To determine whether cats were a risk for transmission of Salmonella to humans, we evaluated the excretion of Salmonella by pet cats. Rectal-swab specimens were taken from 278 healthy house cats, from 58 cats that died of disease, and from 35 group-housed cats. Group-housed cats were kept in one room with three cat trays and a common water and feed tray. Eighteen (51.4%) of 35 group-housed cats, 5 (8.6%) of 58 diseased cats (5/58), and 1 (0.36%) of 278 healthy house cats excreted Salmonella. Salmonella isolates were of serotypes Typhimurium, Enteritidis, Bovismorbificans and 4:i:-. Acquired antimicrobial resistance was found in serotype Typhimurium (resistance to ampicillin, chloramphenicol, and tetracycline; to ampicillin; and to chloramphenicol) and 4:i:- strains (resistance to ampicillin, chloramphenicol, sulfonamides, trimethoprim, and sulfamethoxazole/trimethoprim). Cats that excrete Salmonella can pose a public health hazard to people who are highly susceptible to Salmonella, such as children, the elderly, and immunocompromised persons.

Salmonella infections are still a leading cause of human foodborne infections in the world (1,2). These infections primarily originate from eating contaminated food, especially chicken eggs and egg products, and also meat products from pigs and chickens (3,4). Considering the high frequency of food contamination and the emergence of multidrug–resistant Salmonella strains, control of Salmonella in food-producing animals has become a worldwide challenge. Other environmental sources can lead to accidental human infections with Salmonella as well. The role of pet animals as a source of Salmonella has not been fully investigated, but severe human infections originating from reptiles, especially pet turtles, have been reported (5).

Cats and dogs are the most widely kept pet animals, yet the incidence of Salmonella in these animals is largely unknown, and the risk that these animals pose for transmission of Salmonella to humans is unclear. In particular, cats that can freely roam outside, and are therefore able to scavenge or hunt food of unknown quality, are potential candidates for Salmonella carriage. Most reports concerning Salmonella and cats are case studies of clinical salmonellosis, which resulted in septicemia and death (6,7). Subclinical infections and carrier animals, however, are much more important with respect to transmission to humans. In this study, rectal swabs from cats of different origin (house cats, group-housed cats, diseased cats) were cultured for Salmonella. The serotype and phage type of the Salmonella isolates were determined, and the isolates were characterized with respect to their antimicrobial drug–resistance pattern and interaction with human intestinal epithelial cells.

Methods

Collection of Fecal Samples

A total of 278 rectal swab samples from house cats of different age, sex, and breed were taken between July and November 2003. All house cats came from different owners. The animals came from all over the Dutch-speaking part of Belgium, i.e., north of Brussels. Rectal swab specimens were also taken from 58 cats that were submitted for autopsy to the Faculty of Veterinary Medicine, Ghent University. The latter died or were euthanized because of incurable disease. All cats came from different owners, except three cats that had feline immunodeficiency virus (FIV), which came from one owner. Finally, rectal samples of 35 kittens (all <4 months of age) were taken at a facility.
where the animals were group-housed, waiting to be adopted. These animals came from 16 different owners.

**Bacteriologic Analysis**

Bacteriologic analysis was performed by enrichment of the rectal swabs. The samples were first pre-enriched in buffered peptone water (BPW) (Oxoid, Basingstoke, Hampshire, UK) overnight at 37°C, after which 1 mL of this suspension was added to 9 mL of tetrahionate brilliant green broth (Oxoid) (enrichment). After incubation overnight at 37°C, a drop of this suspension was spread on brilliant green agar (BGA) (Oxoid). Both the serotype and phage type of positive isolates were determined.

