

ORIGINAL ARTICLE

Extraintestinal Pathogenic *Escherichia coli*-Induced Acute Necrotizing Pneumonia in Cats

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Keywords:

E. coli; ExPEC; extraintestinal; pneumonia; cats; feline; zoonosis

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Received for publication July 18, 2007

Summary

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are pathogens involved in several disease conditions, ranging from urinary tract infection to meningitis in humans and animals. They comprise epidemiologically and phylogenetically distinct strains, affecting most species and involving any organ or anatomical site. Here, we report fatal cases of necrotizing pneumonia in cats. Over a 1-week period, 13 cats from an animal shelter in Stamford, Connecticut were presented for necropsy. All had a clinical history of acute respiratory disease. The gross and microscopic findings for all the cats were consistent. *Escherichia coli* was uniformly isolated from the lungs of all the tested cats. All the isolates were haemolytic, genetically related as determined by enterobacterial repetitive intergenic consensus PCR, and harboured genes encoding for cytotoxic necrotizing factor-1 and fimbriae and adhesions that are characteristic of ExPEC, implying a point source clonal outbreak. As cats are common household pets, this report raises concerns regarding zoonotic potential (in either direction) for these ExPEC strains.

Introduction

Escherichia coli is one of the most common bacteria found in the gastrointestinal tract of humans and animals (Bettelheim, 1997). Based on their genetic and clinical manifestations, strains of *E. coli* are classified into three major groups: commensal strains, intestinal pathogenic strains and extraintestinal pathogenic strains (Russo and Johnson, 2000). Commensal strains of *E. coli* originate in the gut and lack specialized virulence traits that are essential for pathogenicity (Johnson et al., 2001a). Intestinal pathogenic strains of *E. coli* cause gastroenteritis of humans and animals. These strains are subdivided into enteropathogenic, enterotoxigenic, enteroinvasive, enterohaemorrhagic, enteroaggregative and diffusely adherent *E. coli* (Russo and Johnson, 2000). *Escherichia coli* that produce disease of organ systems other than the gut are designated as extraintestinal pathogenic *E. coli* (ExPEC)

(Russo and Johnson, 2000). This group has been implicated in urinary tract infection (UTI), meningitis, septicemia and pneumonia in humans and animals (Russo and Johnson, 2000). *Escherichia coli* strains have been classified into serotypes on the basis of lipopolysaccharide (O), capsular (K) and flagellar (H) antigens (Johnson et al., 2001a). The ExPEC strains of dogs and cats have been found to carry virulence attributes such as cytotoxic necrotizing factor 1 (CNF-1), P-fimbriae and certain adhesins (Johnson et al., 2001b; Freitag et al., 2005). Because of shelter colony health implication, respiratory disease is a major concern. While ExPEC-induced bacterial pneumonia has been reported in dogs (Handt et al. 2003), it has not been reported in cats. We describe cases of fatal necrotizing pneumonia in 13 cats that were housed at the shelter prior to contracting the disease. We have characterized the *E. coli* strain causing pneumonia in these cats and found them to contain traits typical of ExPEC

isolated from extraintestinal infections of various host species (Johnson et al., 2001b; Handt et al., 2003; Breitschwerdt et al., 2005). As the same strain of *E. coli* was uniformly recovered from all the tested cats, this raises concern for the clonal spread of this pathogen among animals living in close proximity, as in shelters.

Materials and Methods

Animals

Thirty five cats, kittens and adults, male and female, had been housed in a single room at the Stamford, Connecticut Animal Shelter. The majority of cats were abandoned, either by neglectful owners or by natural catastrophes. Over a 4–6-week period starting in the month of June 2006, a large proportion of the cats (25) at this shelter developed dyspnoea, lethargy and anorexia. Animals died within 48 h of the onset of clinical signs or recovered with antibiotic therapy and supportive care. These 25 cats were not vaccinated and <2 years of age. All other cats (10) on the premise were vaccinated for rabies and feline panleukopenia. Prior to this episode, there had been no history of illness. Thirteen cats (nine kittens and four adults) were presented dead to the Connecticut Veterinary Medical Diagnostic Laboratory. The interval from death to postmortem examination varied from 1–8 h. Necropsy, histopathology and microbiology with bacterial isolations were performed.

