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Molecular epidemiology of *Salmonella* spp. isolates from gulls, fish-meal factories, feed factories, animals and humans in Norway based on pulsed-field gel electrophoresis

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SUMMARY

The molecular epidemiology of 98 isolates of *Salmonella* serovar Agona ($n=27$), *S.* Montevideo ($n=42$) and *S.* Senftenberg ($n=29$) from wild-living gulls, fish-meal factories, feed factories, humans and domestic animals was investigated using pulsed-field gel electrophoresis (PFGE) and computerized numerical analysis. Two of the *S.* Agona profiles were identified both in gulls and in two of the factories. In addition, one of these profiles was detected in two infected poultry farms. Two of the *S.* Montevideo profiles were also identified both in gulls and in two of the factories, and one of these profiles was observed in a human isolate. Four factories shared an identical *S.* Senftenberg profile. The *S.* Senftenberg profile found in gulls was not identified in any other source investigated. The presence of isolates with identical PFGE profiles indicates potential epidemiological links between different factories, as well as between gulls and factories.

INTRODUCTION

The occurrence of *Salmonella* spp. in feed and feed ingredients is a well-recognized problem worldwide, and feed ingredients are believed to represent a major risk of *Salmonella* contamination in feed factories [1–4]. In addition, wild birds, rodents and insects may carry *Salmonella*, but the significance of these species as sources of contamination in factories is unclear [2, 5–7]. The fact that gulls can act as carriers of *Salmonella* bacteria has been well documented in surveys carried out since the 1960s. Prevalence rates ranging from 0% to 31% have been reported in studies from Great Britain [8–11], Germany [12–14], Czech Republic [15], Canada [16] and Norway [17]. Gulls have been considered as indicators of environmental contamination [10, 13], and have also been suggested to be transmitters of *Salmonella* bacteria from one site to another, mainly from abattoirs, refuse tips and sewage to other environmental sites [18].

In a recent study, 23 different *Salmonella* serovars were identified in gulls from eight locations in Norway [19]. Three of the serovars (*Salmonella* serovar Agona, *S.* Montevideo and *S.* Senftenberg) had also been detected in fish-meal and fish-feed factories [20, 21], and the locations of the gulls harbouring these serovars were close to some of these factories. This raised the question of possible cross-contamination between the gulls and the factories. However, the same serovars had also been isolated from other factories, humans and poultry in the same time period.

The aim of the present study was to investigate possible indications of epidemiological relationships between the isolates of *S.* Agona, *S.* Montevideo and *S.* Senftenberg isolated from reported sources in Norway within the time period 2000–2001. Pulsed-field gel electrophoresis (PFGE) analysis was used

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to genotypically characterize the isolates, as this method has previously been successfully applied in epidemiological investigations on these serovars [21–25].

MATERIALS AND METHODS

Salmonella isolates

The samples represented reported cases of the *Salmonella* serovars Agona, Montevideo and Senftenberg in Norway in the years 2000–2001. All the isolations had been carried out at different private or official laboratories and verified at the National Salmonella Reference Laboratory at the Norwegian Institute of Public Health. In addition, National reference strains for all three serovars were included in the study. These strains were originally obtained from L'Institut Pasteur, France.

The 28 isolates from the fish-feed factories have been described previously [21]. In addition, 23 isolates from two factories producing feed for terrestrial animals (animal feed factories) and six fish-meal factories were studied. Nearly all the factories had a history of recurrent isolations of one or a few *Salmonella* serovars. The isolates from the factories were from environmental samples, or fish-feed and fish-meal samples tested before release to the market. None of the feed or meal batches that tested positive for *Salmonella* spp. had been released to the market.

During 2000–2001, a total of 1107 samples from gulls were collected along the Norwegian coastline. A number of different serovars were identified [19], of which all isolates of *S*. Agona ($n=4$), *S*. Montevideo ($n=14$) and *S*. Senftenberg ($n=13$) were included in the present study because these were the serovars earlier identified in the fish-feed factories. The isolates were recovered from birds at three different locations within a distance of 100 km. Location 1 hosted a breeding colony of great black-backed gulls (*Larus marinus*) and herring gulls (*Larus argenatus*) where *S*. Montevideo isolates were identified in 11 of 310 live chicks sampled by cloacal swabs. At location 2, one *S*. Montevideo isolate was recovered from one herring gull out of 40 adult birds killed. At location 3, *S*. Montevideo was isolated from two, *S*. Agona from four, and *S*. Senftenberg from 13 out of a total of 72 adult herring gulls killed. Bacteriological examinations of cloacal swabs (chicks), and intestine and viscera samples (adults) were performed at the National Veterinary Institute. The *Salmonella* isolates were verified at the National Salmonella Reference Laboratory.

The human isolates ($n=10$) included all reported cases during 2000 and 2001 from persons that were infected in Norway or where the place of infection was unknown. The only reported isolations from domestic animals were from two poultry farms, where *S*. Agona was isolated from faeces ($n=3$). None of the birds showed clinical symptoms.

