Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs

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Feeding raw meat-based diets (RMBDs) to companion animals has become increasingly popular. Since these diets may be contaminated with bacteria and parasites, they may pose a risk to both animal and human health. The purpose of this study was to test for the presence of zoonotic bacterial and parasitic pathogens in Dutch commercial RMBDs. We analysed 35 commercial frozen RMBDs from eight different brands. *Escherichia coli* serotype O157:H7 was isolated from eight products (23 per cent) and extended-spectrum beta-lactamases-producing *E coli* was found in 28 products (80 per cent). *Listeria monocytogenes* was present in 19 products (54 per cent), other *Listeria* species in 15 products (43 per cent) and *Salmonella* species in seven products (20 per cent). Concerning parasites, four products (11 per cent) contained *Sarcocystis cruzi* and another four (11 per cent) *S tenella*. In two products (6 per cent) *Toxoplasma gondii* was found. The results of this study demonstrate the presence of potential zoonotic pathogens in frozen RMBDs that may be a possible source of bacterial infections in pet animals and if transmitted pose a risk for human beings. If non-frozen meat is fed, parasitic infections are also possible. Pet owners should therefore be informed about the risks associated with feeding their animals RMBDs.

In recent years, it has become increasingly popular for dog and cat owners to feed their pets raw meat-based diets (RMBDs), instead of the more conventional dry or canned pet foods. It has been estimated that 51 per cent of dog owners in the Netherlands feed their dogs entirely or partially with raw meat-based products.[1] Given that 36 per cent of households in the Netherlands own either a dog or a cat, it is possible that RMBDs are used in more than one million Dutch households.[2] RMBDs contain raw animal products and/or by-products and can roughly be divided into three different groups: raw dried dog and cat treats (eg, pig ears), home-prepared RMBDs and commercial RMBDs. Ingredients used for home-prepared RMBDs are purchased from butcheries and pet shops, and may include meat, meaty bones and organ meats, but also vegetables, eggs, grains, yeast, milk and yoghurt. The BARF (Bones And Raw Food or Biologically Appropriate Raw Food) diet is probably the most popular example of a home-prepared RMBD. For the commercial RMBDs a variety of products and brands are available in most pet shops and supermarkets.[3–5]

Owners have several motivations for feeding RMBDs to their pets. However, the claimed health benefits attributed to the feeding of RMBDs are mostly anecdotal, and no studies have produced results in support of these statements. On the contrary, several publications have reported risks associated with RMBD feeding, including the development of clinical conditions such as hyperthyroidism, and injuries such as gastrointestinal tract perforation or teeth fractures.[3 6–9]

In nutritional terms, these diets are often deficient in several nutrients and may therefore lead to serious health problems, especially in young animals that are growing.[10] However, the topic most discussed at present is the risk to public or animal health because of possible contamination of RMBDs with—zoonotic—bacteria and parasites. The spread of such bacteria and parasites in the environment, either directly from contaminated RMBDs or by animals infected through consumption of RMBDs, represents a risk for the human population.[11 12] Most of the information published on this topic is about *Salmonella* species in RMBDs, with *Salmonella*

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prevalence in RMBDs thought to range from 5 per cent to even 80 per cent.[13–20]

Other bacteria that have a possible impact on human health and that have been isolated from RMBDs include enterohaemorrhagic *Escherichia coli* (including the serotype O157:H7, which may cause renal failure in human beings), *Listeria monocytogenes*[18] and *Brucella suis*.[21] Another concern in companion animals is the presence of antibiotic-resistant bacteria, especially Enterobacteriaceae, and the associated risk for public health. The presence of antibiotic-resistant bacteria (including extended-spectrum beta-lactamases (ESBL)-producing *E coli*) has also been demonstrated in RMBDs.[22–24] *Campylobacter* spp, which are present in high numbers of fresh poultry meat, were not found in studies that evaluated commercial raw pet foods,[12 19 20] probably because of freezing of the meat which causes a significant drop in mean *Campylobacter jejuni* colony-forming unit (cfu) counts.[25]

Parasites that may be present in RMBDs are protozoa of apicomplexan genera, such as *Toxoplasma*, *Sarcocystis*, *Cryptosporidium*, *Isospora* and *Hammondia*; and tapeworm stages of *Taenidae* such as the intermediate stage of *Taenia saginata* and *Echinococcus granulosus* in cattle and the trichurid nematode *Trichinella*. *Toxoplasma gondii* tissue cysts may occur in meat and tissue products of the main meat-producing animals such as cattle, sheep, pigs and poultry. Although infection in dogs can lead to the development of tissue cysts, this does not pose a risk for other animals. In contrast, infection in cats can result in oocyst shedding, predominantly after primary infections.

