# Microbial risks of commercial frozen Biologically Appropriate Raw Food (BARF) pet food sold in Italy

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## Abstract

**Background** Biologically Appropriate Raw Food (BARF) diet is becoming more popular among pet owners in Europe. Since there are documented microbiological risks associated with raw feeding, this study aimed to determine the presence of human pathogens in commercial frozen BARF products sold in Italy.

**Methods** *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter* spp. were searched. BARF products' general microbiological quality and hygiene were also evaluated. As sample size was limited, it has to be considered that it may be not representative of a larger sample.

**Results** None of the tested samples showed total bacterial count (TBC) higher the limit set to consider the sample unacceptable. However, 14 samples out of 21, showed TBC higher than the limit set to consider the sample marginally acceptable. A high percentage of samples was contaminated by the aforementioned pathogens, highlighting the needing for the pet owners to be aware of the risks to themselves and their pets as a result of this feeding strategy.

**Conclusions** Considering that BARF diet meals can be prepared at home by using hands, tools and spaces that could be shared, guidelines on the safer handling of these pet food should be promoted by veterinarian and nutritionists.


## Introduction

Biologically Appropriate Raw Food (BARF), diet is becoming more and more popular among pet owners[1]. This kind of diet recently gained popularity as a way to provide energy and nutrients to companion animals. It is based on products such as raw meat, organs and bones, fish as well as unpasteurized milk and raw eggs, that can be administered as such or after grinding. Commercial BARF diets are generally supplied as frozen products, available on-line[2]. Several benefits have been proposed for pets fed with BARF diets[2, 3, 4, 5], but the majority of them remain anecdotal and not

47 sustained by highly relevant data[6]. In addition to a lack of studies strongly proving nutritional benefit[7],
48 given the frequency with which raw animal products are contaminated with foodborne pathogens,
49 feeding BARF to pets has been cited as a potential risk factor to human and animal health[8-11]. Humans
50 can be exposed to pet-associated risk factors directly by petting animals or indirectly such as through
51 pet food or handling contaminated objects[12-14]. So far, the focus has mainly regarded the presence of
52 zoonotic bacteria[15-17] and the presence of antibiotic-resistant bacteria. Van Bree et al.[18] also studied
53 the presence of parasites in BARF diets. They detected *Sarcocystis spp.* and *Toxoplasma gondii* DNA
54 in 8 and 2 of 35 samples respectively. Nevertheless, as they concluded, such a finding in frozen
55 products, does not represent a risk neither for humans nor for pets since the parasites are inactivated
56 by freezing.

57 Most studies on bacterial contamination of BARF diet have been conducted in Canada and the USA,
58 while limited information is available regarding products in European countries[11, 18, 19], where the
59 recovery of pathogenic bacteria has been the cause of several withdrawals of raw pet food.

60 Only recently, a study concerning the prevalence of *E. coli* and *Salmonella* and the frequency of
61 occurrence of extended-spectrum β-lactamase producing (ESBL) isolates in raw meat Italian products
62 for pets has been published[20].

63 The number of pets in Italy is estimated to be 60.400.000, including fish, birds, dogs and cats. About
64 67% of Italians have at least one pet, positioning the peninsula in third place in the global ranking of
65 the "pet-friendly" European countries[21]. Differently from USA (APPA2018), objective survey data
66 on BARF use for Europe are scarce, but business and expert opinion indicates substantial and growing
67 raw-feeding practices[22]. In Italy, the spreading of raw- feeding practices is highlighted by the increase
68 of social media groups dedicated to BARF, counting thousands of participants, and by the constant
69 requests to veterinary nutritionists for diets based on fresh food (Barrera, personal communication).
70 Since there are documented microbiological risks to animals and humans associated with raw
71 feeding[6], the aim of this study was to determine the presence of the main pathogenic bacteria

contaminants concerning raw meat, i.e. *Salmonella* spp., *E. coli* O157, *L. monocytogenes*, and *Campylobacter* spp. in commercial BARF products sold in Italy. The general microbiological quality (total bacterial count and coliforms) of the BARF products were also evaluated.

