

## Prevalence of *Salmonella* Typhimurium infection in Norwegian hedgehog populations associated with two human disease outbreaks

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### SUMMARY

Faecal carriage of salmonella was investigated in 320 hedgehogs from Moss municipality in south-eastern Norway, Askøy, Bergen and Os municipalities in central-western Norway, and five municipalities in south-western and central Norway. The sampling in Moss was carried out 1 year after a human outbreak of salmonellosis, whereas the sampling in Askøy, Bergen and Os was carried out during a human outbreak. Both outbreaks were caused by *Salmonella* Typhimurium 4,5,12:i:1,2. No salmonella were detected in the hedgehogs from south-western (0/115) and central (0/24) Norway. Thirty-nine percent (39/99) of the animals sampled on Jeløy, and 41% (34/82) of those from Askøy, Bergen and Os, carried *S.* Typhimurium 4,5,12:i:1,2. The PFGE profile of isolates from hedgehogs and human beings were identical within each of the two outbreak areas. A significantly higher carrier rate of *S.* Typhimurium occurred among hedgehogs sampled at feeding places, compared to those caught elsewhere. The salmonella-infected hedgehog populations most likely constituted the primary source of infection during both of the human disease outbreaks, and the Norwegian hedgehog is suggested as a reservoir host of *S.* Typhimurium 4,5,12:i:1,2.

### INTRODUCTION

During August to October 1996, an outbreak of human salmonellosis occurred on the small island of Jeløy, Moss municipality, in eastern Norway [1]. The outbreak comprised 28 laboratory-confirmed cases caused by *Salmonella* Typhimurium 4,5,12:i:1,2 with a pulsed-field gel electrophoresis (PFGE) profile designated E2. Epidemiological investigations failed

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to detect any common source of infection linked to contaminated food or drinking water. A dense population of hedgehogs (*Erinaceus europaeus*) existed on the island, and the animals commonly frequented private gardens where they, in some instances, were fed. Bacteriological examination of faecal hedgehog samples taken from several sites on the ground during late autumn 1996 (after the start of the hedgehog hibernation period) revealed the presence of *S.* Typhimurium of the same serotype and PFGE profile

as the human isolates. It was suggested that the human outbreak had its origin in an infected hedgehog population.

During July to November 2000, a second human disease outbreak caused by *S. Typhimurium* 4,5,12:i:1,2 occurred simultaneously in the three neighbouring municipalities of Bergen, Askøy and Os in central-western Norway [2; A. Walde, personal communication]. The outbreak comprised 37 culture-confirmed cases and 32 of the isolates showed a PFGE-profile designated E5. Two of the remaining isolates showed a profile identical to the Jeløy strain (E2), whereas the rest showed profiles different from both E2 and E5. Interviews with patients identified contact with hedgehogs as the only common exposure, and *S. Typhimurium* 4,5,12:i:1,2 with the PFGE-profile E5 (four isolates) and E2 (one isolate) were found in faecal hedgehog samples taken from the outdoor environment of patients. It was suggested that the human outbreak in Askøy, Bergen and Os had its origin in salmonella-infected hedgehogs.

This study reports the prevalence of salmonella-infection in hedgehogs on the island of Jeløy 1 year after the human disease outbreak, and in hedgehogs from Bergen, Askøy and Os towards the end of the human disease outbreak. Hedgehogs from five different municipalities in south-western (Stavanger, Sandnes) and central (Trondheim, Ørlandet, Bjugn) Norway with no history of human disease outbreaks, were also examined.

## MATERIALS AND METHODS

Faecal samples were collected from hedgehogs on Jeløy (Moss municipality) and in the municipalities of Askøy, Bergen, Os, Stavanger, Sandnes, Trondheim, Ørlandet, and Bjugn during September 1997, 1998 and 2000 (Table 1, Fig. 1). The animals were caught at night while visiting feeding places in private gardens, or elsewhere (gardens, parks, road edges, groves) (Table 1). Each feeding place was observed for one night, and all animals visiting were captured. The study included 2 feeding sites on Jeløy, 7 in Stavanger and Sandnes, 5 in Bergen, Askøy and Os, and none in Trondheim, Ørlandet and Bjugn. The number of animals captured at the same feeding place varied from 2–19. Some of the animals were observed visiting more than one feeding place, and were sampled only on the first occasion. The sampling on Jeløy and in Bergen, Os and Askøy was carried out on sites with and without reported human cases.