For many *Sarcocystis* species, dogs and other carnivores serve as the definitive host, while livestock might serve as intermediate hosts, carrying cysts in their tissues. Human beings can also become infected with species of *Sarcocystis*, although this seldom leads to clinical disease. In fact, human beings are the definitive host of *S bovihominis* and *S suihominis*, for which cattle and pigs serve as intermediate hosts. Cattle are also intermediate hosts of *Sarcocystis cruzi*, *S hirsuta* and *S sinensis*, but the definitive hosts of this last parasite are not yet known.[26] Sheep are intermediate hosts of *S tenella*.[27] *S tenella*, *S cruzi*, *S hirsuta* and *S sinensis* are examples of non-zoonotic *Sarcocystis* species for which also cats and dogs can serve as definite hosts.

Since most studies on bacterial contamination of RMBDs have been conducted in Canada and the USA, such information regarding products in European countries is limited. The purpose of this study was to determine the presence of four zoonotic bacteria and two parasite species in commercial RMBDs in the Netherlands. The general microbiological quality (total bacterial count) and hygiene quality (*E coli*) of the diets were evaluated as well.

Materials and methods

Sample collection and processing

To get an indication of the most commonly fed products, an overview was made of all commercial RMBD brands available in the Netherlands (203 products from 21 brands) and of the number of retailers selling these brands. Brands sold by more than 20 retailers (n=8) were assumed to be more commonly bought by pet owners nationwide, and thus more frequently fed to cats and dogs. Thirty-five frozen (−18°C) raw meat diets were purchased, representing eight different brands from 14 retailers in and around the city of Utrecht, and stored according to label recommendations until analysis. Separate samples of the products were taken for bacterial and parasitical analyses.

Bacterial methods

Before analysis, frozen products—all packaged in vacuum-sealed plastic—were thawed under running tap water at room temperature. All products were processed while still cold (0°C–4°C) to prevent substantial bacterial growth. Four samples, each weighing 25 g, were obtained from each product using a sterile spoon and transferred into a sterile blender bag (BagPage, Interscience, Saint Nom la Breteche, France). After the addition of 225 ml of a solution specific for the subsequent microbiological method, the samples were homogenised manually for 90 seconds. All products were analysed for the presence of the following four bacterial species (one sample per species): *E coli* O157:H7 (enterohemorrhagic *Escherichia coli* (EHEC)), ESBL-producing *E coli*, *L monocytogenes* and *Salmonella*. The quantity of aerobic bacteria and *E coli* in the products was also measured to get an indication of the microbiological quality.

Quantitative bacteriology

For quantitative bacteriology, Petrifilm Aerobic Count Plates (3M Microbiology, St Paul, MN, USA) were used to culture aerobic bacteria, and Petrifilm *E coli*/Coliform Count Plates (3M Microbiology) were used to culture *E coli*. The inoculated petrifilms were incubated for 48±3 hours at 35°C±1°C according to the Association of Official Agricultural Chemists (AOAC) Official Methods 990.12 and 991.14, respectively.

E coli O157:H7

The samples (25 g of each sample) were analysed for the presence of *E coli* O157:H7 according to the International Organization for Standardization (ISO) 16654:2001 protocol entitled 'Microbiology of food and animal feeding stuffs – horizontal method for the detection of *E coli* O157' (ISO, 2001).

ESBL-producing E coli

For the quantitation and the detection of presence of ESBL-producing *E coli*, samples were homogenised in buffered peptone water (Oxoid Ltd, Basingstoke,

Hampshire, UK) containing 1 mg/l cefotaxime (CTX) (Sigma-Aldrich, St. Louis, MO, USA). For quantitative analysis, a further decimal dilution series was prepared in 0.1 per cent peptone salt solution (Maximum Recovery Diluent, Oxoid). A volume of 0.1 ml of the dilutions 10^{-1}, 10^{-2} and 10^{-3} was plated onto MacConkey's agar no. 3 (Oxoid) with 1 mg/l CTX. The plates were incubated 18±3 hours at 37°C±1°C. The number of presumptive purple/red-coloured ESBL-*E coli* colonies was counted.