## Materials and methods

Twenty-one samples were purchased from three different online BARF products stores among the most popular in Italy. Meat were declared to be butchered in Italy or bred and slaughtered in Germany, produced and commercialized with high quality standards, in compliance with EU regulations. Tested products were made of meat and/or by-products of single or multiple animal species according to Table 1. Products were shipped frozen directly to the laboratory and stored according to label recommendations until analysis. None of the raw meat products were accompanied by instructions for thawing or preparation. Before analysis, samples were thawed at 4°C, and processed cold to avoid microbial growth. Each sample was analyzed in two replicates. Each analysis was made respecting the hygiene / health and safety regulations, maintaining the conditions of sterility and asepsis.

### Total microbial count and coliforms

Twenty-five grams of each sample were collected homogeneously under sterility conditions, by using a sterile spoon and transferred into a sterile blender bag (Oxoid, Basingstoke, Hampshire, UK). After the addition of 225 ml of Ringer's solution (Sigma Aldrich, Milan), samples were homogenized by means of Stomacher® (VWR, Milan, Italy) at 350 rpm for 120 seconds. After homogenization, ten-fold serial dilutions for each sample were made in Ringer's solution up to $10^{-7}$. Dilutions were then inoculated to specific culture media. Total microbial count was obtained by plating onto Plate Count Agar (PCA, Oxoid, Basingstoke, Hampshire, UK) and incubating plates at 37± 1°C for 48 hours. Total coliforms were determined by plating dilutions on violet red bile agar (VRBA, Oxoid, Basingstoke, Hampshire, UK).

96 ***E. coli* O157:H7**

97 The presence of *E. coli* O157:H7 in the samples was evaluated according to the protocol ISO 16654-
98 2:2001[23] with slight modifications. Briefly, 25 g of each sample were aseptically collected, and
99 transferred into a sterile blender bag for the enrichment step with 225 ml of modified tryptone soya
100 broth plus novobiocin (mTSB+N, VWR-Merck, Milan). After homogenization, samples were
101 transferred to a sterile bottle and incubated for 18 to 24 hours at 41.5± 1°C. After enrichment, 0.1 ml
102 were spread onto sorbitol MacConkey agar with cefixime-tellurite supplement (CT-SMAC) (VWR-
103 Merck, Milan) and CHROMID® O157H7 selective agar (Biomerieux Italia, Firenze). Plates were
104 incubated for 24-26 hours at 37±1°C. Typical *E. coli* O157:H7 colonies appearing green-blue on
105 CHROMID® O157H7 agar while smooth and colorless with a possible orange halo on CT-SMAC
106 agar, were streaked onto nutrient agar and incubated at 37°C for 18 to 24 h. Presumptive *E. coli*
107 O157:H7 colonies were confirmed by indole test (VWR Chemicals, Milan) and Microgen® *E. coli*
108 O157:H7 latex agglutination test (Microgen, UK).

109 ***Salmonella* spp**

110 The presence of *Salmonella* spp in the samples was evaluated according to the protocol ISO 6579 :
111 2002[24] with slight modifications. Briefly, 25 g of each samples were aseptically collected and
112 transferred to a sterile blender bag for the pre-enrichment step with 225 ml of Buffered Peptone Water
113 (VWR Chemicals, Milan). After homogenization, samples were transferred to a sterile bottle and
114 incubated for 24 ±2 hours at 37°C± 1°C. An enrichment step was carried out by diluting 1 ml from
115 the pre- enrichment bottle in 10ml of Muller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth
116 (VWR-Merck, Milan) and 0,1ml in 10ml Rappaport Vassiliadis soya (RVS) broth (VWR Chemicals,
117 Milan). Tubes were incubated respectively at 37 ±1°C for 24 ± 3 hours and 41,5 ± 1°C for 24 ± 3
118 hours. Then, 0.1 ml from each enrichment tube, was spread onto 2 selective media, Xylose Lysine
119 Deoxycholate agar (XLD-agar) (VWR-Merck, Milan) and Rambach®-agar (VWR-Merck, Milan).
120 Plates were incubated for 24 ± 3hours at 37±1°C. Suspected *Salmonella* colonies, appearing with

black center and a reddish zone with a slight transparency on XLD-agar and pink on Rambach® agar, were seeded into triple sugar iron (TSI) agar (VWR Chemicals, Milan) for biochemical characterization. *Salmonella* Latex agglutination test (Oxoid, UK) was used to confirm the genus of suspected colonies.