At capture, the hedgehogs were marked with a spot of paint on their spines, weighed and sexed, and classified as juveniles (body weight < 600 g) or adults (body weight > 600 g). Thereafter, they were placed in 30 × 40 × 30 cm large cartons, one carton per animal, with a sheet of ordinary copy paper (295 mm × 420 mm) on the bottom of the carton. The animals were kept in the cartons for about 2 h, during which they had access to canned cat food placed directly on the bottom. The great majority of animals ( $n = 320$ ; 97%) defecated during the period of capture. From the remaining animals ( $n = 10$ ; 3%), no faecal samples could be obtained. During the sampling in Askøy, Bergen and Os, the faecal consistency of individual hedgehogs was recorded, using the classification of solid, soft, or liquid.

The study also included four faecal samples from hedgehogs in Oslo ( $n = 2$ ) and the municipalities of Herøy ( $n = 1$ ) and Tysnes ( $n = 1$ ) in western Norway, received towards the end of the human disease outbreak in Askøy, Bergen and Os (a result of mass media focus on the human outbreak).

After sampling, the sheets of paper containing variable quantities of faeces were removed, folded fourfold and placed individually in small plastic bags, and transported to the laboratory within 2 days. The samples were examined for salmonella according to the Nordic Committee on Food Analysis, Method No. 71 with a few modifications. Briefly, the paper sheets were individually incubated in 200 ml buffered peptone water (BPW)(Oxoid Ltd., Hampshire, England). The four faecal samples from Oslo, Herøy and Tysnes were incubated in BPW in a ratio of 1:10 (w/v). All cultures were incubated at 37 °C for 24 h. Then 100 µl from each culture were transferred to 10 ml Rappaport-Vassiliadis Soy broth (Oxoid Ltd.) and incubated at 42 °C for 24 h. Finally the cultures were spread on brilliant green agar plates (Difco Laboratories, Detroit, USA), which were incubated at 37 °C for 24 h. The cultures were also spread on a bromothymol-blue lactose sucrose agar plate (Bacto heart infusion agar 40.0 g, lactose puriss 120 g, saccharose puriss 120 g, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> × 5H<sub>2</sub>O 12 g, bromothymol-blue 0.96 g, and crystal-violet 0.06 g in 1000 ml aqua dest.). Presumptive salmonella colonies were characterized by inoculation on triple sugar iron (Difco) and urease medium (Difco). All salmonella-isolates were serotyped according to the Kauffmann–White scheme [3], and their PFGE-profiles were determined after plug preparation, *Xba*I restriction enzyme cleavage and electrophoresis [4].

Table 1. Prevalence of *Salmonella Typhimurium* in hedgehogs from nine different municipalities by age, sex and site of capture, Norway 1997–2000

No. of animals*	Moss (Jeløy) 1997	Stavanger Sandnes 1998	Trondheim Ørlandet Bjugn 1998	Askøy Bergen Os 2000
Total	99 (39)	115 (0)	24 (0)	82 (34)
Captured at feeding places	29 (23)	53 (0)	0	30 (19)
Captured elsewhere	70 (16)	62 (0)	24 (0)	52 (15)
Adults	64 (26)	74 (0)	14 (0)	42 (21)
Juveniles	35 (13)	41 (0)	10 (0)	40 (13)
Females	52 (16)	51 (0)	15 (0)	47 (19)
Males	47 (23)	64 (0)	9 (0)	35 (15)

\* The total number of animals examined is indicated. Number of animals with *S. Typhimurium* is given in parentheses.