The presence of the bacterium in the homogenised sample after 18±3 hours at 37°C±1°C incubation was assessed by streaking sample on MacConkey's agar no. 3 plates with 1 g/l CTX. The plates were incubated 18±3 hours at 37°C±1°C. Presumptive purple/red-coloured *E coli* colonies were further identified.

For both methods, the *E coli* identity of probable ESBL-*E coli* colonies was confirmed by transferring a maximum of three colonies per sample to Tryptone Soya Agar plates (Oxoid). After an incubation for 24±3 hours at 37°C±1°C, the *E coli* identity was confirmed by a positive indole and oxidase test.

L monocytogenes

The samples (25 g of each sample) were analysed for the presence of *L monocytogenes* according to ISO 11290-1:1996, with two modifications: UVM Modified Listeria Enrichment Broth (BD Difco, Franklin Lakes, NJ, USA) was used instead of Half Fraser Broth (Oxoid) for primary enrichment; and Compass Listeria Agar (Biokar Diagnostics, Allonne, France) was used instead of Agar Listeria Ottavani and Agosti (ALOA) Oxoid Chromogenic Listeria Agar (OCLA) (ISO) (Oxoid) for selective isolation.

Salmonella species

The samples (25 g of each sample) were analysed for the presence of *Salmonella* species according to ISO 6579:2002. The presumptive *Salmonella* colonies were serologically confirmed for the presence of O-antigens.

Parasitological methods

Only *T gondii* and *Sarcocystis* species were studied, since these protozoa were considered relevant in RMBDs and potentially present in the meat. *Trichinella* and *Taenia* species were not included because of current mandatory meat inspection (EC Regulation No 854/2004) and testing after slaughter of susceptible meat-producing animals (EC Regulation No 2015/1375) and subsequent removal of *Trichinella*-positive animals.

DNA was extracted in duplicate from the 25-mg samples of the 35 BARF products using DNeasy Blood & Tissue Kits (Qiagen). Samples were tested for the presence of *T gondii* DNA using quantitative polymerase chain reaction (qPCR) on the 529 bp repeat element as described by Opsteegh and others.[28] For *Sarcocystis*, conventional PCR was performed using Qiagen HotStarTaq DNA Polymerase and the primers SarcoFext (5'-GGTGATTCATAGTAACCGAACG-3') and SarcoRext (5'-GATTTCTCATAAGGTGCAGGAG-3')[26] at an annealing temperature of 59°C. PCR products of the correct size (893 bp) were purified using ExoSAP-IT according to the manufacturer's instructions and sent for sequencing at BaseClear BV (Leiden, The Netherlands). *Sarcocystis* species were identified by Basic Local Alignment Search Tool (BLAST) analysis of DNA sequences in GenBank.

Descriptive statistics

Mean and 95 per cent confidence interval (CI) for proportions were calculated using IBM SPSS Statistics V.23.0.

Results

Sample collection

Thirty-five commercial raw meat-based diet products from eight different brands (one to nine products per brand) were analysed. The ingredients on the label revealed that 15 (43 per cent) products contained only meat and animal by-products, of which five contained meat and by-products of a single animal species (ie, chicken and rabbit). The remaining 20 (57 per cent) products contained other ingredients (eg, rice or vegetables) in addition to meat and animal by-products. The meat and animal by-product sources in the products analysed were chicken (n=20), beef (n=18), lamb (n=6), duck (n=2), rabbit (n=2), horse (n=1) and turkey (n=1). All RMBDs studied were frozen before testing. Warnings and handling instructions on packages were lacking from all but one brand in our study.

Bacterial analysis

The quantitative scores for aerobic bacteria ranged from 7.9×10^{2} to 5.0×10^{6} cfu/g (mean value=2.3×10^{5}, sd=8.6×10^{5}). In three products, the score was lower than 1.0×10^{3} cfu/g, and in two products it exceeded 1.0×10^{6} cfu/g.

E coli was isolated from 30 (86 per cent) products, and quantitative scores ranged from less than 2.6 to 1.1×10^{4} cfu/g (mean value=8.9×10^{2}, sd=2.2×10^{3}). In seven products, the score exceeded 5.0×10^{2} cfu/g. The mean scores for *E coli* varied between the different brands, and for two brands this bacterial species was not isolated from any of the products (Fig 1).

