norway were traced back to ducks imported from France and Scotland.

It should be noted that pets can acquire *Salmonella*, just as human consumers do. Feeding pets with turkey meat, probably imported from Hungary and Poland, could explain, respectively, the mentioned cat and dog infections. However, an indistinguishable PFGE profile found during a similar time period in an isolate from a turtle in Finland, a person in Scotland and Dutch citizen, who had travelled to Brazil, remains unexplained. Nevertheless, possible subsequent exposure of owners of infected pets should not be forgotten (19).

Usually, *Salmonella* isolates from a single source, within a geographical region or period of time show limited, if any, diversity (4, 25, 30). Although clonally related isolates were found in a number of animal species, production types and different food over the five-year span, a high genetic diversity was observed in the tested *S.* Saintpaul isolates, presumably as a result of the length of the collection period. The results confirm the existence of multiple reservoirs and vehicles of transmission to humans, involving not only animals and food of animal origin, but also water (29), seafood (2), fruits, and vegetables (9, 11, 21-24), and even wildlife reptiles (17). Unlike some other serovars; however, feed could be discounted as important vector of *S.* Saintpaul transmission route (16, 30). We draw this conclusion as, despite the broad strain selection criteria, only one feed isolate was collected and its unique PFGE profile diverged from those observed in any other isolate. No association between *Salmonella*-contaminated feed and flock infection has already been reported (3).

The major contribution of our study is the confirmation of *S.* Saintpaul as a turkey-associated *Salmonella* serovar that appears to spread in several countries mostly due to subclinical infections of breeding flocks (11). This is in agreement with the findings of Foley *at al.*(16), who estimated that 85% of *S.* Saintpaul isolates from animal sources and 20% of clinical human isolates were associated with turkey products. Infections in turkey breeders are reflected in those seen in fattening flocks and, *via* food, may reach human population (6, 8, 30). We consider that efficient control measures taken at the top of production pyramid may interrupt the major transmission routes and thus reduce the public health impact of *S.* Saintpaul. However, the ubiquity of the pathogen – demonstrated in our study by the variety of sources – may be assumed and other sources, as well as international trade and travel, will continue to compromise consumers' safety, in spite of the control measures taken on primary animal production.

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