

When Pet Snacks Look Like Children's Toys! The Potential Role of Pet Snacks in Transmission of Bacterial Zoonotic Pathogens in the Household

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Abstract

Recent foodborne pathogen outbreaks associated with dry pet food and treats have focused the attention on these products as vehicles of pathogens for both pets and their owners. This study investigates the purchasing habits of dog owners and determines if and in what form pet snacks can be potentially dangerous for humans, especially for children. For these purposes, questionnaires collected from 406 dog owners were evaluated and microbiological analyses were performed on 120 dry pet snacks. The shape of the pet snack affects the purchase (median score, Mdn=6.4) and the star bone was the preferred one (Mdn=7.1). Most of the participants (76.0%; $p < 0.001$) stated that snacks are attractive to their children and that the possibility for children to get in contact with pet snacks is rare, but not implausible (Mdn=3.1). Indeed, more than 6% of respondents admitted that there had been incidents of involuntary ingestion of pet snacks, with fever and gastrointestinal manifestations. Microbiological analyses showed that 119 dry pet snacks analyzed of a total of 120 (99.2%) were in good sanitary condition except for one sample where the presence of *Listeria ivanovii* was confirmed. The upward trend in the presence of pets in households and the strong and continuous growth in value and volume of the pet food market led researchers to analyze possible public health issues. Children and infants in particular are the most exposed subjects as they are more likely to come into contact with pet snacks, attracted by a shape similar to that of a toy. For this reason, although our findings did not highlight important microbiological contamination of pet snacks, it would be useful to standardize food safety criteria with those for human food from a One Health perspective.

Keywords: one health, pet food, pet snack, food safety

Introduction

THE ONE HEALTH approach is, to date, an action underpinning the protection of public health. In this context, the problems related to foodborne diseases not only affect humans but also pets who are also susceptible to a large number of bacterial zoonotic pathogens. Unfortunately, the safety of pet food, including pet snacks, has been poorly investigated. There is little quantitative information available on the risk of infectious disease transmission among pets, humans, and the domestic environment that they share (Lambertini *et al.*, 2016). More specifically, no information is available on the role of pet food as a vehicle for direct and indirect exposure to pets and humans, particularly children and infants, who can get in contact with pet food or snacks with their hands or by direct ingestion, especially when the snacks have an attractive shape similar to that of toys.

The psychological and health benefits of having a pet at home are well known (Brodie and Biley, 1999; Diverio *et al.*, 2016). Dogs and cats are the most common companion animals. The American Pet Products Association (APPA) found that 68% of U.S. households owned some sort of pet in 2016, among those pets were about 90 million dogs and 94 million cats (APPA, 2020). In Europe, 80 million households own at least one pet and there is a population of 75.3 million cats and 65.5 million dogs (Assalco-Zoomark, 2019; FEDIAF, 2019).

The high presence of companion animals in families has triggered a revision of their social dimension; they are more often considered real members of the family unit and this has led, in the last decade, to the occurrence of a social phenomenon of humanization (Boya *et al.*, 2012; Menchetti *et al.*, 2018).

This phenomenon is the key trend driving the pet food market. Many pet owners are looking for pet food that reflects their taste.

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Commercially prepared pet foods may contain a wide range of ingredients from many raw materials. In the market place, it is also easy to find diverse types of dog treats with differing shapes, flavors, and for different uses (Fig. 1).

As a result, there has been an increase in introduction of new products such as natural, raw, organic, and vegan food in the diet of pets, which on one hand gives lots of choice to the owners, but on the other hand exposes the home environment to possible contamination from the use of raw food (Walia, 2019).

The changing role of pets in society and the increased attention to their health and welfare have led the pet food industry to undergo a strong change with significant and steady growth. Indeed, in the EU context, pet food products show an annual sales volume of 8.8 million tons and turnover is 21 billion Euros (FEDIAF, 2019). As an important family member, pet owners want to give their animals food of high quality to improve their standard of living and welfare (Walia, 2019). Taking care of a pet and giving it a special snack can generate in pet owners positive feelings of affection for them (Griffin *et al.*, 1984).