In eight products (23 per cent; 95 per cent CI: 9 to 37 per cent) from three different brands *E coli* O157:H7 was found (Table 1). Meat and animal by-product sources included in these products were chicken (n=5), beef (n=4), lamb (n=1) and turkey (n=1).

ESBL-producing *E coli* was isolated from 28 (80 per cent; 95 per cent CI: 67 to 93 per cent) products from seven different brands (Table 1). The meat and animal by-product sources included in these products were chicken (n=17), beef (n=14), lamb (n=6), rabbit (n=1) and turkey (n=1). The quantity of ESBL-producing *E coli* could only be determined in one of the products; this product contained 12.0 cfu/g.

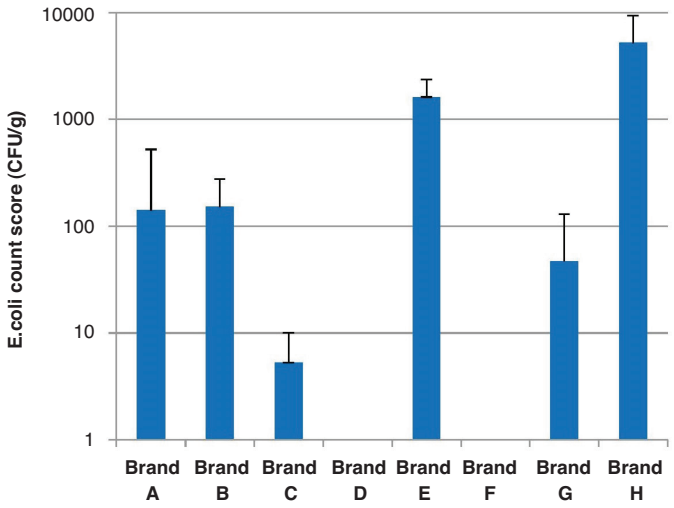

FIG 1: Bar chart showing the mean *Escherichia coli* scores and its upper sd for the different brands of commercial raw meat-based diet tests in this study. cfu, colony-forming units.

L monocytogenes was isolated from 19 products (54 per cent; 95 per cent CI: 37 to 71 per cent) from five different brands, and 15 other products (43 per cent; 95 per cent CI: 27 to 59 per cent) from all eight different brands were confirmed to be positive for other *Listeria* species (Table 1). These two groups of products contained all meat and animal by-product sources mentioned in this study.

Salmonella species were isolated from seven products (20 per cent; 95 per cent CI: 7 to 33 per cent) from four different brands (Table 1). The meat and animal by-product sources included in these products were chicken (n=4), beef (n=3), duck (n=2), horse (n=1) and lamb (n=1). In one product (3 per cent) all four bacterial species were found (ie, *E coli* O157:H7, ESBL-producing *E coli*, *L monocytogenes* and *Salmonella*), eight products (23 per cent) were confirmed to be positive for three of them, 13 products (37 per cent) for two of them, and eight products (23 per cent) for one of them.

Finally, there were only five products from which none of the four bacterial species could be isolated (14 per cent).

Parasitic analysis

In 10 out of 35 different RMBD samples (29 per cent) parasite DNA was detected. Eight products were positive for *Sarcocystis* species. Two *Sarcocystis* species were identified that showed high DNA sequence homology (97–100 per cent) with known sequences in GenBank: *S tenella* (GenBank entry number KP263755) and *S cruzi* (KC209738 and KP640133). In four products *S cruzi* (11 per cent, 95 per cent CI: 0,9 to 22 per cent) and in four other products *S tenella* (11 per cent, 95 per cent CI: 0.9 to 22 per cent) were identified. *S tenella* was detected in three products that had lamb as their main component and one product containing beef. All four products containing *S cruzi* had beef as their main component (Table 2). Two products (6 per cent, 95 per cent CI: 0 to 13.4 per cent) were infected with *T gondii* (Table 1). *T gondii* was detected in a chicken product and in a horse meat product that also contained duck (Table 2).