***Listeria monocytogenes***

Samples were analyzed for the presence of *Listeria monocytogenes* according to ISO 11290-1:2017 [25] with slight modifications. Briefly, for the primary enrichment step, 25 g of each samples were aseptically collected, and transferred to a sterile blender bag, homogenized with 225 ml of Half concentrated Fraser Broth (HFB) (Oxoid, UK) and incubated at 30 ±1 °C for 24 h. After the primary enrichment, 0.1 ml of the cultures were transferred to 10 ml Fraser Broth (FB) (Oxoid, UK) and incubated at 37 ± 1°C for 48 h for a secondary enrichment. From both enrichment steps, 0.1 ml were spread onto Agar Listeria Ottaviani and Agosti medium (ALOA, Biolife Italiana, Milan. Plates were incubated for 48 hours at 37 ±1 °C. Suspected *L. monocytogenes* colonies, appearing with a green-blue color surrounded by an opaque halo were identified by using the micromethod Mono Confirm Test (Biolife Italiana, Milan)[26].

***Campylobacter* spp**

Samples were analyzed according to the ISO 10272-1: 2017[27] with slight modifications. Briefly, 25 g of each sample were aseptically collected and transferred to a sterile blender bag for the primary enrichment step with 225 ml of Bolton broth base (Oxoid, UK). After homogenization, samples were transferred to a sterile bottle and incubated in microaerophilic atmosphere (Oxoid™ CampyGen™ 2.5L Sachet, Oxoid, UK) at 37 °C for 4 h to 6 h and then at 41,5 °C for 44 hours. After enrichment, 0.1 ml were spread onto Blood Free Campylobacter Selectivity Agar base (mCCDA, Oxoid, UK) at 41.5 °C for 44 ± 4 hours in a microaerophilic atmosphere, Putative *Campylobacter* spp. colonies appearing as flat/slightly raised, gray and wet/dry/hue, spreading colonies were analyzed under phase contrast microscopy (100X, Olympus) and M46 MICROGEN® Campylobacter latex agglutination

146 test (Microgen, UK) that is able to detect the following species: *Campylobacter jejuni , C. jejuni*
147 subsp. *doylei , C. coli , C. upsaliensis , C. laridis , C. fetus*

148 **Results**

149 Total aerobic bacteria count (TBC) ranged from mean value of 4,22 x $10^4$ of the sample 9, to mean
150 value of 3,77 x $10^6$ cfu/g of the sample 16 (Figure 1).

151 Total coliforms mean values for the tested samples ranged from a mean value of 1,72 x $10^3$ for the
152 sample 8 to a mean value of 7,2 x$10^4$ for the sample 7 (Figure 2). Presumptive *E. coli* O157:H7 was
153 isolated from 61 % of the total samples. However, after confirmation tests, 23% of the samples were
154 found to be contaminated by *E. coli* O157:H7. For brand A, 22% of samples were confirmed to be
155 contaminated by *E. coli* O157:H7 (Table 2). 16% of samples from brand B resulted to be
156 contaminated by *E. coli* O157:H7. Finally, brand C showed *E. coli* O157:H7 in 33% of samples.

157 *Salmonella* species were isolated from 71% of the samples: 56% of samples from brand A, 83% both
158 from brand B and C (Table 2).

159 *Listeria monocytogenes* was isolated from 90% of tested samples: 88% of samples from brand A,
160 100% from brand B and 83 % from brand C (Table 2).

161 Finally, *Campylobacter* spp. was isolated from 29% (22% of samples from brand A, 33% both from
162 brand B and C; Table 2) of samples despite the frozen status of samples that is known to limit the
163 viability and cultivability of *Campylobacter* spp.[28].

164 **Discussion**

165 The results of TBC are in agreement with Van Bree et al.[18], who analyzed the presence of zoonotic
166 bacteria and parasites in BARF diets for cats and dogs in the Netherlands, revealing TBC ranging
167 from 7.9 x $10^2$ to 5.0 x $10^6$ cfu/g. In the present study the overall microbiological quality of the tested
168 commercial products is acceptable according to the hygienic criteria applicable to both minced and
169 mechanically separated meat intended for human consumption (Regulation EC No. 2073/2005).

