

Contaminated commercial dehydrated food as source of multiple *Salmonella* serotypes outbreak in a municipal kennel in Tuscany

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Summary

The authors describe a large outbreak of canine salmonellosis in a municipal kennel in Tuscany. During the outbreak, 174 samples of 'diarrhetic' and 'normal' faeces and two batches of commercial dehydrated dog food were cultured for pathogenic bacteria. The results of 25, out of a total of 41 dogs (60.9%) revealed at least one faecal sample as being positive for *Salmonella*; incidence per sampling ranged from 12.5% to 34%. Nine of 10 samples of dehydrated food were positive. Ten totally different serotypes were isolated from dry food and faeces: the results of the pulsed-field gel electrophoresis referred to similarity between the *Salmonella* Montevideo, Muenster and Worthington isolates recovered from both the food and canine faecal samples.

Keywords

Canine, Dog, Italy, Kennel, Mantel test, PFGE, Pulsed-field gel electrophoresis, *Salmonella*, Salmonellosis.

Introduction

Salmonella organisms are primarily motile, non-spore forming, Gram-negative aerobic bacilli of the family Enterobacteriaceae. Over 2 500 serotypes of *Salmonella* have been

associated with both human and animal disease (9).

The subclinical carrier state of *Salmonella* is common in dogs and is frequently caused by the ingestion of contaminated food or carrion, or coprophagia (2, 15). On the contrary, clinical cases are extremely rare, but can be observed in puppies and in kennel populations. These cases can exhibit fever (40°C-41.1°C), anorexia, diarrhoea, bloody diarrhoea, abdominal pain and abortion (14). Dogs are able to shed the organism in their faeces for six weeks or more, continuously during the first week and intermittently thereafter (14) and asymptomatic dogs can serve as sources of salmonellosis for other dogs and for humans. *Salmonella* is commonly isolated in dog kennels and the administration of contaminated food is the most important source of introduction of *Salmonella* infection.

The diet used in the kennels can include unprocessed or raw dog food, a home-made diet or dehydrated food. Raw meat used for dog food is generally considered to be an important risk factor in *Salmonella* infection. The meat used for the production of diets can originate from several sources, including human food or products that are no longer deemed suitable for human consumption.

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Often, these diets do not undergo any form of heat processing or sterilisation and existing bacteria can be present at the time of consumption. The risk of salmonellae shedding by dogs fed with a raw food diet including meat contaminated with salmonellae has been widely demonstrated (2, 7). Dehydrated food is generally considered to be less hazardous as it is easier to administer and to preserve. However, animal-derived products are often contaminated with salmonellae and the dehydration procedure might not be effective in eliminating the organism. Furthermore, these procedures provide an opportunity for food to be contaminated by the environment, from workers who might be carrying or might be infected by the organism. Contaminated dehydrated dog food is reported to be responsible both for outbreaks in kennels (18) and as a potential source of human *Salmonella* infections through handling of pet foods and treats (1, 3, 19). This aspect highlights the risk of infection for dogs and owners who handle dog food. Furthermore, if a dry diet is used, this should be reported to public health officials when interviewing human cases of salmonellosis.

In Italy, an extensive study of the prevalence of *Salmonella* infections in kennel dogs has never been conducted. However, Corradini and colleagues (5) reported that the 7.16% of a total of 2 138 faecal samples collected from dogs yielded 30 different serotypes of *Salmonella*, confirming the fact that the canine subclinical carrier state is common across Italy. According to these data, the expectation of prevalence of salmonellosis in a kennel is close to the level of 15% reported by other authors (8). However, the prevalence in kennels can vary (8, 14), depending on the opportunities of transmission that relate to type of diet, structural features (i.e. density of dogs, availability of a common area, presence of isolation rooms, etc.) and kennel management (i.e. veterinary care, sanitation programmes, monitoring of new arrivals).

Our study covered a *Salmonella* outbreak that showed unexpected health risks of multiple serotype infection for dogs that consumed

commercial dry food. We studied the risk of dogs being infected by carriers and by the environment, in relation to direct transmission from the diet. We analysed space-time clustering and the restriction patterns identified by pulsed-field gel electrophoresis (PFGE) to establish the degree of similarity between the strains isolated from the diet and those circulating in the kennels.