TABLE 2: Composition of the products in which parasites were detected

Parasite	Composition of the product
Sarcocystis tenella	Meat and animal by-products (beef 96%; heart 26%), minerals
S tenella	Meat and animal by-products (lamb 99%; heart 70%), minerals
S tenella	Fresh lamb, fresh lamb bones, fresh lamb organs, fresh chicken, fresh chicken bones
S tenella	Fresh lamb meat, fresh chicken, fresh vegetables, rice, vegetable oil, natural vitamins and minerals
S cruzi	Beef, beef heart, beef tripe, beef liver, sheep fat, rice, oils (sunflower oil, linseed oil), vitamins, minerals, trace elements
S cruzi	Category three animal by-products (eg, beef heart)
S cruzi	Beef, chicken, beef organs, chicken organs, chicken bones
S cruzi	Fresh beef, fresh chicken, fresh vegetables, rice, vegetable oil, natural vitamins and minerals
Toxoplasma gondii	Chicken (100%); meat, bones, organs
T gondii	Fresh horse meat, fresh duck bones, fresh duck organs

Discussion

The low sample size and no randomised selection in this study do not allow generalisation of infection rates or to perform a risk analysis. However as high infection rates with bacteria were already found in the 35 samples examined, it allows to assume a high contamination of RMBDs with zoonotic bacteria and parasitical pathogens. This is in contrast with dry, semimoist and canned pet food, which is rarely contaminated with pathogens.[18 19]

TABLE 1: Detection of *Escherichia coli* O157:H7, extended-spectrum beta-lactamases (ESBL)-producing *E coli*, *Listeria monocytogenes*, other *Listeria*, *Salmonella* species and *Sarcocystis* species, and *Toxoplasma gondii* in 35 frozen commercial raw meat-based diets of eight brands

Brand	Products positive for (%)							Total tested
	E coli O157:H7	ESBL-producing *E coli*	*L monocytogenes*	Other *Listeria* spp	*Salmonella* spp	*Sarcocystis* spp	*T gondii*	
A	0 (0)	6 (67)	7 (78)	2 (22)	1 (11)	2 (22)	0 (0)	9
B	5 (63)	7 (88)	5 (63)	3 (37)	4 (50)	2 (25)	0 (0)	8
C	1 (20)	4 (80)	2 (40)	3 (60)	1 (20)	1 (20)	1 (20)	5
D	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1
E	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	1 (50)	0 (0)	2
F	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)	2
G	2 (50)	4 (100)	3 (75)	1 (25)	0 (0)	0 (0)	0 (0)	4
H	0 (0)	4 (100)	2 (50)	2 (50)	1 (25)	2 (50)	0 (0)	4
Total	8 (23)	28 (80)	19 (54)	15 (43)	7 (20)	8 (23)	2 (6)	35

The overall microbiological quality of the commercial RMBDs tested in this study was acceptable since none contained more than 5×10^6 cfu total aerobic bacteria/g meat, and only two contained more than 5×10^5 cfu/g. These two limits are hygiene criteria applicable to both minced and mechanically separated meat intended for human consumption, that is, $>5\times10^6$ cfu/g meat is considered unacceptable and $>5\times10^5$ cfu/g meat is considered marginally acceptable.[29] However, based on the hygiene criteria for *E coli*, 40 per cent of the tested RMBD products (14/35) did not meet the minimum hygiene thresholds for human consumption (less than 500 cfu/g *E coli* in four out of five samples). Seven diets contained more than 500 cfu/g *E coli* and seven other diets contained between 50 and 500 cfu/g *E coli*. Previous studies have reported frequencies and quantities of *E coli* in RMBDs that are comparable or even higher.[19 20 23] RMBDs are intended as pet food and therefore they often contain animal by-products that are not restricted to such strict hygiene regulations as products intended for human consumption. This increases the risk for a higher contamination of the pet food.