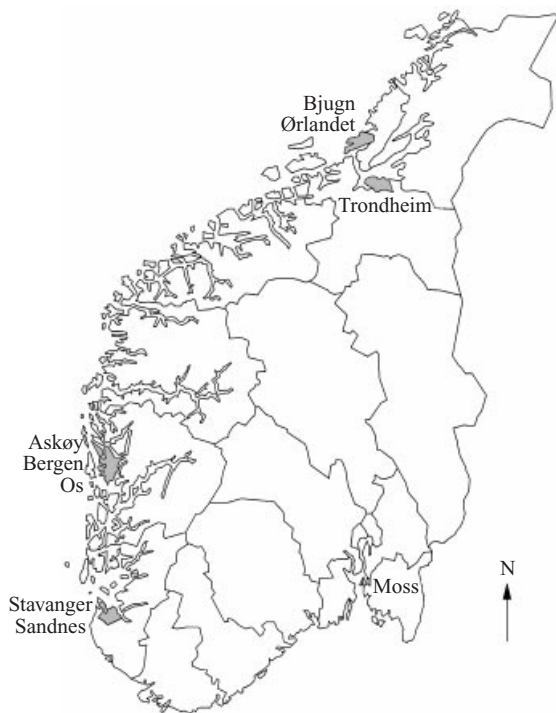


Fig. 1. Map of southern and central Norway (northern Norway not shown), showing the locations of Jeløy (Moss municipality), and the municipalities of Stavanger, Sandnes, Askøy, Bergen, Os, Trondheim, Ørlandet and Bjugn.

## RESULTS

The results of the study are summarized in Table 1. *Salmonella Typhimurium* 4,5,12:i:1,2 was isolated from 39% of all hedgehogs examined on Jeløy, and from 41% of the hedgehogs from Askøy, Bergen and Os. The percentage of salmonella-carriage was significantly higher among hedgehogs sampled at feeding places (71%), compared to those captured elsewhere (25%). Salmonella-positive animals occurred in 8 of the 12 sampling sites on Jeløy and in 8 of 13 sampling

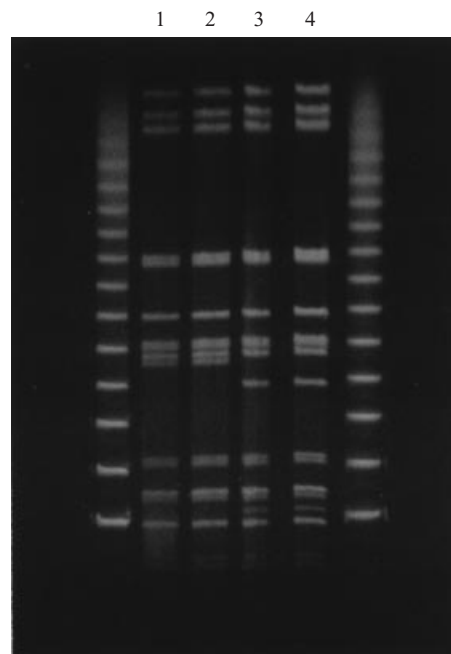


Fig. 2. PFGE-profiles of *XbaI*-digested DNA from 4 *S. Typhimurium* isolates. Lane 1, human isolate from the outbreak on Jeløy; lane 2, hedgehog isolate from the same area. Lane 3, human isolate from the outbreak in Askøy, Bergen and Os; lane 4, a contemporary hedgehog isolate. Unmarked lanes are DNA size markers (Bacteriophage Lambda ladder; New England Biolabs, Beverly, MA, USA).

sites in Askøy, Bergen and Os, and included sites both with and without reported human cases. The frequency of infection on salmonella-positive sampling sites varied between 25% and 100%. The percentages of infected juvenile and adult animals were 35% and 44% respectively, whereas the percentages of infected males and females were 46% and 35%. The faecal consistency of hedgehogs from Askøy, Bergen and Os was solid, soft, or liquid in 41%, 53%, and 6% of salmonella-positive animals, and in 37%, 46%, and

17% of salmonella-negative animals. No salmonella was isolated from the faeces of the hedgehogs sampled in south-western or central Norway.

All hedgehog isolates from Jeløy showed the E2 profile by PFGE-examination, whereas those from Askøy, Bergen and Os belonged to the profile E5 (Fig. 2). *Salmonella* Typhimurium 4,5,12:i:1,2 with the PFGE-profile E5 was also isolated from the hedgehog sample received from the Tysnes municipality, central-western Norway. No salmonella were detected in the three hedgehog samples from Oslo and Herøy.

## DISCUSSION

The present study found the hedgehog populations on Jeløy, and in Askøy, Bergen and Os to be heavily infected with *S. Typhimurium* of the same serotype and PFGE-profile as isolated from patients during the human outbreaks in the same areas. These findings strongly support the earlier suggestions that both of the human outbreaks had their origin in infected hedgehogs that had contaminated the outdoor environment (private gardens) [1, 2]. The results also suggested that hedgehog feeding places may serve as sites of salmonella transmission between animals. The fact that the same serotype (4,5,12:i:1,2) was present in the hedgehog populations both from eastern and western parts of the country, may indicate that the Norwegian hedgehog is a reservoir host for this specific serotype of *S. Typhimurium*. In England and Denmark, the hedgehog has been suggested as a reservoir host for specific phage types of *S. Enteritidis*, and these phage types are isolated from sporadic cases of human salmonellosis in the two countries [5, 6].