Material and methods

Case history

Lucca's municipal kennel, situated in north-western Tuscany, is a modern, solid brick/block construction (Fig. 1) managed by the Italian Organization for Animal Protection (*Ente Nazionale Protezione Animali*: ENPA). It has 36 single boxes that are usually occupied and four rooms that are used for isolation and the nursery; a common green exercise area is also available. The kennel fills the role of 'animal control office' for seven cities in the Province of Lucca and houses approximately 300 dogs each year.



Figure 1
Lucca's municipal kennel situated in north-western Tuscany

In December 2007, several dogs housed at the municipal kennel of Lucca had profuse and often hemorrhagic diarrhoea, in the absence of any other clinical signs. Routine analyses for parasitic research in faeces gave negative results. Subsequently, on 31 December, new faecal sampling was performed on 5 symptomatic dogs to detect *Salmonella* spp.

and *Salmonella enterica* was detected in 4 of the 5 samples collected.

As soon the outbreak was reported, the kennel was closed by-law (10 January 2008). At that time, 41 dogs were housed in the kennel and were confined to their own shelters. Thirty-five of the 41 dogs were confined in individual shelters, but three shelters (Box 3, CP and CA) housed two dogs each (Fig. 2).

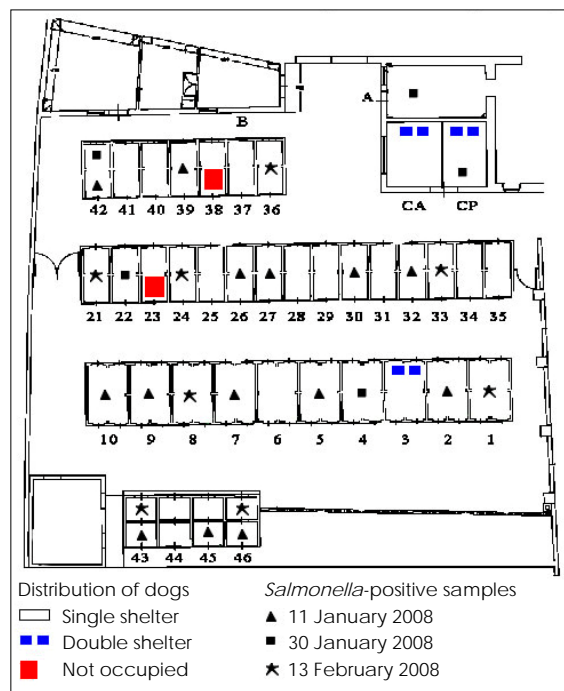


Figure 2
Localisation of *Salmonella*-positive canine samples
Collection times are indicated for each shelter
Rooms A, B, CA and CP are used for isolation, nursery and infirmary

Collection of specimens

During the study, four sampling sessions were performed about every 14 days (from 11 January to 27 February 2008), using the same sampling method each time. Faecal samples (including blood or mucus, if present), were collected from the floor of the shelters using the scoop inside the lid of a sterile container and refrigerated for no longer than 24 h until laboratory analysis. Faecal samples were collected as pooled samples in the three shelters that housed 2 dogs and in the event of a positive result both dogs were considered responsible for shedding the micro-organism.

Two different batches of the commercial dehydrated dog food were sampled from unopened packages on 11 January and 14 February 2008. The first batch covered the feeding period from 25 December to 7 February and the second from 8 to 13 February. In line with Annex VII of the European Council Regulation EC 1774/2002 (6), official sampling criteria were used and five different packages were tested for each lot. At least 200 g samples were delivered to the laboratory in sterile containers. Prior to 25 December, a different batch of food of the same brand was in use, but was not tested.

Stool cultures were taken from personnel and veterinarians working in the kennels.

Salmonella identification and serotyping

Each faecal sample was cultured for *Salmonella* in accordance with the *Manual of diagnostic tests and vaccines for terrestrial animals* (21). The isolation of salmonellae from commercial dehydrated dog food was conducted in accordance with ISO 6579:2002 (10) and ISO 6579:2002/Amd 1:2007 (11). All strains were identified biochemically using the miniaturised commercial system BioMérieux API 20E test and serological identification with commercial antisera in accordance with the White-Kauffmann-Le Minor scheme.

Salmonella isolates were serotyped and tested for susceptibility to 14 antimicrobials using the relevant Clinical and Laboratory Institute Standards (4).