All these aspects have also led to the spread of food supplements as snacks and treats used as a pet reward or for pet health such as snacks for dog's oral hygiene. Indeed, in 2018, the main sale segments showed a positive trend (+5.4%), with the best performance found in pet shop chains where dog snack sales grew by 15.2% (Assalco-Zoomark, 2019). Despite the importance of the pet food market, there is no binding legislation on microbiological criteria for finished pet food.

Enjoyment of the benefits of having a pet, therefore, needs to be coupled with control of the risk of infectious diseases that could be acquired through pets and their food.

Recent *Salmonella* outbreaks associated with dry pet food and treats raised the level of concern for these products as vehicles of the pathogen not only for pets but also for their owners (Lambertini *et al.*, 2016). Contaminated pet food use could cause human illness through different routes: direct contact with pet food or indirect contact that can occur between humans and objects (e.g., bowls) that have come in contact with pet food (Lejeune and Hancock, 2001). In both situations, humans come in contact with contaminated objects and surfaces primarily with their hands. Children and infants can also come in contact with household surfaces with their hands or mouths, such as the floor where treats/snacks are usually eaten by the animals.

This study aims to analyze the purchasing habits of dog owners and determine if and in what form the pet foods and/or snacks can be potentially dangerous for humans, especially for children and infants.

For these purposes, a survey was conducted to investigate the purchasing habits of dog owners and potential impacts of pet snacks in the transmission of pathogens between pets and humans. Moreover, microbiological analyses were carried out on different types of dry pet snacks available from the main distribution segments to outline their hygienic profile.

Materials and Methods

Questionnaire structure

The survey was conducted for 4 months from February to May 2019 through the distribution of the questionnaire to a total of 500 pet owners. The questionnaire was distributed to dog owners in waiting rooms of different veterinary clinics and in several pet shop chains in the Lombardy territory. Dog owners were informed about the study by people who were working in veterinary clinics and pet shop chains, who were previously instructed about the objectives of the study, requirements to participate (dog owners over 18 years old, who habitually buy snacks for their pets, and with at least one child in their household), and structure of the questionnaire.

The questionnaire was left available for dog owners who were free to fill them and participate in the study. The questionnaire (Table 1) designed for the study consisted of five parts focusing on the following issues: (1) the structure of the nuclear family, (2) the presence of pets and children in the household, (3) the habit of buying snacks, (4) the influence of the snack's shape on the purchase, and (5) the occurrence of gastroenteric or febrile episodes concerning ingestion or manipulation by children of animal snacks found in households.

Microbiological analyses

The microbiological analyses were performed to evaluate the hygienic profile of 12 different snack types (from different brands from the largest European manufacturing companies) chosen from the main categories of dry pet snacks collected from pet shop chains. For each type of snack, 10 different samples were collected for a total of 120 samples subjected to microbiological analysis. In particular, enumeration of *Escherichia coli*, enumeration of sulfite-reducing clostridia, and the research of *Salmonella* spp. and *Listeria monocytogenes* were investigated.

The microbiological characteristics of a 10-g sample were obtained after homogenization in 90 mL of sterile diluent solution (0.85% NaCl and 0.1% peptone, OXOID) and homogenized in a stomacher (Star Blender® Digital—VWR) for 1 min at room temperature, and then serial 10-fold

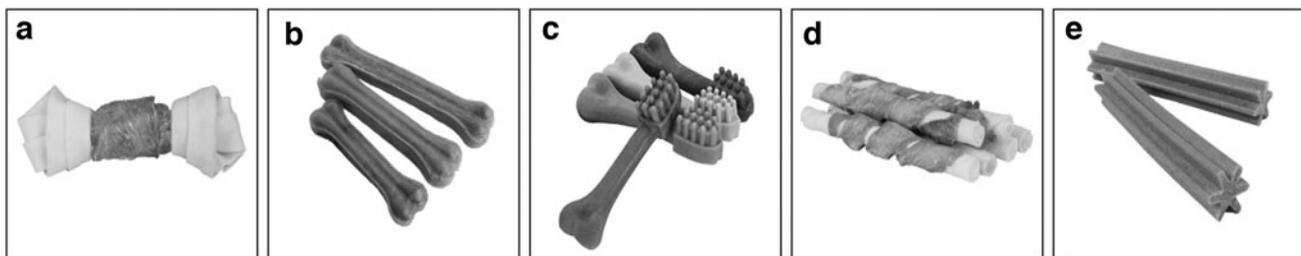


FIG. 1. Main types of snacks considered in the study: (a) knotted bone; (b) pressed bone; (c) shape similar to a human toothbrush; (d) stick bone; and (e) star bone.