While pets are directly exposed to foodborne pathogens when they ingest food, there are several ways in which pet owners and other household members can also encounter such pathogens. This can be through direct contact with the food; through contact with a contaminated pet, such as sharing the same bed and allowing licking of the face and hands; through contact with household surfaces; or by ingesting cross-contaminated human food. Cross-contamination may occur after preparing RMBDs or cleaning infected food bowls on the kitchen sink.[30–32]

Although both cats and dogs can become infected with *E coli* O157:H7, illness rarely occurs, and most of them are asymptomatic carriers that shed these bacteria in their faeces.[11 33 34] The finding that 23 per cent of our samples contained *E coli* O157:H7 is comparable with the finding of 20 per cent reported in a previous study.[3] Five out of eight positive products in our study were from the same brand, and one of these five products contained beef. Infections with *E coli* O157:H7 in human beings have been associated with serious diseases such as haemorrhagic colitis and haemolytic uraemic syndrome (Hamburger disease),[35] and it has been reported that low doses or even a single cfu can lead to infection and disease.[33] While most cases of *E coli* O157:H7 infections in human beings have been associated with raw or undercooked beef, cats and dogs are known to be short-term shedders of these bacteria.[36]

L monocytogenes can be found in the faeces of both cats and dogs, but systemic infection with this pathogen is rare and usually asymptomatic.[33 37] Abortion in the bitch, however, has been described where raw meat products were the source of infection.[38] Another study that investigated raw food samples found that 16 per cent tested positive for *L monocytogenes* and 17 per cent for other *Listeria* species.[18] Compared with these levels, the isolation frequencies in our study were twice as high, indicating a high contamination rate. Unlike in companion animals, *L monocytogenes* can cause serious illness in human beings. Infection of healthy adults usually leads to influenza-like symptoms, but can be life-threatening, especially in neonates and pregnant women where it may cause abortion. Contaminated food products, including raw meat, are common sources of infection[33] and the bacteria replicate easily in food bowls at room temperature.

In studies of RMBDs, *Salmonella* species have received the most attention. Although subclinical infections do frequently occur in animals, salmonellae can cause gastroenteritis and even septicaemic disease. For example, Stiver and others[39] reported fatal septicaemic salmonellosis after RMBD feeding in two cats. Contaminated RMBDs have also been identified as a source of gastroenteritis in greyhounds[40–42] and of diarrhoea in young puppies.[43] Several studies have found the faeces of home-made RMBD-fed dogs to be contaminated with different *Salmonella* serotypes: prevalence varied from 14 per cent to 61 per cent, whereas this bacterial species could not be isolated from the faeces of non-RMBD-fed dogs.[12 15 16] While faecal shedding of salmonellae is generally thought to last up to one week after feeding a contaminated RMBD only once,[13] shedding may last for up to eight months if animals are fed contaminated RMBD over a longer period.[16] Our finding that 20 per cent of our samples contained salmonellae is comparable to the prevalence reported elsewhere for RMBDs, varying from 7 per cent to 80 per cent in Canada and 5 per cent to 45 per cent in the USA (Table 3). A systematic review of case–control studies has shown that direct contact with pets plays a major role in human salmonellosis[44] and direct transmission has been reported frequently.[45–48] Human outbreaks of *Salmonella* infections have been associated with both contaminated dried pig ears and contaminated chicken jerky pet treats, with direct contact as the route of transmission.[49 50] *Salmonella* species are known to be widespread in the environment, and the bacteria can survive for a considerable period outside a host. The results of experiments performed by Weese and Rousseau[51] suggest that *Salmonella* bacteria can persist at room temperature in food bowls contaminated by RMBDs, and that cleaning and disinfection of contaminated food bowls have little effect on the elimination of *Salmonella*. Vacuum cleaner waste from households with RMBD-fed dogs has also been shown to be more frequently contaminated with *Salmonella* species than waste from other households.[12]

A major problem in terms of both animal and human health is the emergence and increase in antibiotic resistance. Different *Salmonella* strains isolated from RMBDs have been reported to be resistant to up to seven antimicrobials.[14] It has been proposed that the resistance of

TABLE 3: Overview of proportions of bacterial contamination in raw meat-based diets described in the literature

Bacterial species	Country	Number of investigated samples	Proportion of contaminated RMBDs (%)	References
Escherichia coli O157	USA	5	20	Freeman and Michel[3]
VT-producing *E coli* (non-O157)	USA	576	4	Nemser and others[18]
Listeria monocytogenes	USA	576	16	Nemser and others[18]
Other *Listeria* species	USA	576	*L monocytogenes* 17	Nemser and others[18]
Salmonella species	USA	576	8	Nemser and others[18]
	USA	60	7	Mehlenbacher and others[17]
	USA	240	7	Strohmeyer and others[19]
	USA	112	45	Chengappa and others[40]
	Canada	42	5	Lenz and others[12]
	Canada	25	20	Weese and others[20]
	Canada	245	21	Finley and others[14]
	Canada	16	37	Finley and others[13]
	Canada	10	80	Joffe and Schlesinger[15]
ESC-producing *E coli*	Sweden	39	23	Nilsson[23]