The PFGE method was the standard in use at the Centers for Disease Control and Prevention (CDC) in the Pulse-Net project (17), using XbaI restriction enzyme. Analysis of PFGE patterns was performed using a Bionumerics version 4.1 software package (Applied Maths, Sint Martens-Latem) with 1% tolerance and 1% optimisation. *Salmonella* serotype Braenderup strain (ATCC H9812) was used as a reference standard. Dice similarity coefficients and an unweighted pair group method with averages (UPGMA) were used to calculate similarity coefficients. The pulsotypes were considered to be highly correlated with a similarity index that exceeded 80%, according to the criteria in

use at the Department of Infectious, Parasitic, and Immune-Mediated Diseases of the *Istituto Superiore di Sanità* in Rome.

Statistical analysis

The Mantel test was used to evaluate the spatial and temporal correlation between cases (13), comparing matrices based on nearest neighbour's spatial distances and temporal distances (in days).

Animal sex, size and ownership status were evaluated as risk factors using the Chi-square test.

All statistical tests were performed by using the R computer program (16).

Results

Sampling of five symptomatic dogs (31 Dec), gave four positive results. As a consequence, the kennel was closed and the dogs were housed in isolation in their shelters. Official procedures, including appropriate strategies for control of faecal/oral transmission of the disease, were introduced to minimise the risk of transmission to other dogs and from dogs to humans.

During the study session, samples of 'diarrhetic' and 'normal' faeces were cultured for pathogenic bacteria: 24 out of the

36 shelters and 25 out of the 41 dogs revealed at least one faecal sample that was positive for *Salmonella* (66.6% CI 50.7-82.3 and 61.0% CI 44.5-75.8, respectively). Infection prevalence per sampling ranged from 12.5% to 34%. The dogs were treated orally with enrofloxacin for 5 days (5 mg/kg) and checked again 10 days after each antibiotic cycle. Good clinical response to the antibiotic was observed. The distribution of the positive cases in the shelters of the kennel per sampling session is given in Figure 2.

Both dehydrated dog food lots (9 out of 10 samples) were positive for *Salmonella*.

In total, from 174 faecal samples and 10 dehydrated food samples were obtained. A total of 31 isolates and were identified, belonging to 10 different serotypes. In the faecal samples, *S. Livingstone* was isolated four times, *S. Montevideo* and *S. Worthington* three times, *S. Muenster* twice and *S. Derby*, *S. Give* and *S. Isangi* once each. In the dehydrated food samples, *S. Thompson* was isolated six times; *S. Montevideo* three times, *S. Anatum* and *S. Seftenberg* twice *S. Livingstone* and *S. Muenster* and *S. Worthington* once each. The distribution of the serotypes identified is presented in Table I and the similarities among PFGE restriction patterns is shown in Figure 3.

Table I
Description of *Salmonella* serotypes identified in food and faecal samples (31 December 2007-27 February 2008)

Date of identification	<i>Salmonella</i> serotype	
	Faecal sampling session	Food lot sampling session
31 December	<i>S. Isangi</i>	
11 January	<i>S. Give</i> <i>S. Livingstone</i> ⁽¹⁾	<i>S. Thompson</i> <i>S. Anatum</i> <i>S. Montevideo</i> ⁽²⁾ <i>S. Muenster</i> ⁽³⁾
30 January	<i>S. Livingstone</i> ⁽⁴⁾ <i>S. Montevideo</i> ⁽⁵⁾	-
13-14 February	<i>S. Derby</i> <i>S. Worthington</i> ⁽⁶⁾ <i>S. Montevideo</i> ⁽⁷⁾ <i>S. Muenster</i> ⁽⁸⁾	<i>S. Worthington</i> ⁽⁹⁾ <i>S. Livingstone</i> ⁽¹⁰⁾ <i>S. Seftenberg</i>
27 February	<i>Salmonella</i> -negative	Introduction of a new dehydrated dog food brand in use

Superscript letters different serotype strains progressively isolated from faeces and food by pulsed-field gel electrophoresis (see matching figures in ID No. column in Figure 3)