Isolation of bacteria

Tissue samples, including lung from seven different cats (three adults and four kittens) were collected aseptically. Cotton-tipped swabs or loops were pierced into the fresh tissues, then streaked onto blood agar plates and incubated at 37°C under 10% CO₂ atmosphere and examined after 24 and 48 h. A tap water sample was also submitted and processed similarly. Bacterial identification was performed by the Biolog system (Hayward, CA, USA), and antimicrobial susceptibilities were determined by disc diffusion susceptibility assays (Clinical and Laboratory Standards Institute). Antimicrobials tested included amoxicillin, cephalexin, gentamicin, ceftiofur, enrofloxacin, clindamycin, streptomycin, tetracycline, chloramphenicol, amikacin, erythromycin, neomycin and sulfisoxazole (Sensi-Disc, Becton Dickinson, NJ, USA). *Escherichia coli* isolated from affected tissues were serotyped and subjected to virulence genotyping at the *E. coli* Reference Center at Pennsylvania State University.

Virus isolation

Lung lobes from eight cats (8/13) were also sent to the Cornell Animal Health Diagnostic Center for virus isolation.

Tissue supernatants were inoculated into cat kidney cortex epithelial cells and pneumocytes for the isolation of feline panleukopenia virus, feline calicivirus and feline herpesvirus. It should be noted that only five of these cats also had corresponding lung microbiological testing performed.

Serotyping

The presence of O antigens was tested for by using the standard method as described by Orskov et al. (1977). Serotyping was based on agglutination reaction. The H typing was performed by *fliC* PCR-(restriction fragment length polymorphism) RFLP technique as reported by Machado et al. (2000).

DNA isolation and virulence genotyping

DNA was extracted from overnight-grown cultures by resuspending in water (150 µl) and heating the suspension at 100°C for 10 min. The suspension was centrifuged at 12 000 g for 5 min, and the resulting supernatant containing the DNA was used as template for PCR. Initially genes encoding for virulence factors such as heat stable toxins (STa, STb) heat labile toxin (LT), shiga toxins (Stx -I and Stx-II), cytotoxic necrotizing factors (Cnf-1, Cnf-2) and intimin (Eae) were detected by PCR to determine if these strains of *E. coli* were potentially pathogenic. Since, the strains carried Cnf-1, further testing for the presence of genes for ExPEC strains such as those encoding for fimbriae (Sfa, Foc, Fim), P fimbriae adhesins (PapG allele I and III) and haemolysin were performed by PCR. These genes were selected on the basis of reports of common virulence genes associated with *E. coli* O4:H5 ExPEC strains in dogs (Johnson et al., 2001d). While the primers used for detecting genes encoding for LT, STa, STb, Stx-I, Stx-II Cnf1, Cnf-2 and Eae were conducted using the laboratory procedures described by DebRoy and Maddox (2001), other virulence genes were detected by using the primer sets shown in Table 1. Positive samples were identified based on the presence of bands of expected sizes compared with results of positive control strains J96 for *sfa/focG*, *papG* allele I, *fim* and *hlyD* (Johnson et al., 1997) and BUTI-3-1-4 for *papG* allele III that was obtained from Dr James R. Johnson. *Escherichia coli* K12 was used as negative control strain.

Enterobacterial repetitive intergenic consensus (ERIC) PCR was conducted to determine relatedness between the strains (Versalovic et al., 1991). Genotyping was performed using the ERIC2 fingerprinting assay, which uses one 22-bp primer designed to the conserved ERIC region (Johnson, 2000).

Table 1. Primers for virulence genes

Gene	Primers (5'-3')	Amplicon	Reference
<i>papG</i> Alleles I	F: TCG TGC TCA GGT CCG GAA TTT R: TGG CAT CCC CCA ACA TTA TCG	461 bp	(Johnson et al., 1997)
<i>papG</i> Allele III	F : GGC CTG CAA TGG ATT TAC CTG G R: CCA CCA AAT GAC CAT GCC AGA C	258 bp	(Johnson et al., 1997)
<i>fim</i> (Type 1 fimbriae)	F: GCC GGA TTA TGG GAA AGA R: AGT GAA CGG TCC CAC CAT	600 bp	(Roe et al., 2001)
<i>focG</i>	F: CAG CAC AGG CAG TGG ATA CGA R: GAA TGT CGC CTG CCC ATT GCT	364 bp	(Rodriguez-Siek et al., 2005)
<i>sfa</i>	F: CTC CGG AGA ACT GGG TGC ATC TTA C R: CGG AGG AGT AAT TAC AAA CCT GGC A	410 bp	(Rodriguez-Siek et al., 2005)