ESC, extended-spectrum cephalosporinase; RMBD, raw meat-based diet; VT, verocytotoxin.

these salmonellae is a result of gene transfer from other bacteria such as *E coli*. Indeed, one study has reported the *Salmonella* resistance phenotype of one dog as being similar to that of an *E coli* strain isolated from this dog's feed.[19] A recent Swedish study[23] reported that in 23 per cent of the tested RMBD samples, extended-spectrum cephalosporins-producing *E coli* were found (Table 2). In recent years, ESBL-producing Enterobacteriaceae are being isolated from companion animals with increasing frequency, and the types seem to resemble those found in human beings. Owning companion animals is therefore considered to be a risk factor for infection with ESBL-producing bacteria in human beings.[22 24 52] Cats and dogs that eat raw meat are much more likely to become infected with such antibiotic-resistant bacteria than animals on conventional diets.[53] A Dutch study also found that shedding of ESBL-producing Enterobacteriaceae was more likely in dogs that ate raw meat.[52] The presence of antibiotic-resistant bacteria in RMBDs could therefore pose a serious risk to both animal health and public health—not only because infections with these bacteria are difficult to treat, but also because of the potential of it contributing to a more widespread occurrence of such bacteria.

Finally, our results suggest that the risks posed by parasites of RMBDs are far lower than the risks described above for bacterial contamination. *S cruzi* was found in four products (11 per cent) and *S tenella* in another four products (11 per cent). Zoonotic *S hominis* or *S suihominis* was not present. In two products (6 per cent) *T gondii* was found. The protozoan parasites *T gondii*, *S cruzi* (from beef) and *S tenella* (from sheep) are known to infect cats and dogs, but seldom cause clinical illnesses. Naïve cats infected with *Toxoplasma* bradyzoites from undercooked meat produce billions of oocysts that subsequently pollute the environment via cat faeces. While most human infections with *Toxoplasma* are benign, a generalised infection can occur with mild to severe symptoms. Intrauterine infections, however, are quite severe and cause retinochoroiditis, encephalomyelitis and hydrocephalus or microcephaly in newborns.[54] Dogs that are infected with *Sarcocystis* spp shed continuous infective sporocysts in their faeces for several months. In cattle, *S cruzi* produce microscopic cysts, principally in myocardium, and can affect 100 per cent of some cattle populations. It may cause acute disease in calves, eosinophilic myositis in cattle, and abortions, stillbirths and deaths in pregnant cows.[55] Bovine eosinophilic myositis is primarily responsible for cattle condemnation after meat inspection.[56] Inflammation, hepatitis and myocarditis are the main lesions of acute and subacute ovine sarcocystosis caused by *S tenella*.[57] Despite the finding of DNA from *T gondii* and *Sarcocystis* species in the RMBD products in our study, there is no risk of these parasites being transmitted to either animals, human beings or the environment, as all of these products were sold frozen and freezing at −20˚C for one to two days inactivates *Sarcocystis* spp and *T gondii*.[58 59] However, this study does show that if raw pet food is purchased fresh and prepared at home without freezing, there is a potential risk of parasitic infections in pet animals, which can result in shedding of oocysts in the environment, thereby leading to potential additional exposure to human beings. Feeding raw meat to pets has been practised all over the world as shown by the several reports from Australia, the USA, Canada and Europe. This means that this issue is of global importance.

Conclusions

Despite the relatively low sample size (n=35) of frozen products in our study, it is clear that commercial RMBDs may be contaminated with a variety of zoonotic bacterial and parasitic pathogens. Feeding of freshly prepared, non-frozen RMBDs to companion animals can not only result in infection and disease in the animals, but also poses a risk to public health and livestock farming through shedding of pathogens

into the environment. Moreover, infected companion animals can transmit pathogens to their owners by direct contact, while human infection may also occur by cross-contamination of foods in the kitchen. It is important to encourage awareness of the possible risks associated with feeding RMBDs to companion animals, and pet owners should be educated about personal hygiene and proper handling of RMBDs. In addition, warnings and handling instructions should be included on product labels and/or packages.

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