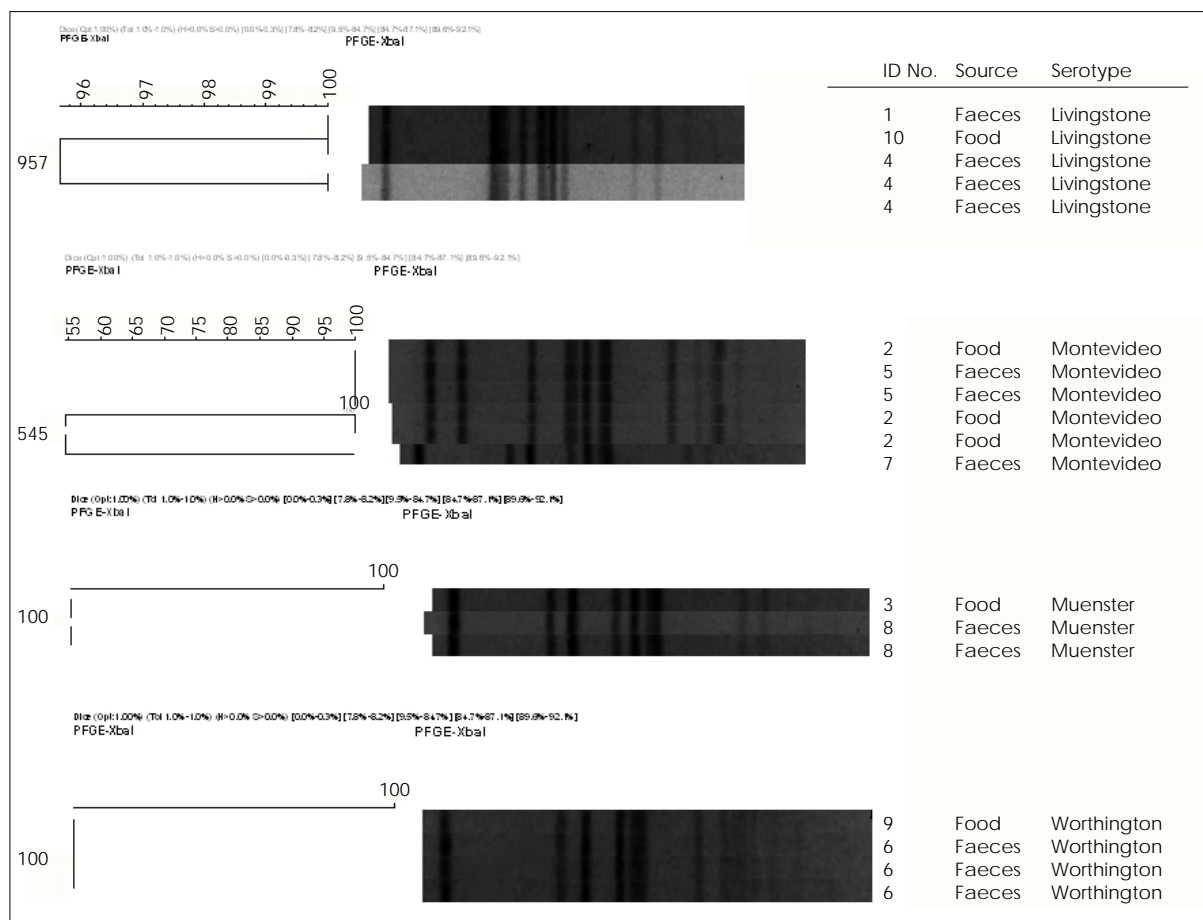


Figure 3 Comparison of XbaI pulsed-field gel electrophoresis pattern of *Salmonella* isolates from faeces and food. The ID code enables identification in chronological order, in relation to the time of isolation of the different strains in the sampling session

All isolated strains did not show resistance to individual antimicrobials, with the exception of *Salmonella* serotype Worthington isolated from faeces that showed multiple resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, trimethoprin-sulphametoxazole and tetracycline.

During the study session, no association was observed among positive dogs in relation to sex, size and ownership status ($\chi^2 p > 0.05$ in all tests). No spatio-temporal correlation of the cases was found that supported the absence of direct transmission among animals (Mantel test $p > 0.05$).

Veterinarians and kennel personnel were tested for *Salmonella* and all results were negative.

Discussion

The results revealed that several different *Salmonella* serotypes from food and faeces were present. The PFGE showed similarities between the serotypes isolated (Muenster, Worthington, Montevideo and Livingstone) from both food and canine faecal samples.

