

Comprehensive microbiological evaluation of dry foods for growing dogs marketed in Poland

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Abstract: *Comprehensive microbiological evaluation of dry foods for growing dogs marketed in Poland.* Microbiological safety is one of the most important parts of qualitative assessment and monitoring of commercially available products intended for canine nutrition. Twenty commercial dry dog foods formulated for growing dogs were surveyed for the prevalence of bacterial contamination. To assess total plate counts of mesophilic strains, yeasts and molds, *Enterobacteriaceae* family and *Enterococcus* ISO standards were applied. Moreover, the presence of major pathogenic bacteria was evaluated. The growth of molds was detected in five products. *Enterobacteriaceae* strains were identified in 12 examined foods. *Escherichia coli* was identified in four samples. Half of the analyzed foods showed apparent presence of enterococci. All analyzed samples were free from *Staphylococcus*, *Salmonella* and *Listeria* spp. contamination. During microscopic confirmation of suspicious colonies *Bacillus* spp. were identified in seven products. The results of our pilot study allowed to conclude that the principles of good manufacturing practice and hygienic regime were generally respected during factory processing, resulting in a relative low risk, with a clear necessity for continued control.

Key words: microorganism, dog, pet food, safety

INTRODUCTION

The evaluation of the microbiological status of pet foods is an important element of the nutritional safety for animals and health security for owners. Between the harvesting of pet food ingredients, handling and preparation at home, and finally the consumption of the product, there appear multiple opportunities for microbial populations to proliferate.

Modern techniques of dry dog food production aim at preventing any microbial contamination of the final product. But still numerous recalls are being announced, resulting in withdrawal (often voluntary) of particular batches of products, potentially contaminated with *Salmonella* sp., the main bacteriological concern of the pet food sector (Behravesh et al. 2010).

Not many reports have been published to date related to the general microbial safety of specifically dry kibble diets for dogs (Behravesh et al. 2010, Nemser et al. 2014). However, in Poland some compound feed surveys have been previously conducted (Wojdat et al. 2004).

In the literature, the prevailing experimental setup was to compare all types of pet food for both dogs and cats in one study. In our opinion, it is more clear to present results of comprehensive analysis of a particular category of products intended for one species, on the basis of randomly selected group of samples, similar to the approach recently proposed by van Rooijen et al. (2014).

The aim of the present, preliminary study was to ascertain the potential presence of multiple foodborne pathogens in commercial dry products intended for feeding growing dogs.

MATERIAL AND METHODS

Sample collection

Twenty dry dog food products of various brands were purchased in specialized retail stores and through an Internet distribution channel. All examined items were randomly chosen from the previously compiled inventory of extruded products, labeled as intended specifically for young and growing dogs, and are currently available on the market.

Weights of collected bags were from the range of 0.4 to 1.4 kg. After purchasing, all factory-sealed bags were stored in room temperature (approx. 18–22°C) until analysis. The remoteness of best before date from all labels was carefully checked before opening. Right before the moment of sampling, all bags were precautionary gently washed with alcohol according to PN-EN ISO 6887-2:2005.

Procedures of sample analysis

Preparation of test samples and dilutions for microbial examination.

The general preparation for analyses of tested dog foods was performed basing on International Standard ISO regarding microbiology of food and animal feeding stuffs PN-EN ISO 6887-1:2000. Samples of 20 g were aseptically weighed consecutively from each bag and transferred with sterile spoon into 180 ml of aseptic peptone water (bioMérieux, Warsaw, Poland). Of each initial dilution 1 ml was taken for further processing and consequently inoculated into Petri's dishes.

Enumeration of the total count of aerobic microorganisms. This assay aimed to reveal the number of colonies grown on the plate count agar (PCA) (Bio-Rad Laboratories, Hercules, USA) medium after incubation under aerobic conditions in 30°C for 72 h. The procedure was conducted according to PN-EN ISO 4833-2:2013 standard.

Enumeration of the total count of yeasts and molds. Deep culture plate method using Saubouraud agar with chloramphenicol (BTL, Warsaw, Poland) was applied according to PN-ISO 21527-2:2009 standard. Inoculated plates were incubated at 25°C for 5 days. The morphological identification of colonies was performed following the description in Martins et al. (2003).