High carrier rates of salmonella have been recorded in hedgehog populations in many countries, the most prevalent serovar reported being *S. Enteritidis* followed by *S. Typhimurium* [5–9]. On this basis it may seem strange that there are in fact no reports of epidemic outbreaks of human salmonellosis traced to a hedgehog reservoir from countries other than Norway. One possible reason is the very favourable salmonella-situation in Norway that facilitates epidemiological tracing of domestic outbreaks. Norwegian food production animals are virtually free from salmonella [10], and thus do not represent a complicating source of infection when investigating outbreaks.

Norwegian hedgehog populations have not previously been surveyed for faecal carriage of salmonella. However, salmonella-infection, most often

*S. Typhimurium*, is diagnosed sporadically in various wild mammals and birds submitted for post mortem examination in Norway, including two hedgehogs with *S. Typhimurium* 4,12:i:1,2 and *S. Enteritidis* infection [11]. The only significant manifestation of salmonellosis known to occur in Norwegian wildlife is septicaemic salmonellosis, which occurs among passerine birds at feeding places during winter [11]. This infection is invariably associated with *S. Typhimurium* 4,12:i:1,2. This bacterium also causes sporadic cases of human salmonellosis in Norway, affecting mainly young children. A case-control study identified contact with wild birds and their faeces as risk factors, in addition to consumption of untreated drinking water [12]. Young children also constituted the main group of patients during the two hedgehog-associated outbreaks, about half of the patients were under 6 years old [1, 2]. The dominance of disease among young children presumably is due to a higher susceptibility to infection with low inoculums and a greater risk of faecal-organ acquisition of infection from a contaminated outdoor environment in this age group, compared to older persons [12].

During the hedgehog sampling on Jeløy, 20 birds including 5 common gulls (*Larus canus*), 6 hooded crows (*Corvus corone*), 5 pigeons (*Columba livia*) and 4 magpies (*Pica pica*) were shot and examined for intestinal carriage of salmonella. One each of the crows and gulls examined were infected with *S. Typhimurium* of the same serotype and PFGE-profile as the hedgehogs from Jeløy [I.Tjernsbekk and S. Race, personal communication]. It is not unlikely that these birds had acquired their infection from infected hedgehogs, either by eating hedgehog carcasses or food contaminated with hedgehog faeces (including food available on the hedgehog feeding places). In Askøy, Bergen and Os no birds were examined. However, in 1999 a human waterborne outbreak caused by *S. Typhimurium* of the same serotype and PFGE-profile as found in the hedgehogs from Askøy, Bergen and Os occurred in the municipality of Herøy, north-western Norway, and this bacterium was isolated from seagull feathers found in the drinking water reservoir [13]. This reservoir is located at an altitude that makes the presence of hedgehogs unlikely. However, one hypothesis could be that gulls had brought the infection from a primary hedgehog reservoir. Opportunistic birds like gulls are found to be sporadic carriers of various salmonella in our country [11], infections that may reflect sources in the local environment (e.g. dead salmonella-infected hedgehogs). A recent investigation of the molecular

epidemiology of *S. Typhimurium* from human cases revealed that the PFGE profile E5 was the second most prevalent pattern (22% of human domestic cases) observed among sporadic infections acquired in Norway during the period 1996–9 [14]. Moreover, the domestic E5 profile isolates were exclusively from cases in central-western Norway, and these cases were reported between April and August, i.e. during a period when hedgehogs are active. In combination with the present data this suggests hedgehogs are a primary source of this specific *S. Typhimurium* clone in central-western Norway.

There are several reports of disease and death following salmonella-infection (*S. Enteritidis*) in hedgehogs [5, 15, 16]. The disease normally is associated with enteritis (diarrhoea), which did not seem to occur during the salmonella-infection in the present study. As a matter of fact the highest percentage of hedgehogs with liquid faeces occurred among salmonella-negative animals, and animals with signs of general illness were not observed. The mode of transmission of salmonella between hedgehogs is unknown, but both faecal spread and transmission via vectors like fleas and slugs have been suggested [5, 17].

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