Results

The cats (nine kittens and four adults) that were presented for necropsy had similar gross lesions. A majority were in thin body condition with minimal fat reserves. The cats had a watery blood-tinged discharge at the nares. The tracheal mucosa was hyperaemic and contained variable amounts of pink frothy fluid. Approximately 30–40 ml of serosanguinous fluid was present in each thoracic cavity. The lungs were moist, oedematous and hyperaemic with red to dark red mottling (Fig. 1), and there were focal areas of consolidation. Some consolidated lung sank in 10% neutral buffered formalin. On cut sections, the regions of discolouration (mottled appearance) extended deep into the pulmonary parenchyma. Livers of several animals had an accentuated lobular pattern. One cat had a left ventricular cardiomyopathy and another had a biliary-cyst adenoma on the capsular surface of the

liver. Three kittens had segmental reddening of the intestines. The mesenteric lymph nodes of these kittens were moderately enlarged.

Microscopically the lungs of the various cats showed a progression of pneumonia, from peracute to subacute (Fig. 2). In all the sections, the alveolar spaces were variably filled with oedema, fibrin and sparse numbers of leucocytes. There were extensive areas of coagulative necrosis of the alveolar septae, with partial to complete parenchymal dissolution and replacement by necrotic cellular debris. Colonies of short rod-shaped Gram-negative bacteria were present free in the proteinaceous fluids. Bacteria were also seen within the cytoplasm of foamy macrophages; more frequently the bacteria were seen colonizing the necrotic alveolar septae. Randomly, there were foci of thrombosis of pulmonary capillaries, some containing bacterial aggregates. In some cats, the subacute lesion included a neutrophilic and a histiocytic infiltrate. Large

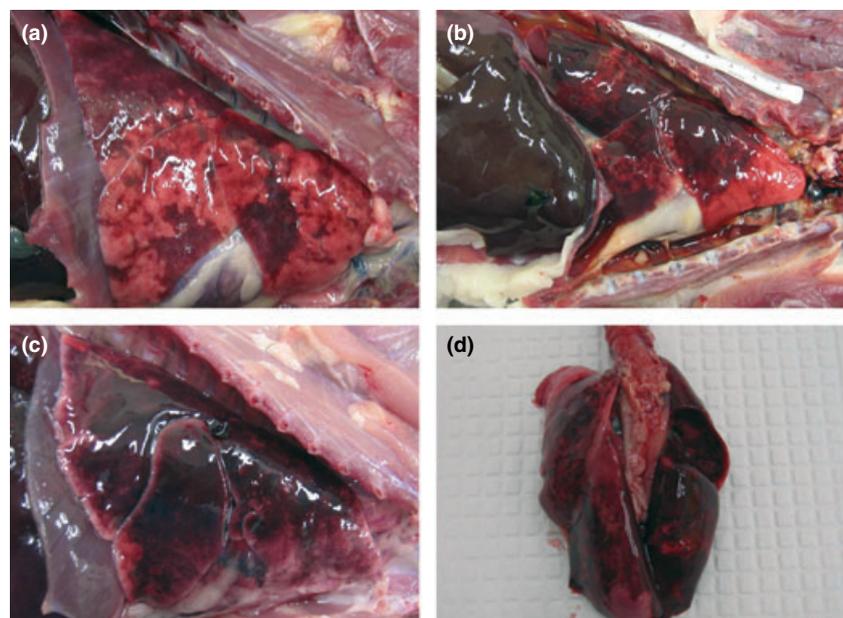


Fig. 1. Pulmonary lesions from four extraintestinal pathogenic *Escherichia coli* (ExPEC)-infected cats (a, b, c and d). Note the variable hyperaemia and red to dark-red mottling with areas of consolidation in multiple lobes.