**Antimicrobial Susceptibility Testing**

Resistance to antimicrobial agents was tested by using the disk diffusion assay on Mueller-Hinton agar with commercial antimicrobial susceptibility disks (Oxoid) according to the international standards of the National Council for Clinical Laboratory Standards (NCCLS) (8). The following antimicrobial agents were tested: ampicillin (A, 10 µg), chloramphenicol (C, 30 µg), streptomycin (S, 10 µg), sulfonamide (Su, 300 µg), tetracycline (T, 30 µg), ciprofloxacin (Cip, 5 µg), kanamycin (K, 30 µg), gentamicin (Gn, 10 µg), sulfamethoxazole-trimethoprim (Sxt, 25 µg), cefotaxime (Cxt, 30 µg), nalidixic acid (Na, 30 µg), and amoxicillin-clavulanic acid (Amc, 30 µg). *Salmonella enterica* serovar Typhimurium 8420 (resistance type ASSuT), 6237 (sensitive), 3520 (resistance type T), 2200 (resistance type ASSuT), and 5833 to invade human intestinal epithelial cells was determined. Cells of the human colon carcinoma cell line T84 were seeded in 96-well cell culture plates (Greiner, Frickenhausen, Germany) at a density of 5.10⁵ cells/mL culture medium (DMEM + 10% fetal calf serum + 2% L-glutamine, without antimicrobial drugs) and grown for 24 h. Bacteria were grown for 20 h in LB-medium, after which the suspension was diluted 1:50 in fresh LB-medium. After 4 h of incubation at 37°C, suspensions were centrifuged and resuspended in DMEM with 10% fetal calf serum (FCS). The number of colony-forming units (CFU)/mL was determined by plating 10-fold dilutions on BGA. The suspensions were stored overnight at 4°C. The next day, 10⁶ CFU in 200 µL were added to the T84 cell cultures, which were then centrifuged for 10 min at 1,500 rpm to make close contact between the bacteria and the colon cells. The plates were incubated for 1 h at 37°C and 5% CO₂. The cells were then rinsed three times with Hanks’ Balanced Salt Solution (HBSS, Life Technologies, Paisley, Scotland). Cell culture medium with gentamicin (50 µg/mL) was added, and plates were incubated for 1 h at 37°C and 5% CO₂. Hereafter, the cells were rinsed three times with PBS and analyzed with 1% Triton X-100 (Sigma, St. Louis, MO) in distilled water. From this lysate, 10-fold dilution series were made. From each dilution, 6 x 20 µL was added to BGA, to determine the number of CFU *Salmonella* per mL. The assays were performed in triplicate. The percentage of intracellular bacteria, relative to the number of *Salmonella* bacteria, initially incubated with the cells, was calculated. The previously mentioned human isolates of *S. Typhimurium* were used for comparison.
between the cat isolates and human isolates. Statistical analysis was performed by analysis of variance methods using the SPSS 11.0 software.

Results

Characterization of Salmonella Isolates from Cats

Of 278 healthy house cats, 1 Salmonella strain was isolated, an S. Enteritidis phage type 21 strain, sensitive to all tested antimicrobial drugs. Five strains were isolated from cats that died from or were euthanized because of incurable disease. Feline AIDS (caused by feline immunodeficiency virus [FIV]) was diagnosed in three cats, one died due to feline panleukopenia parvovirus infection, and one was poisoned. Three isolates were identified as being ampicillin-resistant S. Typhimurium phage type 193, harboring the blaTEM gene. They had the same pulsed-field gel electrophoresis (XbaI) pattern, indicating that the isolates were of clonal origin (Figure 1). The three cats came from the same owner. One isolate was an antimicrobial drug–sensitive Salmonella Bovismorbificans strain. One isolate was Salmonella 4:i:-, which was resistant to ampicillin, chloramphenicol, sulfonamides, tetracycline and sulfamethoxazole-trimethoprim (ACSuTSxt), harboring the blaTEM, cat, sul2, tet(A), and dfrA1 antimicrobial drug–resistance genes. Eighteen strains were isolated from

the group-housed cats. All of these were S. Typhimurium phage type 120/ad. Fourteen of these strains showed acquired resistance to ampicillin, chloramphenicol and tetracycline and harbored the bla<sub>TEM</sub>, cat, and tet(A) antimicrobial drug–resistance genes, while four isolates were resistant to chloramphenicol only and only harbored the cat gene (Table 2). Pulsed-field gel electrophoresis showed that the isolates from the group-housed cats were of the same XbaI PFGE type, and that three subtypes within this type were present, indicating a clonal origin (Figure 1). One subtype contained the 14 strains that were resistant to the three mentioned antimicrobial drugs. All Salmonella strains harbored the SopB gene.

### Invasion of the Human Intestinal Epithelial Cell Line T84

All isolates invaded T84 cells, with the cat isolates of S. Typhimurium PT193 (strains 1147, 1145, and 55, which belong to the same clone) and the human isolate S. Typhimurium strain 2200, the most invasive, yielding a percentage of invasion of 8% to 10%. The multidrug–resistant cat isolate Salmonella 4:i:- (strain 11) was the least invasive strain, having an invasion percentage of about 0.5%. Invasion percentages of the different isolates are shown in Figure 2. Of the strains of the same PFGE type, only one was shown in Figure 2, since no significant differences were detected between the invasion percentages of these strains. Statistically significant differences are shown in the figure.

### Discussion

This study concluded that, although cats can transmit Salmonella strains, healthy house cats are generally safe. Earlier reports regarding isolation of pathogens from healthy cats showed low percentages (mostly around 1%) of Salmonella-positive rectal swabs (18,19). In our study, 1 of 278 healthy cats was found to be positive. Immunodeficiency and nonhygienic housing can be predisposing factors for cats to shed Salmonella in the feces, resulting in contamination of the environment. Rectal swabs from 18 of 35 group-housed kittens were Salmonella-positive in our study. The fact that the 35 kittens were derived from more than 10 different owners before being group-housed and that one PFGE type (three subtypes) of S. Typhimurium 120/ad was isolated, indicates spread of the Salmonella strain between the cats or a common source. The age of these animals may also play a role, since all animals in this group were <4 months. Young animals are more susceptible to Salmonella infection. Also immunodeficiency can result in Salmonella excretion. One outbreak of fatal salmonellosis in cats has been reported after mild immunosuppression induced by live panleukopenia virus vaccination (7). In our study, animals infected with FIV and one animal that had panleukopenia shed Salmonella. Three animals that were infected with FIV were derived from the same owner, which indicates that the animals were infected with Salmonella from the same source or that one animal contaminated the others.