TABLE 1. STRUCTURE OF THE QUESTIONNAIRE DISTRIBUTED TO DOG OWNERS

| <i>Question</i> | <i>Answers</i> | <i>Type of answer</i> |
|---|---|--|
| First part | | |
| 1 Place of distribution of the questionnaire | Veterinary clinic Pet shop chains | Multiple-choice single-answer question |
| 2 Gender of the pet | Male Female | |
| 3 Pet's age | Puppy (0–1 year) Adult (1–10 years) Senior (more than 10 years) | |
| 4 Size of pet | Mini/toy Small Medium Big | |
| Second part | | |
| 5 Where do you buy pet snacks? | Grocery shop Traditional pet shop Pet shop chain Other | 10-Point scale ranging from “never” to “always” |
| 6 What are the pet snacks used for? | As a game As a pastime As a training support As an oral hygiene product | |
| 7 What kind of shape do you usually buy? | Knotted bone Pressed bone Shape similar to that of a human toothbrush Stick bone Star bone Other (specify) | |
| 8 How much does the shape of the pet snack affect the purchase? | | 10-Point scale ranging from “does not affect” to “greatly influences” |
| Third part | | |
| 9 How many children are in the nuclear family? | 1 2 3 >3 (specify) | Multiple-choice single-answer question |
| 10 What is the age range? | From 0 to 1 year From 2 to 3 years From 4 to 6 years More than 6 years | |
| 11 Are pet snacks attractive to children? | Yes No | |
| 12 If yes, how much does the shape of the pet snack attract children and/or infants? | | 10-Point scale ranging from “does not attract” to “greatly influences” |
| 13 How often do children have the opportunity to come into contact with pet snacks? (e.g., manipulated and/or put in the mouth) | | 10-Point scale ranging from “never” to “frequently” |
| 14 Have there been any incidents of illness? | Yes No | Multiple-choice single-answer question |
| 15 If yes, what kind of illness? | Fever Gastroenteric episodes | |
| Fourth part | | |
| 16 Gender of respondent | Male Female | Multiple-choice single-answer question |
| 17 Occupation of respondent | Student Employee Freelancer Pensioner Other | |
| 18 Age of respondent | | Open-ended question |

dilutions were prepared in a sterile saline solution. *E. coli* was then enumerated using a Petrifilm plate (3M, St. Paul, Minnesota, USA), following the AFNOR 3M 01/08-06/01 method. Moreover, the sulfite-reducing clostridia were determined on iron sulfite agar (OXOID) after incubation in an anaerobic atmosphere at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24–48 h, while *Salmonella* spp. detection (analytical unit: 25 g) was carried out using UNI EN ISO 6579-1:2017 and the presence was confirmed by an API 20E system (BioMerieux, Marcy l'Etoile, France).

The detection of *L. monocytogenes* (analytical unit: 25 g) was performed according to AFNOR BRD 07/4-09/98 and the presence was confirmed according to the AOAC N.060402 (MID 67) method. All analyses were done in duplicate.

Statistical analysis

Descriptive statistics were used to present data as numbers and frequencies or median, first (Q1) and third (Q3) quartiles, or interquartile range (IQR), and means and standard deviations. Distributions within categorical variables were analyzed using chi-square (χ^2) goodness-of-fit tests. For questions 5–8, 12, and 13, each participant assigned a score to all the possible answers proposed. Therefore, the scores of the answers to each question were treated as paired data and analyzed by Friedman tests, followed by Dunn's test for multiple comparisons. The answer "other" was excluded from the statistical analysis because 0 is almost constant. We used graphic tests to verify the assumptions.

The sample size for microbiological analyses was determined by the formula derived from the Wald confidence interval for binomial proportions and large populations.

Data were analyzed using SPSS Statistics, version 25 (IBM, SPSS, Inc., Chicago, IL, USA), and GraphPad Prism, version 7.0 (GraphPad Software, San Diego, CA, USA). The level of statistical significance was set at <0.05 .