PFGE

Genomic DNA was prepared in agarose plugs and subjected to *Xba*I macrorestriction cleavage as described previously [26]. Images of PFGE gels obtained using GelDoc 2000 and Quantity One software (Bio-Rad, Hercules, CA, USA) were saved in TIFF format and transferred to GelComparII software (Applied Maths, Kortrijk, Belgium) for computer-assisted analyses. Similarity between fingerprints was determined by the Dice coefficient using a band position tolerance of 1%. Fragments in the range 48·5–776 kbp were included. Dendrograms were generated by the unweighted pair group method with arithmetic averages (UPGMA). Restriction profiles differing by one or more bands were designated by a capital letter indicating the serovar, combined with numerical suffixes. The profiles of the reference strains were designated by two capital letters, the first indicating the serovar and the second being 'R'.

RESULTS

In all, 12 *S*. Agona PFGE profiles were identified [Fig. (*a*)]. Two distinct *S*. Agona profiles (A1, A2) were identified in two fish-feed factories (A, B) and in gulls from location 3 (Table). In addition, the profile A2 was identified in the two poultry farms. A third profile (A15) obtained from one gull isolate, was more than 90% similar to the A1 profile (one band different). The two factories and the two poultry farms were located close to each other and within a distance of 40 km from the positive gull location. The factory that displayed a different profile (A16) was located in another part of the country. All the six human cases displayed different *S*. Agona profiles, none of which was identical to the gull and factory profiles.

Of the nine *S*. Montevideo PFGE profiles that were identified [Fig. (*b*)], two distinct profiles (M1, M2) were detected in isolates from two fish-feed factories (A, C), one fish-meal factory (E), one human case,

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Table. *Number of* Salmonella *Agona,* S. *Montevideo and* S. *Senftenberg isolates of each PFGE profile in the years 2000–2001*

Source	Total no. tested	*S.* Agona profiles					*S.* Montevideo profiles						*S.* Senftenberg profiles							
		A1	A2	A15	A16	Other	M1	M2	M10	M11	M12	Other	S1	S2	S3	S10	S11	S12	S13	Other
Gull locations																				
1	11						11													
2	1							1												
3	19	1	2	1			1	1								13				
Fish-feed factories																				
A	7	1	4					2												
B	7	3	4																	
C	7						7													
D	7												4	3						
Fish-meal factories																				
E	11						10							1						
F	2													1			1			
G	1													1						
H	3														1			2		
I	2								2											
J	2									1	1									
Animal feed factories																				
L	1				1															
M	1																		1	
Poultry farms																				
a	2		2																	
b	1		1																	
Humans	10					6	1					3								
National reference strains	3					1						1								1
Total	98	5	13	1	1	7	30	4	2	1	1	4	4	6	1	13	1	2	1	1

and in gull isolates from all three locations (Table). Two of these factories (A, E) were located within a range of 10–100 km from the positive gull locations, whereas, the third was located approximately 600 km away. The infected person lived approximately 800 km from the locations where *S.* Montevideo was found in gulls, and approximately 200 km from the nearest factory (C) positive for the same serovar profile. There was no information on possible epidemiological contacts between this person and the other sources of this PFGE profile. The two fish-meal factories (I, J) with other *S.* Montevideo profiles, were located more than 500 km away from the positive gull locations. The PFGE profiles of the additional three human *S.* Montevideo isolates were distinct and separate from profiles observed among gull and factory isolates.

Eight *S.* Senftenberg PFGE profiles were identified [Fig. (*c*)]. Four factories (D, E, F, G) harbouring the *S.* Senftenberg profile S2 were located within 100 km of each other, whereas two factories (H, M) housing other *S.* Senftenberg profiles were located more distantly. All the isolates from gulls displayed the same PFGE profile, which was not seen in any of the isolates from other sources (Table).

DISCUSSION

In the present study, the PFGE results suggest epidemiological links between different factories

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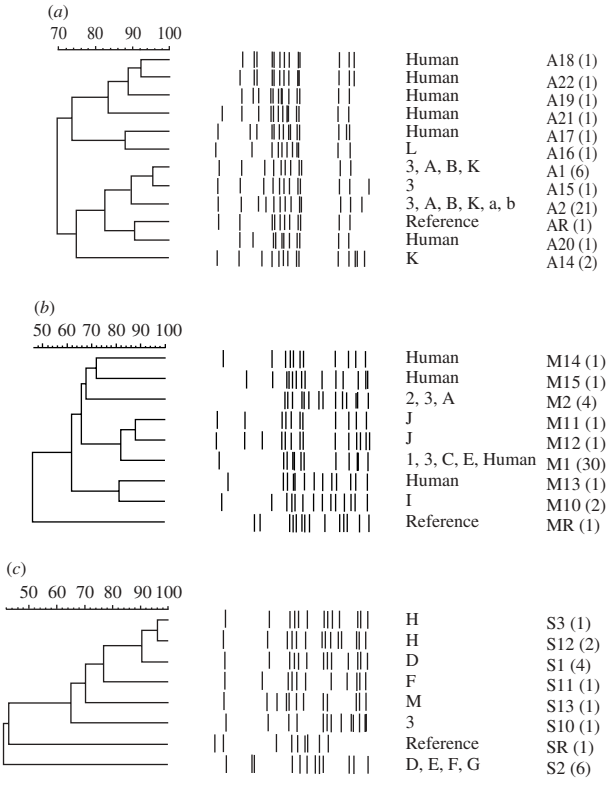