Enumeration of *Enterobacteriaceae*. These bacteria have ability to ferment glucose with acid production. Two sets of plates with Violet Red Bile Lactose (VRBL) medium (Bio-Rad Laboratories, Hercules, USA) were deep cultured in compliance with PN-ISO 21528-2:2005 standard. The counting was performed after 48 h incubation at 37°C.

Enumeration of coliforms. Endo agar (BTL, Warsaw, Poland) was used to enumerate coliforms in samples accord-

ing to PN-ISO 4832:2007 standard. Lactose fermenting bacteria after incubation at 37°C for 48 h present growth in deep red colonies.

Isolation and enumeration of *Enterococcus* spp. (*E. faecium*). In compliance with PN-EN 15788:2009 standard, Slanetz and Bartley medium (Oxoid, Basingstoke, UK) was used as a selective medium. Plates were incubated at 37°C for 24 h.

Detection of staphylococci. The differentiation of coagulase-positive bacteria is based on Baird Parker Agar with egg-yolk tellurite emulsion (bioMérieux, Warsaw, Poland) according to PN-EN ISO 6888-1:2001 standard. Species that contain lecithinase cause clear zones around the colonies whereas the reduction to elemental Te stains the bacteria black. The potential presence of *Staphylococcus aureus* was tested using catalase test.

Salmonella detection. The following procedure was applied according to PN-EN ISO 6579:2003 standard. Carefully weighed 25 g of the sample was transferred to 225 ml of buffered peptone water and incubated in 37°C for 18 ± 2 h, for pre-enrichment. Approximately 0.1 ml of the suspension was then incubated on Rappaport-Vassiliadis Soy broth (RVS) (Bio-Rad Laboratories, Hercules, USA) in 42.5°C for 24 ± 3 h. Subsequently, the plating on the selective Brilliant Green Agar (BGA) (Oxoid, Basingstoke, UK) and Xylose Lysine Deoxycholate (XLD) (Bio-Rad Laboratories, Hercules, USA) was performed, followed by an incubation at 37°C for 48 ± 3 h and 24 ± 3 h, respectively.

Listeria detection. The procedure for *Listeria* spp. detection was as follows

(PN-EN ISO 11290-1:1999/A1:2005 modified standard): 25 g of the sample was transferred to 225 ml of Half-Fraser broth (Bio-Rad Laboratories, Hercules, USA) (for incubation in 30°C for 24 h), then transferring 1 ml into Fraser's broth (Bio-Rad Laboratories, Hercules, USA) (for incubation in 37°C for 48 h). Subsequently, one loopful from each tube was streaked onto Oxford Listeria Selective Agar Base supplemented with Oxford Listeria Selective Supplement (Merck, Darmstadt, Germany), (in amount of 1 vial/500 ml), and incubated at 37°C for 48 h.

Macroscopic and microscopic identifications. The following features were evaluated in all obtained cultures: color, size and surface type of the colony as well as description of color changes of the diagnostic (Martins et al. 2003, Pitt and Hocking 2009). Mold colonies and questionable bacterial cultures (subsequently to Gram stain test) were screened with MB300 microscope (OPTA-TECH, Warsaw, Poland).

Results were statistically modified using PS IMAGO 3.0 software.

RESULTS AND DISCUSSION

Twenty commercially available dry extruded products intended for young and growing dogs were analyzed for the potential presence of foodborne pathogens of bacterial and fungal origin (Fig.), showing the growth of at least one strain. The highest number of different microbial and/or fungal strains (4) was revealed in samples 4, 17 and 18. Subsequently, in products 1, 3, 8 and 12 the presence of three various pathogenic microorganisms was revealed. In the remaining