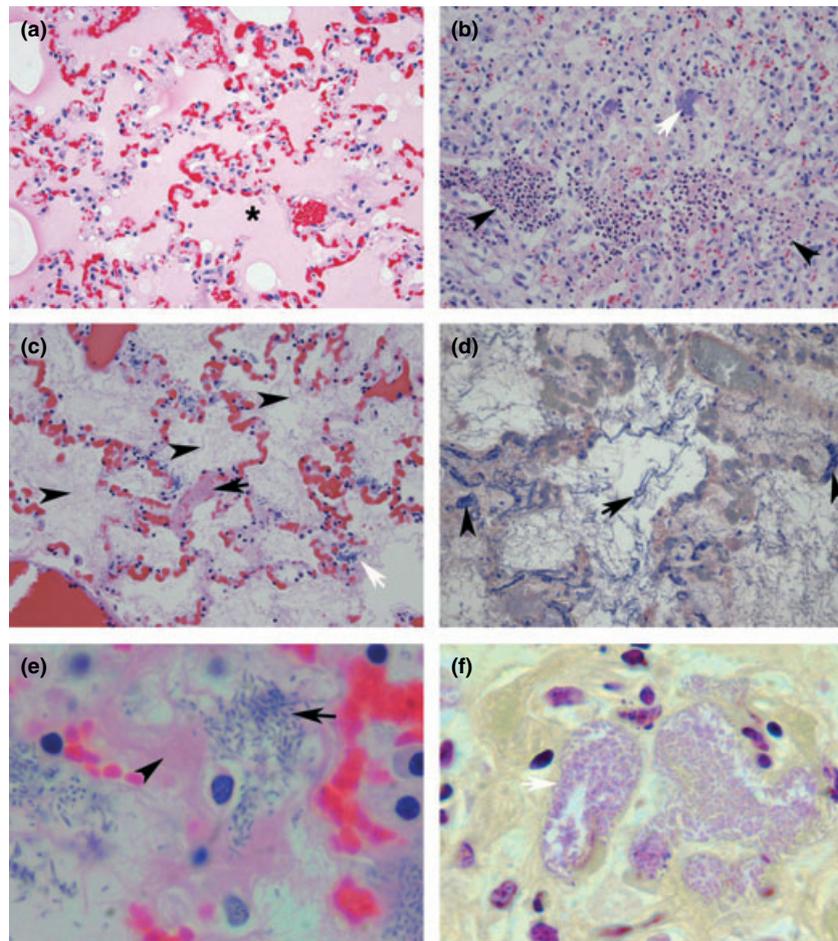


Fig. 2. Photomicrographs of lung sections showing a progression of lesions and histopathology. (a) Peracute change; alveolar spaces are filled with eosinophilic proteinaceous fluid (oedema)*; [Haematoxylin and eosin (H&E), 400 \times]. (b) Subacute change; the majority of alveoli are filled with proteinic fluid, fibrin and scattered mononuclear infiltrates (arrowheads). There is extensive alveolar septal necrosis with accumulation of degenerate leucocytes, capillary thrombosis and intralesional Gram-negative bacteria (white arrow); (H&E, 400 \times). (c) Acute change; intra-alveolar fibrin exudation (arrowhead) and capillary thrombosis (arrow) and occasional bacterial aggregates (white arrow); (H&E, 400 \times). (d) Acute change; phosphotungstic acid haematoxylin (PTAH) stain highlighting alveolar fibrin (arrow) and capillary thrombosis (arrowhead); (PTAH, 600 \times). (e) Alveolus; intra-alveolar rod-shaped bacterial aggregates (arrow) and fibrin (arrowhead); (H&E, 1000 \times). (f) Brown and Brenn tissue gram stain, intra-alveolar Gram-negative bacteria (white arrow); (1000 \times).

bacterial colonies were admixed with fibrin and oedema and were surrounded by necrotic and degenerate neutrophils.

Bacteriological testing of the lungs was performed on seven of the submitted 13 cats, of which five yielded a pure culture of haemolytic *E. coli*. In one cat, a mixed colony of *E. coli* and *Bordetella bronchiseptica* were recovered. In one cat there was no growth. Apart from the lungs, haemolytic *E. coli* was also recovered from visceral organs (intestine, gall bladder, liver and faeces) in two cats. All *E. coli* isolates recovered belonged to the O4:H5 serotype. The *E. coli* strains were characterized for the presence of virulence attributes. All isolates carried genes encoding for CNF-1, but did not carry genes for shiga toxins or heat stable or

heat labile toxins. Isolates were typed for diarrhoea-associated virulence factors initially to screen for the pathogenicity of the bacterial strain. However, the isolates carried allele I and allele III *papG* adhesin, S-fimbriae (*sfa*) and D-mannose specific adhesin, type 1 fimbriae (*fimH*) and haemolysin (*hlyD*) (Table 2). To determine if all the isolates were related, ERIC-PCR was performed. All the strains had identical ERIC-PCR results reflecting that the same strain infected all the cats (point source outbreak).

