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ORIGINAL RESEARCH ARTICLE

Hygiene quality and presence of ESBL-producing *Escherichia coli* in raw food diets for dogs

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Background: Raw food diets are popular among some dog owners, even though there are concerns regarding the infectious disease risk and public health implications. Hence, the two aims of this study were to investigate the hygiene quality of raw food diets for dogs in the Swedish market and if *Escherichia coli* with transferable resistance to extended spectrum cephalosporins (ESC) was present in such products.
Methods: Samples of raw food diets were suspended and further diluted in 0.9% saline. Appropriate dilutions were 1) cultured on Petrifilm™SEC to quantify the amount of *E. coli* in the samples and 2) mixed with cefotaxime to a final concentration of 1 mg/L and cultured on Petrifilm™SEC to quantify the amount of ESC-resistant *E. coli* in the samples. Furthermore, undiluted suspensions were mixed 1:1 with double strength MacConkey broth with cefotaxime, enriched overnight and finally cultured on MacConkey agar with cefotaxime (1 mg/L). Suspected ESC-resistant *E. coli* were screened by PCR for genes encoding extended spectrum beta lactamases and plasmid-mediated AmpC and their susceptibility to a panel of antimicrobials was performed by broth microdilution using VetMIC GN-mo.
Results: *Escherichia coli* was isolated from all samples ($n = 39$) and ESC-resistant *E. coli* was isolated from nine samples (23%). All ESC-resistant *E. coli* were PCR-positive for the $bla_{\text{CMY-2}}$ group and only one of them was also resistant to a non-beta-lactam antibiotic.
Conclusion: The results of this study indicate that raw food diets could be a source of ESC-resistant *E. coli* to dogs and highlight the need for maintaining good hygiene when handling these products to prevent infection.

Keywords: *BARF*; *raw food diet*; *ESBL*; *AmpC*; E. coli

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Raw food diets are popular among some dog owners, despite concerns regarding infectious disease risk and public health (1, 2). The focus has mainly been regarding the presence of *Salmonella* spp. as a potential pathogen (3–7). Another concern would be the presence of antibiotic-resistant bacteria, for example, Enterobacteriaceae with transferable resistance to extended spectrum cephalosporins (ESC) due to extended spectrum beta lactamases (ESBL) or plasmid-mediated AmpC (pAmpC). Bacteria with such resistance are a problem in human medicine and have been identified in farm animals as well as in raw food diet products (3, 8). In Sweden and Norway, ESBL and pAmpC are only occasionally isolated from farm animals other than broilers (9, 10). In Finland, the occurrence of ESBL and pAmpC in poultry is lower than in many other countries (11).

As by-products from broiler slaughter are sometimes used to manufacture raw food diet products, we identified such diets as a potential source of ESBL and pAmpC to dogs (12). It could also act as a source of antibiotic-resistant microorganisms for people caring for the dogs (13). The aim of this study was to investigate if *Escherichia coli* with transferable resistance to ESC was present in raw food diets for dogs in the Swedish market. In addition the hygiene quality of the products was assessed by quantification of *E. coli* present in the raw food diets.

Materials and methods

Frozen samples of raw food diet products containing poultry were purchased in shops in and around Uppsala, Sweden, and from one Swedish internet shop, and stored at -20°C until analysed. Samples were thawed at 3°C; 25 mL of saline (0.9%) was added to 25 g of feed and treated for 30 sec at 230 rpm in a Stomacher (Stomacher 400 circulator, Seward, UK) before ≥ 7 mL of liquid was removed. Dilutions of 1:10 and 1:1,000 were prepared in 0.9% saline for quantitative isolation of *E. coli* and 1 mL of each dilution was cultured on Petrifilm™SEC (3M Health

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Care, St Paul, USA) and incubated at 37°C overnight. For quantitative isolation of ESC-resistant *E. coli*, a 1 mL aliquot of the undiluted suspension was mixed with 10 µL of cefotaxime solution (0.1 mg/mL; Sigma Aldrich, China) to create a final concentration of 1 mg/L before the mixture was cultured on Petrifilm™SEC as above. For qualitative isolation of ESC-resistant *E. coli*, 5 mL of double strength MacConkey broth (Lab M, Lancashire, UK) with cefotaxime (2 mg/L) was added to a 5 mL aliquot and incubated at 37°C overnight before 0.1 mL was streaked on MacConkey agar (Difco, Hampshire, UK) with cefotaxime (1 mg/L) and incubated at 37°C overnight.