Indeed, none of the samples showed TBC higher than 5 x $10^6$ cfu/g, which is the limit to consider the sample unacceptable. However, 14 samples out of 21, showed TBC higher than $5\times10^5$ cfu/g which is the limit to consider the sample marginally acceptable. As for coliforms, our results are in agreement with Weese et al.[29], who in analyzing 25 commercial raw diets for dogs and cats found coliforms contamination ranging from 3.5 x $10^3$ to 9.4 x $10^6$ cfu/g. Also other previous studies have highlighted high frequencies and levels of coliform contamination in raw meat-based diets[30,31]. Coliforms give an indication of general microbiological condition of a food and among them, *E. coli* represents an indicator of fecal contamination, informing on the hygienic quality of the sample.

When it comes to the detection of *E. coli* O157:H7, our results are in good agreement with the study of Van Bree et al.[18], where *E. coli* O157:H7 was found in 23% of the tested samples and almost 80% of the samples were contaminated by extended spectrum beta-lactamase producer (ESBL) *E. coli*. Similar results were also obtained by Nilsson[31], who isolated from all the tested samples *E. coli* positive for the *bla* $_{\text{CMY-2}}$ family of the ampC beta-lactamase genes, which are known to confer broad-spectrum resistance to beta-lactamases antimicrobials[32]. Some studies have reported a rise in the antimicrobial resistance patterns of *E. coli* O157:H7[33-35]. Therefore, the number of positive *E. coli* O157:H7 samples found in the present study, confirms that, together with the risk associated to the presence of one of the most important food-borne pathogens among shiga toxin-producing *E. coli* (STEC), the use of BARF products could also be involved in spreading antibiotic resistance genes among pets and owners[20, 31, 36]. *E. coli* O157: H7 has a very low infective dose, <50 cells/g for human[37], thus, simply manipulating contaminated pet-foods could expose the owners to a relevant risk of infection. Cross contamination is a quite likely event when preparing food[38], even if it is likely that owners do not prepare food simultaneously for themselves and their pets, and they wash their hands and clean the kitchen table before they start to prepare food for themselves. Furthermore, infected pets can be asymptomatic carriers and could directly infect their owners[39].

194 Results obtained for the detection of *Salmonella* spp. were in agreement with Joffe and Schlesinger[40],
195 who found 80% of raw pets' diets contaminated by *Salmonella* spp. However, our results are much
196 higher than those reported by Van Bree et al.[18] and by Fredriksson-Ahomaa et al.[10], where only 20%
197 and 2% of samples respectively, tested positive for *Salmonella* spp. This discrepancy could be due
198 the lower prevalence of *Salmonella* spp. in Finnish and Dutch farm animals compared to Italy and
199 Germany where the meat sampled for this study came from[41-43].
200 Previous studies suggest that *Salmonella* spp. can persist at room temperature in contaminated food
201 bowls, and that cleaning and disinfection of these bowls may not achieve the elimination of
202 *Salmonella*[44]. Furthermore, as for *E. coli,* pets that consume contaminated raw food diets can be
203 colonized with *Salmonella* spp. without exhibiting clinical signs, making them a possible source of
204 contamination for owners[15, 45]. It has to be noted that also animals fed with dry foods could carry
205 *Salmonella* in their faces, yet its transmission from dogs to humans has rarely been reported[46].
206 However, a systematic review of case–control studies has shown that direct contact with pets plays a
207 major role in human salmonellosis, and direct transmission has been reported frequently[47].
208 Therefore, our results highlight that, also concerning *Salmonella* spp, BARF products sold in Italy
209 could represent a potential threat for owners' health if products are not hygienically handled. Our
210 results were in good agreement with other studies[18], also regarding the presence of *L. monocytogens*
211 in BARF-diet samples. It is not surprising that *L. monocytogenes* is the most widespread pathogen in
212 this type of food, as the conditions of production, storage and use of the product are such to allow the
213 development and uncontrolled proliferation of this microorganism. *L. monocytogenes* is in fact, a
214 psychotropic and ubiquitous microorganism[48]. The ability to survive and grow under the refrigeration
215 temperatures means that products that do not undergo heat treatment, such as BARF diet products,
216 can be a source of listeriosis. In addition, once raw pet food items are purchased, they may be exposed
217 to raised temperatures during transport and after arrival at home, encouraging the potential growth of
218 pathogens.

Listeriosis is a serious disease for humans and being possibly asymptomatic in domestic animals, infected pets could represent a direct source of infection for owners.