Tap water tested negative for microbes

Antibiotic susceptibility testing demonstrated that the *E. coli* isolates were resistant to several antimicrobials

Table 2. Serotype and virulence attributes of the *Escherichia coli* isolates from cats with necrotizing pneumonia

Cat no.	1	2	3	4	5	6	7
O-type	4	4	4	4	4	4	4
H-type	5	5	5	5	5	5	5
LT	-	-	-	-	-	-	-
STa	-	-	-	-	-	-	-
STb	-	-	-	-	-	-	-
Stx1	-	-	-	-	-	-	-
Stx2	-	-	-	-	-	-	-
Cnf-1	+	+	+	+	+	+	+
Cnf-2	-	-	-	-	-	-	-
Eae	-	-	-	-	-	-	-
Foc	-	-	-	-	-	-	-
Sfa	+	+	+	+	+	+	+
HlyD	+	+	+	+	+	+	+
Fim	+	+	+	+	+	+	+
PapG allele I	+	+	+	+	+	+	+
papG allele III	+	+	+	+	+	+	+

(amoxicillin, clindamycin, streptomycin, tetracycline, chloramphenicol, amikacin, erythromycin, neomycin and sulfisoxazole). However, they were susceptible to cephalexin, gentamicin, ceftiofur and enrofloxacin, which is consistent with previous reports (Handt et al., 2003).

In four out of eight cats, viruses were not detected; in three cats feline panleukopenia virus was detected and one had feline herpesvirus.

Discussion

This study documents *E. coli*-induced acute necrotizing pneumonia in cats. *Escherichia coli* isolates uniformly recovered from the tested cats were characterized for their serotypes and virulence attributes. These isolates were haemolytic and belonged to O4:H5 serotype. All the isolates harboured the genes encoding for the virulence factors S-fimbriae (*sfa*) adhesin (*papG* allele I and III), type I fimbriae (*fim*) haemolysin and CNF-1 that are associated with ExPEC strains. These ExPEC strains have been implicated in cases of necrohaemorrhagic pneumonia in dogs (Handt et al., 2003). They belonged to O4 and O6 serogroups and harboured the virulence genes alpha haemolysin, *cnf-1* and *papG* allele III (Handt et al., 2003). These pathogenic strains are known to possess specialized virulence traits, which are absent in commensal *E. coli*. The ExPEC may possess a full repertoire of virulence factors that are involved in pathogenicity, such as type I-fimbriae (*fim*), S-fimbriae (*sfa*), alpha hemolysin (*hlyA*) and CNF 1, lipopolysaccharides, proteases, invasins, etc. Some of the virulence factors of ExPEC are encoded on certain 'pathogenicity associated islands' (PAIs) and contribute to the disease-causing nature of the

strains (Blum et al., 1994; Groisman and Ochman, 1996). The production of disease requires bacterial adherence to host epithelial cells by their fimbriae or pili, which promote colonization and stimulation of a host inflammatory response and the production of cytotoxic factors (Johnson, 1991; Donnenberg and Welch, 1996). The P-fimbriae enable ExPEC to adhere to the host by virtue of their adhesin encoded by *papG* that expresses variably in different alleles, of which allele III is often associated with ExPEC, resulting in cystitis in women and children (Johnson et al., 2001c). This particular allele is reported to be identical in human and canine isolates (Johnson et al., 2000, 2001c).