Results

Participants, questionnaires, and demographic characteristics of pets

A total of 406 questionnaires were collected (response rate=81.2%). Of those, 337 were correctly compiled and analyzed (83.0%). Most of the completed questionnaires had been distributed in pet shop chains (231/337, 68.5%; $p < 0.001$), while 106 (31.5%) were completed in veterinary clinics.

Male and female participants were equally distributed ($p > 0.1$; Table 2) and their ages ranged from 18 to 75 years. Most of the participants were employed or students ($p < 0.001$). Most of the children in their households were 1–6 years old ($p < 0.001$; Table 2).

There was no difference in the gender of pets, but most of them were adults ($p < 0.001$) and of small and medium sizes ($p < 0.001$; Table 2).

Pet snacks: shopping habits

Owners usually buy snacks in a grocery shop ($p < 0.001$), especially to be used as a pastime and for oral hygiene for their pet ($p < 0.001$; Table 3). They prefer the star-shaped snack, while the one similar to the toothbrush shape was least preferred ($p < 0.001$; Table 3).

The median score for the question "How much does the shape of the snack affect the purchase?" was 6.4 (IQR = 5.2–

TABLE 2. DEMOGRAPHIC CHARACTERISTICS OF DOG OWNERS PARTICIPATING IN THE SURVEY AND THEIR PETS

| Characteristics of participants | | Number and percentage or mean and standard deviation | χ^2 | p |
|---|-------------------|--|----------|--------|
| Gender of respondent (n, %) | Male | 165 (49.0%) | 0.145 | 0.703 |
| | Female | 172 (51.0%) | | |
| Age of respondent, years (mean \pm SD) | | 38 \pm 16 | — | |
| Occupation of respondent (n, %) | Student | 90 (26.7%) | 101.739 | <0.001 |
| | Pensioner | 31 (9.2%) | | |
| | Employee | 129 (38.3%) | | |
| | Freelancer | 50 (14.8%) | | |
| | Other | 37 (11.0%) | | |
| No. of children in the nuclear family (mean \pm SD) | | 2 \pm 1 | — | |
| Age range of children (n, %) | 0–1 Year | 38 (11.3%) | 76.923 | <0.001 |
| | 2–3 Years | 113 (33.5%) | | |
| | 4–6 Years | 134 (39.8%) | | |
| | More than 6 years | 52 (15.4%) | | |
| Gender of the pet | Male | 179 (53.1%) | 1.309 | 0.253 |
| | Female | 158 (46.9%) | | |
| Pet's age | Puppy | 31 (9.2%) | 366.522 | <0.001 |
| | Adult | 278 (82.5%) | | |
| | Senior | 28 (8.3%) | | |
| Size of the pet | Toy | 62 (18.4%) | 25.267 | <0.001 |
| | Small | 89 (26.4%) | | |
| | Medium | 120 (35.6%) | | |
| | Big | 66 (19.6%) | | |

SD, standard deviation.

TABLE 3. PET SNACKS: SHOPPING HABITS OF DOG OWNERS

| Question | Answer | Median | Q1 ^a | Q3 ^b | p ^c |
|--|---|--------|-----------------|-----------------|----------------|
| Where do you buy snacks? | Traditional pet shop | 4.8b | 3.5 | 6.4 | <0.001 |
| | Grocery shop | 6.8a | 4.9 | 8.3 | |
| | Pet shop chains | 5.4b | 3.8 | 7.1 | |
| | Other | 0.0 | 0.0 | 0.0 | |
| What are the pet snacks used for? | As a game | 4.5c | 3.3 | 6.1 | <0.001 |
| | As a pastime | 6.4a | 4.9 | 7.6 | |
| | As a training support | 5.7b | 4.1 | 7.1 | |
| | As an oral hygiene product | 6.3a | 4.9 | 7.3 | |
| What kind of shape do you usually buy? | Knotted bone | 5.6b | 4.1 | 7.4 | <0.001 |
| | Pressed bone | 5.1bc | 4.2 | 6.7 | |
| | Shape similar to that of a human toothbrush | 4.3d | 3.3 | 6.2 | |
| | Stick bone | 4.8cd | 3.5 | 6.6 | |
| | Star bone | 7.1a | 5.8 | 8.1 | |
| | Other ^d | 0.0 | 0.0 | 0.0 | |

^aQ1 = first quartile.