In our study, serotypes Typhimurium, Enteritidis, Bovismorificans, and 4:i:- were isolated from cats. The

### Table 2. Characteristics of Salmonella isolates from cats<sup>a</sup>

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Source</th>
<th>Serotype</th>
<th>Phage type</th>
<th>PFGE pattern&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Resistance phenotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Resistance genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Diseased house cat</td>
<td>4:i:-</td>
<td>–</td>
<td>ND</td>
<td>ACSuTSxt</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;, cat, sul2, tet(A), dhaA1</td>
</tr>
<tr>
<td>40</td>
<td>Diseased house cat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>House cat</td>
<td>Bovismorificans</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>1145, 1147, 55</td>
<td>Diseased house cats</td>
<td>Enteritidis</td>
<td>21</td>
<td>ND</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>89, 165, 174, 198, 320, 326, 352, 355, 358, 359, 368, 390, 392</td>
<td>Group-housed cats</td>
<td>Typhimurium</td>
<td>193</td>
<td>II</td>
<td>A</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
</tr>
<tr>
<td>161, 350</td>
<td>Group-housed cats</td>
<td>Typhimurium</td>
<td>120/ad</td>
<td>la</td>
<td>ACT</td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;, cat, tet(A)</td>
</tr>
<tr>
<td>220, 339</td>
<td>Group-housed cats</td>
<td>Typhimurium</td>
<td>120/ad</td>
<td>lb</td>
<td>C</td>
<td>cat</td>
</tr>
</tbody>
</table>

<sup>a</sup>A, ampicillin; C, chloramphenicol; Su, sulfonamides, T, tetracycline; Sxt, sulfamethoxazole-trimethoprim; ND, not determined; PFGE, pulsed-field gel electrophoresis.

<sup>b</sup>Roman numerals indicate the major types of fragment patterns; lowercase letters indicate minor variations in the respective fragment pattern.
isolate serotypes indicate that the cats were infected from the same sources compared with other animals and man. Indeed, serotypes Typhimurium and Enteritidis are the most widespread serotypes and the serotype Bovismorbificans is not uncommon in other animals, including humans (2,20).

Generally, invasion in the human intestinal epithelial cell line T84 was comparable between the cat isolates and isolates from humans. Invasion in intestinal epithelial cells is the primary step in the pathogenesis of Salmonella that causes gastrointestinal problems (21). This finding implies that the cat isolates are potentially pathogenic for humans. Moreover, all cat isolates harbored the SopB gene, which is involved in blocking the closure of chloride channels in gut epithelium and thus in inducing diarrhea. As in most other animal species, the cat isolates of the serotype Typhimurium harbored antimicrobial drug–resistant genes, raising concerns about spreading antimicrobial drug–resistant strains to humans.

Since the 1990s, concerns have arisen about the emergence and spread of multidrug–resistant Typhimurium strains, especially the multidrug–resistant ACSSuT type, which is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (2). In our study, some S. Typhimurium isolates from cats were resistant to a single drug such as ampicillin or chloramphenicol, while most isolates from the group-housed cats (same clone) were resistant to ampicillin, chloramphenicol, and tetracycline. Resistance genes were found to be blaTEM (ampicillin), cat (chloramphenicol), and tet(A) (tetracycline). The genes in the class 1 integron of the multidrug–resistant genomic island in ACSSuT type S. Typhimurium, required for the resistances to the above three mentioned antimicrobial drugs, are blaPEST, floR, and tet(G) (22). This illustrates that these isolates did not acquire their resistance genes from horizontal transfer from pentadrug–resistant ACSSuT type strains. The isolate Salmonella 4:i:- was resistant to ampicillin, chloramphenicol, sulfonamides, tetracycline, and sulfamethoxazole/trimethoprim (ACSSuT Sext-type), encoded by blaTEM (ampicillin), cat (chloramphenicol), sul2 (sulfonamides), tet(A) (tetracycline), and dfrA1 (trimethoprim). Also the resistance shown by this example had no relationship to the typical S. Typhimurium DT104 multidrug–resistant genomic island.

In conclusion, healthy house cats are generally safe with regard to excretion of Salmonella in the environment. Cats that are sick or are receiving medication resulting in immune deficiencies can potentially pose a threat to public health. Young children, the elderly, and immunocompromised persons are at risk because of their high sensitivity for the infection. All persons should follow good hygiene practices when keeping cats as pets.

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