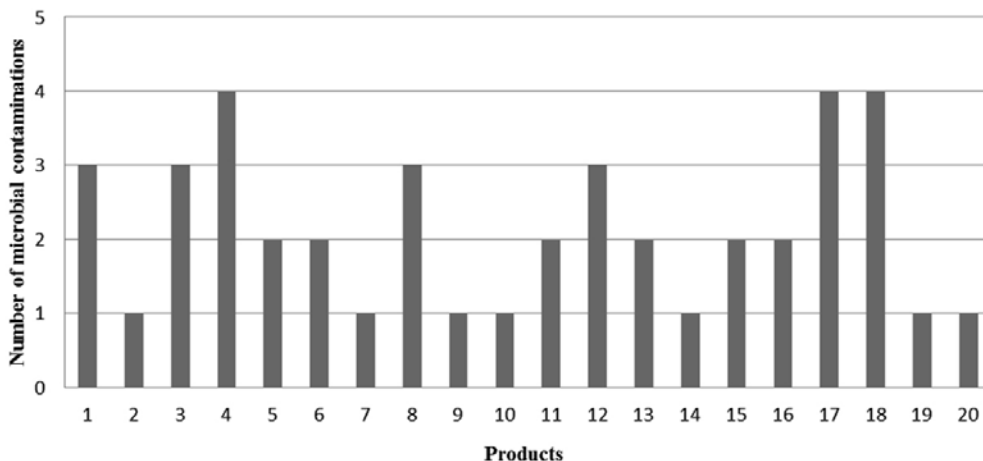


FIGURE. The number of microbiological contaminations detected in dry dog foods

group of products (13) one or two viable microbial strains were detected. In total, 10 different microbiological groups and genera were assayed in the samples.

Typical growth of aerobic bacteria was detected in 15 products studied ranging between 1.0×10^1 to 2.7×10^2 cfu/g (Table). The growth of moulds was detected only in 5 products (25%) with the highest number of 2.0×10^1 cfu/g. The microscopic examination identified *Aspergillus*, *Penicillium* and *Rhizopus* species within the observed colonies. Brewer's yeast were listed as an ingredient on the labels in 8 of 20 analyzed products but no growth of yeast-like colonies was observed. *Enterobacteriaceae* strains were identified in 12 examined products (60%) within the range of 1.0×10^1 – 2.3×10^2 cfu/g. *Escherichia coli* was identified in 4 (20%) samples with relatively low count (10–50 cfu/g). Half of the analyzed foods showed apparent presence of enterococci, with the range of the bacterial presence from 1.0×10^1 to 7.0×10^1 cfu/g. Bacterial

growth, indicating staphylococci presence was noted in 11 samples (55%). However, during macroscopic identification small size of colonies, no changes in the substrate color and the “in substrate” growth were observed. Subsequent negative catalase test results in all 11 samples suggested that the observed growth was more likely a result of anaerobic enterococci presence. All analyzed pet food samples were free from *Salmonella* contamination. No bacterial colony observed both on XLD and BGA media showed typical symptoms of the pathogenic growth. *Listeria* spp. were not isolated from any sample. During microscopic confirmation of suspicious bacterial colonies, stained according to Gram's method, *Bacillus* spp. were identified in 7 products.

It is estimated that currently most of the dry pet foods are produced worldwide with the implementation of extrusion technology. In dry expanded extruded products for pets, the final moisture content has to be lower than 10% to

TABLE. Microbiological presence in dry pet food samples (expressed as log cfu/g)

| Product | TAMBC | Yeasts and molds | <i>Enterobacteriaceae</i> | <i>Escherichia coli</i> | <i>Enterococcus</i> |
|---------|-------|------------------|---------------------------|-------------------------|---------------------|
| 1 | 1.301 | <1 | 2.113 | <1 | 1.477 |
| 2 | <1 | <1 | <1 | <1 | 1.602 |
| 3 | 1.602 | 1.000 | 1.000 | <1 | <1 |
| 4 | 1.301 | 1.301 | 2.301 | 1.477 | <1 |
| 5 | 2.301 | <1 | 2.113 | <1 | <1 |
| 6 | 1.845 | <1 | 2.361 | <1 | <1 |
| 7 | <1 | <1 | 2.361 | <1 | <1 |
| 8 | 1.954 | <1 | 1.000 | <1 | 1.301 |
| 9 | <1 | <1 | 1.602 | <1 | <1 |
| 10 | <1 | <1 | <1 | <1 | 1.000 |
| 11 | 2.000 | <1 | <1 | <1 | 1.698 |
| 12 | 1.000 | 1.000 | <1 | 1.000 | <1 |
| 13 | 1.301 | 1.000 | <1 | <1 | <1 |
| 14 | 1.954 | <1 | <1 | <1 | <1 |
| 15 | 1.301 | <1 | <1 | <1 | 1.845 |
| 16 | <1 | <1 | 1.000 | <1 | 1.301 |
| 17 | 1.903 | <1 | 1.301 | 1.000 | 1.477 |
| 18 | 2.431 | <1 | 1.477 | 1.698 | 1.000 |
| 19 | 1.000 | <1 | <1 | <1 | <1 |
| 20 | 1.602 | <1 | <1 | <1 | <1 |