^bQ3 = third quartile.

^cFor each question, medians, followed by the same subscript letter, do not differ significantly ($p < 0.05$; Dunn's test).

^dNot included in the statistical analysis.

7.6), while it was 4.3 (IQR = 0.0–6.3) for the question “How much does the shape of the pet snack attract children and/or infants?” To the question “How often do children have the opportunity to come into contact with pet snacks?” participants assigned a median score of 3.1 (IQR = 3.1–4.6).

Pet snacks: food safety

Most of the participants (76.0%; $p < 0.001$) stated that these snacks are attractive to children living in their households and more than 6% admitted that there had been incidents of illness, with fever and gastrointestinal manifestations (Table 4).

Results of microbiological analyses

Microbiological analyses showed that *Salmonella* spp. and *L. monocytogenes* were absent (in 25 g) in 119 of 120 samples tested (99.2%). Moreover, regarding the presence of *E. coli* and sulfite-reducing clostridia, all samples were below the detection limit of 1 log CFU/g.

Only one sample, specifically a pet snack for oral hygiene care, was biochemically tested (AOAC N.060402-MID 67 method) for the presence of *L. monocytogenes* suspect colonies selected from RAPID'L mono agars. The biochemical test confirmed the presence of *Listeria ivanovii* with a probability percentage of 96.25%.

Discussion

This study allows us to analyze the purchasing habits of dog owners and to investigate a problem not yet fully addressed in scientific literature concerning the possibility of transmission of pathogens through pet snacks to younger members of the household. The correct communication of potential risks could strengthen the credibility of recommendations on proper handling of pet food in the domestic environment, particularly where children and infants are present.

The findings of the questionnaire confirm that the shape of the pet snack significantly influences the choice of the consumer. In accordance with Brennan *et al.* (2013), for many food products, even for human consumption, characteristics such as the variety of sizes, shapes, colors, and flavors are specifically designed to attract the consumer. In particular, the snack shape preferred by the respondents was the star bone, followed by the knotted and pressed bone. The main channel of purchase was grocery shops. These results are in line with other reports concerning the Italian pet food market, where grocery shops in 2018 accounted for 55.3% of the total value in the cat and dog pet food market (1.151 million Euros), followed by pet shop chains (Assalco-Zoomark, 2019).

The results also showed that the drivers of purchase behavior are mainly to keep animals occupied and for oral hygiene. In any case, functional snacks and treats remain the most dynamic segment: +5.4% in value terms in 2018 (Assalco-Zoomark, 2019).

TABLE 4. PET SNACKS: FOOD SAFETY ASPECTS

| Item | | Count | % | χ^2 | p |
|---|---------------|-------|-------|----------|--------|
| Are pet snacks attractive to children? | Yes | 256 | 76.0 | 82.047 | <0.001 |
| | No | 81 | 24.0 | | |
| Have there been any incidents of illness? | Yes | 21 | 6.2 | 265.128 | <0.001 |
| | No | 316 | 93.98 | | |
| If yes, what kind of illness? | No | 316 | 93.8 | 567.808 | <0.001 |
| | Fever | 9 | 2.7 | | |
| | Gastroenteric | 12 | 3.6 | | |

Importantly, the shape can also attract the attention of children, although the answers to this question were highly variable. To explore this result more deeply, it would be interesting to carry out comparative *in vivo* studies on the behavior of children. Participants also claimed that children rarely have the opportunity to come into contact with pet snacks. However, none of the respondents excludes the possibility. Indeed, most of the participants (76%) admitted that pet snacks are attractive to children. Besides, the owner's response on this topic could be distorted by a misperception of risks/hazards or underestimation of a problem in their household as parents often do not have a complete perception of the risks in the home environment (Ablewhite *et al.*, 2015).

A question about where and how snacks are stored in a home environment to understand the ease of access by children could provide a more bias-free answer.