Salmonella serotypes Muenster, Worthington and Montevideo showed a similarity index (SI) of 100%, while serotype Livingstone an SI of 95.7%. Since the pulsotypes were considered to be highly correlated with a similarity index that exceeded 80% and serotype Livingstone was found in a batch of dog food that was not yet in use at the kennel when it was isolated from the faeces, it was possible to confirm that dehydrated food was the original source of three serotypes (Muenster, Worthington and

Montevideo). The results did not indicate that food was the source of all of the serotypes shed. In fact, dogs also carried *Salmonella* serotype Give, Derby, Isangi, a different pulsotype of Montevideo (SI = 54.5%) and serotype Livingstone. It is not clear how these pathogens were introduced into the kennel. They could have been present in dry food used previously or they might have been circulating already as saprophytic strains in the dogs (20). The high level of qualitative contamination observed in the two batches made isolation difficult for any single serotype.

On the contrary, *Salmonella* serotypes Anatum, Seftenberg and Thompson, were isolated from food but not from faeces. It is possible that there was a difference in the shedding likelihood related to serotypes, as reported for *Salmonella* Thompson (7). Other reasons can justify these results, in particular: these serotypes can be less virulent for dogs, they cannot colonise canine gut or they were present in the food in low bacterial loads. It is also possible that the dogs gave false-negative results because the sampling frequency, once every 15 days was inadequate for cases of intermittent shedding.

A comparison of isolates from faeces and from food revealed that *Salmonella* serotype Worthington had a different antimicrobial susceptibility pattern. In this case, the additional resistance of the canine isolates that was observed in the absence of selective antimicrobial used, appears to have originated from the transfer of resistance genes from viruses or other bacteria housed in the intestinal tract of the dogs. However, the evidence of enteric colonisation by non-resistant *Salmonella* in dogs, the acquiring of resistance plasmids *in loco* and the subsequent spread of specific serotypes harbouring these plasmids, emphasised the fact that a kennel can be a source of additional risk for *Salmonella* infection in humans. Transfer of resistance determinants were not been investigated here.

The causal relationship between food and faecal isolates clarified by molecular typing led to an epidemiological investigation of this outbreak. This likelihood was evaluated in the study introducing the Mantel test which

allows for statistical testing of the interaction of incidence of infectious diseases in space and over time, demonstrating that there was no space and time interaction in the evolution of *Salmonella* cases in this outbreak. This result, confirmed by the good clinical response to the antibiotic cycle, suggested that the incidence evaluated in the study agreed with the shedding rate in dogs fed with the contaminated food in the kennels.

Since this study is based on a field investigation, the results point to a problem of contamination of dehydrated dog food which is largely used in Italy to feed kennel dogs for reasons of safety, nutritional value and handiness in administration and storage. The shedding rate observed, considering the confidence interval for sampling (2%-23% and 19%-49%), included the value reported by other authors in dogs fed with a commercial and home-made raw food diet (7, 12). Since the shedding rate relates to the total load of salmonellae in the food, the results of our study suggest that the microbial load in dehydrated food can be comparable to the load in raw food. This outcome was remarkable because one may expect the microbial load to be lower in processed feed materials, such as dehydrated food.

Conclusions

In this study, dogs fed with *Salmonella*-contaminated dry food, shed salmonellae for at least two weeks. We noted that certain serotypes spread particularly in dogs which serve as an amplifier and intermediate source for humans, sometimes modifying the resistance pattern of the serotypes. This result suggests that dogs play an active role in the transmission of salmonellosis to humans. The canine isolate of *Salmonella* serotype Worthington showed resistance against trimethoprim-sulphamethoxazol. This aspect is important for Italian public health management because this antibiotic is still in use as a second choice in the treatment of intestinal infection of young patients.

In this study, the epidemiological links between the outbreak observed in the kennels

and the intake of infected food was demonstrated as being the unique source of transmission of *Salmonella* to the dogs. At the same time, the risk of transmission among dogs and from dogs to humans, appeared to be controllable by introducing appropriate health strategies.

As a consequence of the outbreak, an official investigation was conducted in the premises of origin and all necessary checks were made by local health authorities. Later, the self-monitoring plan manager of the plant reported

that, although the absence of *Salmonella* contamination was confirmed in the raw materials, the final product remained contaminated, therefore accurate investigations of all the sections of the plant were conducted and corrective action was taken, resulting in the manufacture of *Salmonella*-free food.

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