Suspected *E. coli* colonies were confirmed by subculture on horse blood agar (Oxoid, Basingstoke, UK) and an indole test conducted. Furthermore, suspected ESC-resistant *E. coli* were screened by PCR for genes encoding ESBL and pAmpC, that is, the gene groups CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25/26, MOX, CIT, DHA, ACC, EBC, FOX, OXA-1, SHV, and TEM (14). The number of *E. coli* or ESC-resistant *E. coli* on each Petrifilm™SEC was recorded.

Susceptibility testing of all ESC-resistant *E. coli* to a panel of antimicrobials was performed by broth microdilution using VetMIC GN-mo (SVA, Uppsala, Sweden) according to standards of the CLSI (15). The *E. coli* reference strains ATCC 25922 were used for quality control and the results were interpreted according to epidemiological cut-off values issued by EUCAST (www.eucast.org, retrieved 2015-09-17; Table 1).

Results

Altogether, 39 samples of eight different raw dog food brands (1–11 samples/brand) were analysed. Depending on the brand, the products contained by-products from animals slaughtered in Finland ($n = 10$), Norway ($n = 11$), or Sweden ($n = 18$). Of the 39 samples, 22 contained only by-products from poultry, whereas 17 contained by-products from several animal species including poultry.

Escherichia coli was isolated from all 39 samples. In 34 (87%) of the samples, the amount of *E. coli* was higher than 5×10^1 CFU/g, in 19 (49%) the amount was higher than 5×10^2 CFU/g, and in 2 (5%) the amount was higher than 5×10^4 CFU/g.

ESC-resistant *E. coli* was isolated from nine samples (23%) and all isolates were PCR-positive for the $bla_{\text{CMY-2}}$ group. The products with ESC-resistant *E. coli* originated from Norway ($n = 3$, 27%) or Sweden ($n = 6$, 33%) and contained either only poultry by-products ($n = 7$, 32%) or by-products from several animal species ($n = 2$, 12%). The number of colony forming units (CFU) of ESC-resistant *E. coli*/gram could be calculated in five of the nine samples, all of which were ≤ 10 CFU/g. All of these samples were products that contained by-products exclusively from poultry.

Only one of the ESC-resistant *E. coli* was also resistant to a non-beta-lactam antibiotic (Table 1). That isolate was also resistant to kanamycin.

Discussion

It was not unexpected that ESC-resistant *E. coli* could be isolated from raw food diet products containing poultry. ESC-resistant *E. coli* bacteria is commonly isolated from poultry in Europe and ESC-resistant Enterobacteriaceae in raw food diet products has previously been described (3, 8). Furthermore, studies have demonstrated an increased risk of shedding of ESC-resistant Enterobacteriaceae

Table 1. Antibiogram of the nine *Escherichia coli* isolates with transferable resistance to extended spectrum cephalosporins isolated from raw food diets for dogs

	Resistance pattern													
Isolate	Am	Ctx	Caz	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Trim	Cm	Km
1	**64**	**>2**	**8**	0.06	4	1	16	≤1	8	≤0.5	16	0.25	4	≤8
2	**128**	**>2**	**8**	0.06	2	1	8	≤1	≤4	≤0.5	32	0.25	≤2	≤8
3	**128**	**>2**	**8**	0.06	2	0.5	8	≤1	≤4	≤0.5	16	0.5	4	≤8
4	**64**	**>2**	**4**	0.06	2	1	8	≤1	≤4	1	≤8	0.25	4	≤8
5	**64**	**>2**	**8**	0.06	4	1	8	≤1	≤4	2	16	0.5	4	≤8
6	**64**	**>2**	**16**	0.06	2	1	8	≤1	≤4	2	16	0.5	4	≤8
7	**>128**	**>2**	**>16**	0.06	4	1	8	≤1	≤4	1	≤8	0.5	4	≤8
8	**128**	**>2**	**8**	0.06	2	1	8	2	8	1	16	0.5	4	≤8
9	**64**	**>2**	**4**	0.06	4	0.5	8	≤1	8	1	16	0.25	4	**16**

Bold and shaded figures indicate MIC above the epidemiological cut-off values issued by EUCAST (www.eucast.org, retrieved 2015-09-17).