Finally, our results revealed a presence of *Campylobacter* spp, higher than expected, considering the frozen nature of the samples. However, several studies showed that *Campylobacter* spp may be more robust than previously thought and it can survive freezing and thawing[49-51]. There is still uncertainty about minimum infectious doses for *Campylobacter* spp.[52], but some estimates are as low as 500 cells/g and therefore simply manipulating contaminated pet-foods could expose owners to an infection risk.

Taken together, our results show that, the analyzed frozen BARF products had high levels of microbial contamination, beyond the microbiological limits set by the EU Regulation for products that are intended for human consumption. However, the limited sample size considered in the present study might not represent the overall situation in all raw food products sold in Italy. Dedicated legislation is not available yet for BARF pet foods, but their microbiological quality should fall, at least, within the specification for human products. Given that raw feeding is currently well established and that BARF diet meals may be prepared at home, probably in the kitchen, by using hands and tools that could be shared, specific microbiological criteria should be set, in order to limit the risk for pet owners. It would even be recommendable to have some EU regulation for such products including specific microbial limits and labelling containing guidelines for consumers. These guidelines should include the suggestion to consult the veterinarian to decide on the most appropriate diet for their pet, but in this context, pet owners should be made aware of the potential risks to themselves and their pets as a result of this feeding strategy. Furthermore, no indication regarding the safe handling of these raw meat products was available on the tested BARF products packaging. This is a significant omission that can only partially be addressed by guidelines on the safer handling on BARF products at home as promoted by veterinarians and nutritionist.

## Competing interest statement

The authors have no competing interest to declare.

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377 TABLE 1 Declared composition of tested BARF samples

| | Brand | Composition |
|---|---|---|
| 1 | A | 35% horse meat off cuts, 25% horse cartilage (sternum), 20% horse offal (lung, heart), 10% horse fat, 10% vegetables (carrots), enriched with salmon oil <1%. |
| 2 | A | 10% omasum, 25% green tripe, 15% beef cartilage, 20% beef cuttings, 25% beef offal (kidney, lung, heart, liver), 5% pureed fruit / vegetables (carrots, apples) |
| 3 | A | 100% beef |
| 4 | A | 100 % Beef Green Tripe (rumen) |
| 5 | A | 100% beef muscles |
| 6 | A | 60% rabbit meat, 40% rabbit carcass |
| 7 | A | 40% organic beef larynx, 40% green tripe, 20% udder |
| 8 | A | 100% organic carcasses and chicken necks |
| 9 | A | 75% poultry carcasses (chicken, turkey), 25% poultry offal (chicken, turkey), enriched with <1% fish oil |
| 10 | B | 100% Chicken necks |
| 11 | B | Horse meat composed of lean cuts of muscle, lung and tripe |
| 12 | B | Beef Liver 40%, Lung 40%, Heart and Spleen 20% |
| 13 | B | 89% Lamb and rabbit meat, 8% of bones and cartilage, 3% internal organs |
| 14 | B | Beef meat and heart 40%, fat 38%, trachea, lung and spleen 20%, fresh blood 2% |
| 15 | B | 100 % Beef Green Tripe (rumen) |
| 16 | C | 100% horse meat |
| 17 | C | 100% beef tripe (rumen) |
| 18 | C | 100% beef muscles |
| 19 | C | 100% chicken back |
| 20 | C | 100% beef meat and cartilage (epiglottis) |
| 21 | C | 100% rabbit muscles |

TABLE 2 Number of samples (%) contaminated by presumptive *E.coli* O157, *E.coli* O157:H7, *L. monocytogenes*, *Salmonella* spp, *Campylobacter* spp. among tested BARF products.

| Brand | Presumptive *E.coli* O157 | *E. coli O157:H7* | *L. monocitogenes* | *Salmonella spp.* | *Campylobacter spp.* |
|---|---|---|---|---|---|
| A | 5(56) | 2 (22) | 8 (88) | 5 (56) | 2 (22) |
| B | 2 (33) | 1 (16) | 6 (100) | 5 (83) | 2 (33) |
| C | 6 (100) | 2 (33) | 5 (83) | 5 (83) | 2 (33) |

**Figures captions**

**FIGURE 1** Total bacterial count (mean values, CFU/g) in tested BARF samples. Bars are standard deviations.

**FIGURE 2** Coliforms (mean values, CFU/g) in tested BARF samples. Bars are standard deviations.

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