TAMBC – total aerobic mesophilic bacteria count; <1 – under the detection limit (10 cfu/g).

prevent mold and bacterial growth. The hygienic quality of the final product is affected by the process of conditioning of raw materials with the use of heat, water, pressure and time (Thomas et al. 1997). The producer has to guarantee that within the shelf-life period that is declared on the label, the product will maintain its microbiological safety at specific storage conditions. This declaration is based on identified hazards for the product, heat or other preservation treatments and packaging methods and materials. Microbiological evaluation is

a part of raw ingredients vendor control programs, routinely implemented in pet food producing plants, as well as finished product quality testing procedures.

The recent highly publicized outbreaks and recalls have caused a major review of microbiological control programs and reinforced the idea of going beyond traditional factory quality management processes.

Since 1920, various additives have been used in animal foods. The terms additive and preservative are often perceived synonymously. The latter has

highly negative implication for consumers (pet owners/parents) who are making purchase decisions. However, Annex I to the Regulation (EC) 1881/2006 on additives used in animal nutrition, lists preservatives in the category of “technological additives” defining them as substances or, when applicable, micro-organisms which protect feed against deterioration by micro-organisms or their metabolites’.

On the other hand, obligatory declaration of the content of micro-organisms that have a positive, stabilizing effect on the gut flora, added to the formulation must be placed on the label according to Regulation (EC) 767/2009.

In fact, regarding merely few additives with the maximum legal limits from “preservatives” and “micro-organisms” groups, the content declaration on the label is mandatory. Another Regulation (EC) 1831/2003 allows using exclusively names of functional groups on the label. Other feed additives used can be voluntary declared on the labels.

In the current study all positive samples of dog foods showed low levels of contamination regardless of the strain or species assayed. These results confirm earlier reports, describing the Polish compound feed market as generally safe (Wojdat et al. 2004). It has to be stated however, that precise determination of the microbiological safety in the category of dry dog foods based on combined results of the studies cited above is unfeasible. Extruded dry canine diets were previously considered not a good substrate for microbial growth (Adelantado et al. 2008).

Considering this, it could be anticipated that in majority of dry products

commercially available, various additives preventing growth of bacterial and fungal cells were present.

An analysis of the labels content of foods assessed in the current study revealed few examples of voluntary declaration of such substances. On the majority of the labels (13 of 20; 65%) the presence of antioxidants and/or preservatives was declared. In two cases an additional note of UE approval of chemicals used was found. One producer declared citric acid used as a “natural” preservative and on another list of technological additives pentasodium triphosphate was reported.

In the current study two products listed “dried *Enterococcus faecium* fermentation product” after the ingredients heading. Interestingly, its presence was experimentally confirmed in only one of the two products. Moreover, the *E. faecium* strain was detected in 8 more products, most likely due to the cross contamination during processing or incorrect labeling.

Among the probiotic bacterial species, those of the genus *Bacillus* are not the most commonly used, but their use and effectiveness are documented in numerous reports (Biourge et al. 1998). Their apparent presence in 7 of the assayed products in this study may be the result of their ubiquity in the environment, as much as an apparent reflection of quality control point weaknesses in the processing plant.

It has to be noted, that the strict regulations overseeing maximum limits of particular bacterial and fungal contamination for pet foods have not been yet established. For example the Regulation (EC) 183/2005 stating the general

rules of feed hygiene does not apply to retail pet food.