The last data presented are very important especially considering the fact that more than 6% admitted that there had been incidents of illness with fever and gastrointestinal manifestations. These data should be confirmed by clinical studies even because the role of pet snacks as a reservoir of potentially damaging bacteria for children has not been sufficiently investigated and it is therefore not possible to exclude that illnesses may also depend on other factors. Rigorous quantification of these variables would significantly advance the development of risk-based prevention and mitigation strategies in the household (Behraves *et al.*, 2010).

There are not much data in the literature regarding the relationship between pet food or snacks and children. Indeed, online literature searches of the PubMed database on "pet food microbiological risks" revealed only 113 articles. However, some studies have described cases where pets have been a vector of zoonotic enteric diseases to humans (Schotte *et al.*, 2007). It is important to highlight that children interact with pets and their environment differently than adults in both frequency and type of contact (Millot *et al.*, 1988; Mathers *et al.*, 2010). The occurrence of zoonotic pathogens in pet food has been observed for decades (D'Aoust, 1978). In the last 10 years, the level of concern has been raised due to detection of several human outbreaks and large-scale recalls linked to commercial pet food and treats (Finley *et al.*, 2006; Buchanan *et al.*, 2011).

For example, between January 2006 and December 2007, an outbreak of *Salmonella* Schwarzengrund caused by dry pet food made 79 people sick in 21 U.S. states (Ferraro *et al.*, 2008). As reported by the CDC in 2012, in the United States, 49 individuals, of which 10 were hospitalized, contracted salmonellosis due to the presence of *Salmonella* Infantis in dry dog food (Rama *et al.*, 2017). In our study, one pet snack (of a total of 120 analyzed) confirmed the presence of *L. ivanovii*. This was found in a sample containing beef, potato starch, and other natural ingredients used for the oral hygiene of dogs. *L. ivanovii*, considered to infect mainly ruminants, rarely infects humans.

In addition, there are different studies (Snapir *et al.*, 2006; Guillet *et al.*, 2010) that indicate the pathogenic potential of *L. ivanovii* in causing gastroenteritis in humans, as reported for *L. monocytogenes*. Furthermore, the presence of a specific strain in food does not exclude the presence of another strain given the documented characteristic of coexistence between several *Listeria* species (Johnson *et al.*, 1990). A further topic to consider is the consumer's tendency to buy new types of

diets, for example, pet food derived by food surplus (sustainable feed) (Castrica *et al.*, 2018) or raw diets consisting of bones and raw food where many bacterial pathogens (especially *Salmonella* spp.) are commonly present.

In this new type of pet food, the microbiological safety of feed is very important because it could be a potential source for several zoonotic pathogens and a public health concern (Weese *et al.*, 2005; Nemser *et al.*, 2014).

The fact that they are dry snacks does not exclude possible bacterial contamination; indeed, strains of *Salmonella* spp., *E. coli*, and *Listeria* spp. inoculated on dry nuts have been observed to decrease exponentially over time, but can still be detectable after 1 year (Blessington *et al.*, 2012; Kimber *et al.*, 2012).

Contamination and bacterial growth are much easier in raw and/or moist pet food; this consideration also applies to food for human consumption. Very often, dry pet food with low water activity (aw: about 0.5, moisture of 10%) remains stable with inhibited bacterial growth until it is accidentally or voluntarily rehydrated (e.g., food rewetted accidentally during storage or by the pet owner to increase the food's softness or palatability or by the pet's drinking water or saliva). The addition of water in contact with the rich substrate of pet food at room temperature can potentially create conditions favorable to bacterial growth.

The European Pet Food Industry Federation (FEDIAF) and the American Feed Industry Association (AFIA) have issued good manufacturing guidelines to minimize microbiological risks in animal feed during manufacturing. It might be prudent to extend this approach to the postproduction phases.

Conclusions

Our findings did not highlight a situation of health and hygiene noncompliance in the pet food industry. However, on analyzing the pet food sector, habits of dog owners, and several case studies and comparing them with the results we have obtained, it emerges that it may be prudent to (1) provide a preventive approach concerning the risk of introduction into the home environment of pathogens transmissible to humans, (2) create awareness in dog owners of the risks associated with incorrect management of feedstuff, and (3) standardize food safety criteria with those for human food from a "One Health" perspective.

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