Real-Time Monitoring of *Salmonella enterica* in Free-Range Geese

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Free-range geese were sampled longitudinally and *Salmonella* isolates characterized to reveal highly diverging colonization dynamics. One flock was intermittently colonized with one strain of *Salmonella enterica* serovar *Enteritidis* from 2 weeks of age, while in another, *S. enterica* serovar *Mbandaka* appeared after 9 weeks, without dissemination but with multiple serovars appearing at later stages.

Intervention and control strategies are implemented in many countries in order to reduce the prevalence of food-borne enteropathogens, such as *Salmonella*, in poultry, and progress has been made in conventional production (4, 6, 7, 8, 11). However, there is an increased production of free-range poultry, and information is limited about the prevalence of *Salmonella* under such production systems (5, 8, 10). In the present study, the colonization dynamics of *Salmonella* and its interaction with the environment were studied in flocks of free-range geese produced on small islands of Denmark.

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Two flocks of day-old Emdener goslings with a Salmonella-free status, flock 1 (2,500 individuals) and flock 4 (500 individuals), were purchased from a hatchery (Bülow’s Aps, Faarevejle, Denmark) and arrived at the island of Sejeroe, Denmark, on 1 April 2009 and 1 June 2009, respectively. The flocks were housed in a stable for the first 2 weeks and then allowed access to 7 ha of pasture. Half of flock 1 was relocated to neighbor premises of 10 ha separated by fences and a road at 6 weeks of age, while the other half (flock 1A) was moved to the remote island of Skaroe, Denmark. Flock 4 was raised on the same premises as flock 1 until 6 weeks of age.

Sampling was done by collecting fresh fecal droppings, and for flocks 1 and 1A, 50 neck skin samples were additionally collected at slaughter.

Isolation of Salmonella strains was performed according to guidelines of the Nordic Committee on Food Analysis (2, 3). One to five presumptive Salmonella colonies were subcultivated onto blood agar (Statens Serum Institute, Copenhagen, Denmark). Salmonella isolates were serotyped by the slide agglutination method according to the White-Kauffmann-Le Minor scheme (9) by using Salmonella antisera (Statens Serum Institute, Copenhagen, Denmark). S. enterica serovar Enteritidis isolates were phage typed according to the Colindale scheme (13).

Pulsed-field gel electrophoresis (PFGE) of S. Enteritidis isolates was carried out by the Pulse-Net USA protocol (http://www.cdc.gov/pulsenet/protocols.htm) using XbaI and XmaI as restriction enzymes.

Pathological investigations were conducted on dead geese according to routine procedures by the Danish Agricultural Advisory Service, Aarhus, Denmark.

Parasitological examinations of fecal droppings were conducted according to accredited routine procedures by the National Veterinary Institute, Copenhagen, Denmark.

Flock 1 was sampled most frequently, and weeks of age, numbers of droppings, and findings for this flock are shown in Fig. 1. Colonization with S. Enteritidis was found to take place at 2 to 3 weeks of age, and its increase in numbers of samples revealed a quick dissemination. Mortality increased at 3 to 5 weeks of age, and polyarthritis was clinically diagnosed. Parasitological examination revealed the moderate presence of coccidia. Treatment at 5 to 6 weeks of age with tetracycline (Terramycin, 20% powder, 1 g per 4 kg body weight per day for 5 days; Pfizer) relieved the symptoms, suggesting an etiological role for S. Enteritidis. A transient shedding at 28 weeks of age of S. Enteritidis cells correlated with an outbreak of pneumonia caused by Pasteurella multocida. The flock was relocated to another paddock, with a subsequent decrease in mortality and an arrest of detectable shedding of Salmonella. Part of flock 1 was slaughtered at 30 weeks of age, and 1 of 50 neck skin samples was found positive for S. Enteritidis. A total of 68 isolates of S. Enteritidis collected from the first three sampling points after introduction and 1 to 2 isolates from each of the other sampling points were found to belong to phage type 29 and have identical PFGE types (see Fig. 3), suggesting that only one strain of Salmonella colonized this flock; it was detectable also in flock 1A 2 weeks after relocation but no later than that.

FIG. 2. Regimens of sampling and the prevalences of Salmonella during the lifetime of flock 4. The number of droppings (N) is indicated at each sampling point. P/N, number of droppings positive for Salmonella per number of droppings.
that disseminated colonization with a single strain of S. Enteritidis in flock 1 protected against spread of other strains of Salmonella in flocks 1 and 1A. The decline of colonization in flocks 1 and 1A likewise might be due to immunity developed, concordant with what has been reported in experimentally colonized and in vaccinated broilers (6, 14). Other parameters, such as health status, coinfections, and relocations, also seem to play roles in the colonization dynamics.

The multiplicity of Salmonella strains colonizing flock 4 and the fluctuation over time in the appearance of strains illustrate the challenge in tracing human outbreaks of salmonellosis to the herd of origin.

Geese are consumed in Denmark mainly on Christmas evening. Although the geese of the present study were distributed through the two largest retail companies, no human disease caused by Salmonella strains observed in the present study was recorded during Christmas of 2009 (1). This might be attributed to the slaughtering of flocks planned at times when levels of Salmonella in the flocks were below the detection limit, which illustrates the risk-mitigating potential of real-time monitoring of Salmonella.

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FIG. 3. Pulsed-field gel electrophoresis patterns produced with Xbal or Xmal of representative isolates of Salmonella from geese flocks 1 and 4.

Sampling was less frequent in flock 4, and weeks of age, numbers of droppings, and findings are shown in Fig. 2. S. enterica serovar Mbandaka was observed at 9 weeks of age. At 27 weeks of age, a relapse of shedding of S. Mbandaka (in one of five droppings) and S. enterica serovar Hadar (in the remaining 3 of 5 droppings) was observed. At 28 weeks of age, two isolates of S. Enteritidis which were sensitive not only to phage 12 (like phage type 29) but also to phage 11 were collected. Yet, these isolates had PFGE banding patterns identical to those of all the other isolates of S. Enteritidis collected from the flocks, as shown in Fig. 3, suggesting that in a particular strain of S. Enteritidis in the environment of this island, sensitivity to phage 11 had developed.

Relapses of Salmonella shedding at the onset of egg production have been reported in hens (12), but due to the colonization dynamics observed, vertical transmission in the present study is considered unlikely. A predominant role of environmental sources in the transmission of infections to these geese flocks is illustrated by the fact that flock 4 at the age of 8 weeks became colonized with a serovar of Salmonella other than that seen in flock 1. Once the organism was introduced to the flock, the colonization dynamics of Salmonella were found to differ significantly in the flocks. As multiple serovars of Salmonella and deviating phage types of S. Enteritidis were indeed present in the environment, appearing in flock 4, it may be suggested