Antimicrobials included and cut-off values (mg/L) used are ampicillin (Am, >8), cefotaxime (Ctx, >0.25), ceftazidime (Caz, >0.5), ciprofloxacin (Ci, >0.06), nalidixic acid (Nal, >16), gentamicin (Gm, >2), streptomycin (Sm, >16), tetracyclin (Tc, >8), florfenicol (Ff, >16), coliston (Cs, >2), sulfamethoxazole (Su, >64), trimetoprim (Trim, >2), chloramphenicol (Cm, >16), kanamycin (Km, >8).

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among dogs fed raw food diets or raw meat in Canada and the United Kingdom (16, 17). Together, this indicates that raw food diets can also be a source of ESC-resistant Enterobacteriaceae for dogs in Sweden. The risk probably relates to feeding non-heat-treated animal products, regardless of whether it is in the form of commercial diets or not, as indicated by the previously reported increased risk of shedding ESC-resistant *E. coli* in dogs fed raw meat (17). In addition, the occurrence of ESC-resistant *E. coli* in the products originating from Norway and Sweden in this study is comparable to their prevalence in samples of broiler meat in these two countries (9, 10).

Poultry from Finland, Sweden, or Norway was an ingredient in all investigated products, and $bla_{\text{CMY-2}}$ is the dominant gene among ESC-resistant Enterobacteriaceae from broilers in those countries (9–11). Therefore, it is likely that most of the isolated ESC-resistant *E. coli* would have a gene in the $bla_{\text{CMY-2}}$ group. Although the isolates were not sequenced in this study, one could speculate that it will be the $bla_{\text{CMY-2}}$ gene that confers resistance as all ESC-resistant *E. coli* isolates from Swedish poultry identified to date has carried $bla_{\text{CMY-2}}$ (9). The resistance pattern of the isolates is also similar to those which have been described for isolates from poultry in Norway and Sweden (9, 10).

The occurrence of *E. coli* in all products is comparable to or higher than in previous studies (5, 18). The isolation frequency of *E. coli* is comparable to that of broiler meat 95%), but considerably higher than that of pork (20%) reported in the Swedish surveillance program Svarm (9). Although *E. coli* could be isolated from all samples, it should be noted that only two of the products contained more *E. coli* than the maximum limit of coliform bacteria (5×10^4 CFU/g) recommended by the Swedish Board of Agriculture for animal feed materials of animal origin (SJVFS 2011:40). Nineteen of the products contained $>5 \times 10^2$ CFU/g *E. coli* and would therefore have been considered of unsatisfactory hygiene quality had they been minced or mechanically separated meat intended for human consumption (EC 2073/2005). According to this regulation, a batch of minced or mechanically separated meat is also classified as having unsatisfactory hygienic quality if more than two of five samples contain between 5×10^1 and 5×10^2 CFU *E. coli* per gram. In this study, 15 of the 39 products tested contained that amount of *E. coli*. However, as only one sample from each batch was analysed, it is not possible to know if they had been classified as having unsatisfactory hygiene quality or not unless additional samples were analysed. The high incidence of *E. coli* in these raw food diets highlights the need for maintaining good hygiene when handling these products to prevent infection.

Raw food diets for dogs are not intended for human consumption. However, it is likely that they are handled in kitchens where food for human consumption is also handled. Thereby there is a possibility of cross-contamination and a subsequent exposure of humans to bacteria from the raw food diet products. The major concern would be transient colonisation with ESC-resistant *E. coli* which may allow gene transfer to *E. coli* adapted to the human gut, resulting in prolonged colonisation with resistant coliforms as a consequence (19). Thus, raw food diets could constitute a source of ESC-resistant *E. coli* not only for dogs but also for humans. However, at this time, there are no indications of spread of ESC-resistant *E. coli* from broiler meat to humans in Sweden (20). Hence, the risk of spread from raw food diets to humans is probably low, but this relies on good hygiene being maintained when handling these products.

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