Upon bacterial adhesion to the host mucosal surface, the O4 antigen, the alpha haemolysin, and CNF-1 reduce the influx of neutrophils into the lungs and thus evade their attack (Russo et al., 2003). This and attenuation of host proinflammatory responses by the O4 antigen, has been demonstrated in a rat model of pneumonia (Russo et al., 2000). In the same model, alpha haemolysin and CNF-1 were found to enhance neutrophil apoptosis or necrosis (Russo et al., 2005). This might contribute to the reduced number of neutrophils observed in the lungs of the diseased cats that were examined. In addition, CNF-1 evokes oedema, necrosis and other inflammatory changes in the affected tissues (Elliott et al., 1998), which is consistent with our findings of marked alveolar oedema and coagulative necrosis of the alveolar wall and the pulmonary vessels. The P-fimbriae, *hlyA* and *cnf-1* are all part of the same PAI's (Breitschwerdt et al., 2005). Cytotoxic necrotizing factor 1 and haemolysin are frequently isolated from human extraintestinal infections such as UTIs and septicaemia (Blanco et al., 1996). De Rycke et al. (1999) reported that alpha haemolysin and *cnf-1* are rarely found in *E. coli* isolated from the faeces of healthy individuals. However, in *E. coli* strains isolated from patients suffering from UTI, prostatitis and other extraintestinal infections the frequency of the presence of these genes reaches 40% to 50%. The ExPEC strains belonging to O4:H5 have been implicated in UTI and exhibit a J96-like clonal group encoding alleles I and III of *papG* (Johnson et al., 1997).

As all the cats were housed in one room at an animal shelter facility and the onset of disease was rapid, we speculate that there must have been a common source of infection. Because all the *E. coli* strains isolated from the seven cats had the same serotype and virulence profiles and were found to be identical as determined by ERIC PCR, we speculate that they may have contracted the strain from faecal flora, cats with subclinical infection, or from contaminated water, feed or infected personnel. Beutin (1999) reported that ExPEC strains in dogs and cats often possess group antigens O4 and O6 and are frequently isolated from normal faecal flora of these animals

(Beutin, 1999). He suggested that these animals may serve as major reservoirs and a source of infection for humans. The frequent isolation of O4 and O6 serogroups from faeces of dogs and cats suggest that the cats' own faecal flora coupled with the compromised immune status of the cats and overcrowding in shelter may have been responsible for the disease outbreak. We tested the water in the shelter for faecal contamination, but *E. coli* were not recovered. We were unable to test the feed and the personnel to ascertain if the bacterial strain which caused the disease outbreak was omnipresent at the shelter or not. Four out of the seven sampled cats had concomitant viral infections. Three harboured feline panleukopenia virus and one had feline herpesvirus. In these cats, the viral infection may have led to immunosuppression, bacteraemia and death. It should be noted however, most of the cats examined did not appear to have a predisposing condition, suggesting that this strain might be a primary pathogen in this context.

Many of the characteristics of ExPEC recovered from cats in this study are similar to those found in dogs (Handt et al., 2003). Further genotyping of these isolates would likely determine the relatedness among these strains. In dogs, like humans, ExPEC are commonly isolated from cases of UTI, pyometra, otitis, mastitis, prostatitis, and pneumonia (Oluoch et al., 2001). ExPEC is the second most common bacterium isolated from dogs with bacterial pneumonia (Hawkins et al., 1989). Several reports have shown that ExPEC isolates from humans and dogs are indistinguishable (Johnson et al., 2001b,d). We now recognize that these ExPEC can cause disease in cats too, and can be clonally spread when housed in close proximity.

Other authors have proposed that domestic pets serve as reservoirs for ExPEC (Westerlund et al., 1987; Low et al., 1988). This assumption is based primarily on the similarities between canine and human isolates with respect to their virulence profiles, O antigen and evolutionary lineage (Westerlund et al., 1987; Yuri et al., 1999). Based on the present findings and work by Yuri et al. (1998), felines must also be considered to harbour these agents. The cats that are domestic pets that come into close contact with humans raises the possibility of zoonotic transmission of these pathogenic strains in either direction (Johnson and Clabots, 2006). Considering the spread of the disease among cats in the shelter, we should try to understand to what extent cats become carriers and have the potential to bring disease into the home.

Public Health Forum

1. This is a newly reported disease.
2. The disease is associated with acute onset and high mortality in cats.

3. The disease has significant zoonotic potential as the agent is reported in multiple species including humans.

Acknowledgements

We thank Dr Priyanka Sura, Dr Steven O. Goldie, Lindsey Marrotte, Ione Jackman, Denise Woodward, Alex Bothell and Laurie Hollywood-Chief Animal Control Officer at Stamford, Connecticut, for all the timely assistance provided.

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