Fig. Dendrograms of (*a*) *S*. Agona isolates, (*b*) *S*. Montevideo isolates and (*c*) *S*. Senftenberg isolates based on PFGE (*Xba*I) patterns with corresponding PFGE profile, number of isolates and source of the isolates indicated.

producing fish-meal or feed. Ingredients for feed production may be contaminated with *Salmonella* [4, 27], and are, therefore, probable sources of feed factory contamination. However, *S*. Agona is rarely isolated from fish-feed ingredients produced in or imported to Norway [21]. The fact that the two factories (A, B) sharing two identical *S*. Agona PFGE profiles are closely located may, therefore, indicate a possible cross-contamination by traffic of people, wild birds, rodents, or vehicles between the factories. The three fish-meal factories (E, F, G) sharing the same *S*. Senftenberg PFGE profile, were located within a distance of 100 km from each other. Probable epidemiological links between these fish-meal factories may be the purchase of raw material batches from the same supplier, or the acquisition of second-hand equipment from each other or from the same source as sometimes occurs between these factories.

The results may also indicate an epidemiological link between factories and wild-living gulls, as the *S*. Agona and *S*. Montevideo PFGE profiles obtained from *Salmonella* isolates of gull origin were also identified in one or more of the factories. Furthermore, four of these five factories were located less than 100 km from the positive gull locations. All the factories harbouring other *S*. Agona and *S*. Montevideo PFGE profiles were more distantly located. This is in agreement with findings of Davies and Wray [5], who reported that the *Salmonella* serovars and phage types identified in droppings from wild-living birds and rodents collected on the premises of animal feed mills were the same as those isolated from the mills. In our study, samples from birds had not been collected at the premises of the factories, but at other locations. However, gulls are capable of carrying the *Salmonella* bacterium over long distances [28]. During the breeding season, parental gulls may move 20–60 km from the colonies, while non-breeding individuals may move even further [29]. The gulls are thus capable of frequently visiting closely located primary sources of infection, and may transmit bacteria back to breeding colonies, as suggested with gull location 1 in our study.

Several reports suggest that gulls carrying *Salmonella* can be indicators of environmental contamination [10, 13, 19]. All the factories in our study were located in costal areas, and gulls were frequently observed near the factories. At some of the premises, gulls had possible access to raw material when this was being loaded as bulk. Access to the final products of the factories was usually more restricted. Contaminated raw material is, therefore, a probable source of *Salmonella* infection in the gulls. The possibility that carrier birds also may constitute a vehicle for cross-contamination between factories cannot be excluded. It has previously been suggested that occurrence of *Salmonella* in feeds of plant origin may be due to transfer from birds, rodents or other pests [2], and contamination of feed mill ingredient intake pits and outloading gantries for finished feed products by wild-bird droppings containing *Salmonella* has been described [5]. Outdoor areas at the Norwegian factories are often contaminated with bird droppings, and samples from these areas and even from the soles of workers' shoes have been proven to be *Salmonella* spp. positive (unpublished observations). Thus traffic of people and vehicles into the factory without proper hygienic barriers may represent a risk of contamination of the production environment.

Several studies have reported that *Salmonella*-contaminated feeds have been associated with infections in food-producing animals and ultimately with

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human foodborne salmonellosis [3, 30–32]. Transmission of infections from gulls to domestic animals has also been suggested, related to contamination of drinking water and pasture by large numbers of birds [28, 33–35]. On the other hand, earlier reports have suggested that gulls as carriers of *Salmonella* constitute little health hazard to humans [10, 36, 37], although gulls washing and roosting in drinking-water supplies may constitute a potential risk [19, 38, 39]. Our results suggest a low risk of transmitting *Salmonella* from either gulls or feed factories to humans or domestic animals in Norway. Isolates which displayed profiles identical to any of those of gull and factory origin were only identified in one human (*S*. Montevideo profile M1) and two poultry flocks (*S*. Agona profile A2).

In conclusion, our study indicates a possible risk of *Salmonella* cross-contamination between factories and wild-living gulls, as well as between different factories producing feed or fish-meal. The results suggest that cross-traffic with possible vehicles of contamination between factories should be minimized. Furthermore, efforts to restrict access of wild-living birds to industrial premises should be emphasized, thus reducing the risk of both environmental spread and transmission of *Salmonella* from the factories to birds and vice versa. One should also aim at hygienic precautions to prevent transmission of *Salmonella* bacteria from outdoor environments (e.g. bird droppings on the ground) to indoor production facilities.

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