Apparently more relevant is the Commission Regulation (EU) 142/2011, concerning health rules of animal by-products and derived products not intended for human consumption. According to this document, in processed petfood *Salmonella* has to be absent in 25 g randomly-taken sample, whereas *Enterobacteriaceae* must not exceed 3.0×10^2 in 1 g. In the current study, contamination with *Enterobacteriaceae* was relatively high (2.3×10^2 in products 6 and 7) in 2 products, yet the threshold was not exceeded. This observation, along with the complete absence of *Salmonella* in the examined products likely confirms previously reported general ascertainment that dry dog foods pose low microbiological threat for animals and humans (Adelantado et al. 2008).

In the fungal flora category, one study reported the contamination of various dry pet foods ($n = 20$) within the range of 10^1 – 10^2 cfu/g underlining low levels of mold presence (Martins et al. 2003). Our assessments are even more encouraging considering that within the products for young and growing dogs in only one sample the cfu count for molds was above 10.

In the recently published paper, both human and pet *Salmonella* exposure associated with dry pet food was estimated. The strengths and weaknesses of current production processes and consumer handling in household were highlighted including a discussion of an ingredients-to-consumer quantitative assessment model. Authors pointed to an urgent need for more information on contamination levels in ingredients and

validation studies of production steps critical to microbial reduction and in-plant cross-contamination prevention (e.g. pre-conditioning, oven drying), as well as increased understanding of cross-contamination routes were would likely improve exposure estimates. Maintaining proper hygienic behavior by consumers can also be crucial in lowering the likelihood or the extent of potential exposure. However, the accuracy, precision, and overall usefulness of these models is highly dependent on the availability and quality of input data. Therefore, conclusions from modeling exercises should be drawn with caution (Lambertini et al. 2016).

CONCLUSION

Microbiological safety control during the production of complete dog foods with low water activity requires scrutinizing the ingredient quality, numerous microbial reduction steps, avoidance of potential cross-contamination and a constant control of moisture. This report presents results that support a relative low risk linked with the dry complete foods for growing dogs. Similar methodological approach, applied to other categories like adult, senior or special pet foods, may improve quality control efficacy of in-plant processing steps that would accordingly improve pets and pet owners safety.

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Streszczenie: *Kompleksowa ocena jakości mikrobiologicznej dostępnych w Polsce suchych karm dla psów rosnących.* Jakość mikrobiologiczna to jedno z najistotniejszych kryteriów kontroli systemu produkcji i dystrybucji rynkowej produktów żywienia psów. Dwadzieścia dostępnych w sprzedaży produktów dla psów rosnących poddano ocenie czystości mikrobiologicznej. Analizy przeprowadzono ściśle według metodyki zgodnej z aktualnymi normami PN-ISO dotyczącymi oznaczania bakterii mezofilnych, drożdży i pleśni, przedstawicieli rodzin *Enterobacteriaceae* oraz *Enterococcus*. W badaniu oznaczono również obecność ważniejszych organizmów patogennych. Wzrost pleśni stwierdzono w pięciu przypadkach. W dwunastu produktach zaobserwowano bakterie z rodziny *Enterobacteriaceae*. Połowa spośród ocenianych karm okazała się pozytywna pod względem enterokoków. Wszystkie oceniane

produkty były wolne od *Staphylococcus*, *Salmonella* i *Listeria* spp. Dla siedmiu produktów przeprowadzono obserwacje mikroskopowe podejrzanych kolonii, identyfikując je jako *Bacillus* spp. Uzyskane w niniejszym, pilotażowym badaniu wyniki pozwalają stwierdzić, że kluczowe elementy dobrych praktyk produkcyjnych są na ogół zachowywane podobnie jak kryteria higieniczne podczas procesów technologicznych. Ze stosowaniem karm suchych wiąże się relatywnie małe ryzyko wystąpienia u psów poważnych schorzeń o podłożu mikrobiologicznym, co nie wyklucza konieczności stałego monitoringu jakości produktów dostępnych na polskim rynku.

Słowa kluczowe: mikroorganizm, pies, karma dla pupila, bezpieczeństwo

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