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Investigation of microbiological safety of dry cat foods marketed in Poland

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Pets are inhabiting more and more human homes every year. In 2020, the cat population in Europe was 110 million, including 6.8 million in Poland. Dry food is the most popular dietary model for cats because of its easy storage and efficient satisfaction of pet needs. The high processing temperature of dry food reduces the chance of microbial contamination, but this can occur later, during post-production or storage in the pet's caregiver's home or, in the case of weighed foods, in the store. The purpose of this study was to investigate the microbiological safety of dry feed sold in the original manufacturer's packaging and the same feed from the same manufacturers sold in a retail store by weight. Six discriminants, presence of *Salmonella* spp., number of coliforms, number of coagulase-positive staphylococci, determination of yeast and mould counts, *Enterobacteriaceae* count, *Listeria monocytogenes* and determination of total aerobic microbial count were used for the analysis. Then, cat food was then stored for 45 days according to the manufacturer's recommendations. Based on the samples tested both after opening and after storage, it was concluded that the dry cat food analyzed posed a law microbiological risk to animals and humans.

Keywords: microbiological safety, dry cat food, pet food safety, microbiology of food, cat

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Abbreviations: CFU, colony-forming unit; FEDIAF, European Pet Food Industry Federation; GMP, good manufacturing practices; MRSA, Methicillin-resistant *Staphylococcus aureus;* MKTTn, Muller-Kauffmann Tetrathionate-Novobiocin; RASFF, Rapid Alert System for Food and Feed; RSV, Rappaport Vassiliadis Soya; XLD, Xylose Lysine Deoxycholate; SS, Salmonella Shigella; VRBL, Violet Red Bile with Lactose, VRBG, Violet Red Bile with Glucose; TAMC, total aerobic microbial count

INTRODUCTION

According to statistics presented by FEDIAF, within the European Union, 88 million households had pets and the cat population was 110 million in 2020. In Poland, there are 6.8 million cats and more than one-third of households in Poland have at least one cat (FEDIAF, 2020). By 2020, pet food sales were 21.8 billion Euros, representing 8.5 million tons of products annually. Proper feeding of animals, including cats, is essential for a healthy and long life. The development of research and access to information has increased pet owners' awareness of the quality of food provided to their pets (FEDIAF, 2020). In the European Union, the Rapid Alert System for Food and Feed (RASFF, 2021) is responsible for controlling the safety of raw materials and food products. According to this organization, pet food can be a significant source of many risks, both biological, chemical, and physical (RASFF. The Rapid Alert System for Food and Feed. Annual Report, 2020). These hazards may be related to diseases and injuries that occur in pets. The foundation for maintaining nutritional safety is compositional and nutrient analyses, as well as microbiological evaluation. Despite the use of the latest dry food production methods to prevent contamination, recalls of a particular batch of products due to microbiological contamination are still evident (Kępińska-Pacelik & Biel, 2021).

Dry food is a regular part of the diet of both cats and dogs. This type of food dominates the market due to its ease of storage and efficient satisfaction of the pet's needs. It is processed at a temperature of 80–160°C (Meineri *et al.*, 2019) which significantly reduces the number of pathogenic microorganisms, however, the product may be contaminated at a later stage of production (Kazimierska *et al.*, 2021). The occurrence of pathogenic microorganisms is associated with cross-contamination and deviation from good manufacturing practices (GMP) (Meghwal *et al.*, 2017). Good microbiological quality of food is a major factor, along with the nutritional value of food, to produce healthy and safe food (Chlebicz & Śliżewska, 2018). In recent years, reports of pathogenic microorganisms (bacteria, fungi, and the toxins they produce) have accounted for about 20% of all RASFF food and feed reports, showing, in particular, the presence of *Salmonella*, *Listeria*, *Escherichia,* and others (Pigłowski, 2019).

There have been studies on the microbiological safety of dry dog food (Hołda *et al.*, 2017; Kazimierska *et al.*, 2021) and livestock feeds (Hoszowski *et al.*, 2012; Kukier *et al.*, 2012) in Poland, but no studies have focused on dry cat food in Poland. Previous international studies have also focused on the study of animal foods in general (Błajet-Kosicka *et al.*, 2014; Leiva *et al.*, 2019), without distinguishing between cat and dog foods. However, it was reported that there is a need to distinguish between studies on these two species (Hołda *et al.*, 2017; van Rooijen *et al.*, 2014), which differ not only in their nutritional needs but also in their behavioral patterns towards humans and other animals, which affects the possibility of infection risk.

The study aimed to evaluate dry food for adult cats, with a focus on (1) assessing their microbiological safety, (2) comparing microbiological safety in food sold in a

sealed pack and by weight, and (3) assessing their microbiological safety after 45 days.

MATERIALS AND METHODS

Materials

Microbiological analysis was performed on 6 commercially available dry, whole-food cat foods, including 5 international brands and 1 available on the local market. The criterion for the selection of a particular pet food was its availability for sale both in the form of manufacturer-sealed packages and the availability of the same food sold by weight and packaged at points of sale. All feeds were purchased from a specialized pet store located in the city of Szczecin. To obtain reliable results, five packages (n=5) of each feed were purchased for analysis in five replicates. Prior to purchase, the shelf life was checked and the condition of the packaging was assessed for damage that could affect the microbiological quality of the product tested. The purchased assortment for testing was divided into two groups. The first group consisted of pet food purchased in the manufacturer's original packaging. While the second group consisted of pet foods from the same manufacturers sold by weight. The weight of the finished packages ranged from 340 g to 500 g. After microbiological analysis, the tested cat food was stored for 45 days in accordance with the manufacturer's recommendations, the average shelf life of the food after opening was calculated and after that time the food was again subjected to microbiological tests.

Storage of samples for tests

In the first stage of microbiological tests, both commercial feed from the original manufacturer and feed purchased by weight (collected in sterile bags closed with string) were stored at room temperature (18–22°C). In the second stage of the study (after opening the package), the shelf life of the food was 45 days.

Preparation of samples for testing

All packages were washed with alcohol before opening to exclude product contamination by microorganisms on the surface of the package. The general preparation of samples and dilutions to perform microbiological tests were carried out by the International Standard PN-EN ISO 6887-1:2017-05 "Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions". Depending on the standard, 25 g or 10 g of feed was used for analysis in five replicates.

Microbiological analysis

Each sample, depending on the discriminant to be tested, was weighed accordingly and mixed with an appropriate diluent according to the standard and homogenized (time 30 seconds, speed 8 strokes/second) in Star-Blender™ Digital Homogenizator (VWR, Pennsylvania, USA).

Detection of *Salmonella* spp.

The test was performed according to the PN-EN ISO 6579-1:2017-04 standard. By mixing 25 g of the sample with 225 mL of buffered peptone water (Scharlab, Barcelona, Spain), a stock suspension was obtained, which was incubated at 37°C±1°C for 18 h±2 h for pre-enrichment in non-selective liquid medium. Then 0.1 mL of the obtained culture was transferred to 10 mL of Rappaport Vassiliadis Broth (RVS) medium (Scharlab, Barcelona, Spain) and 1 mL to 10 mL of Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) Broth medium (Graso, Starogard Gdański, Poland). The inoculated RVS medium was incubated at 41.5°C for 24 h±3 h, while the inoculated MKKTn medium was incubated at 37°C for 24 h±3 h. The material obtained from RVS and MKKTn cultures was seeded onto two selectively isolating media, Xylose Lysine Deoxycholate (XLD) agar (Scharlab, Barcelona, Spain) and Salmonella Shigella (SS) agar (Scharlab, Barcelona, Spain), which were incubated at 37°C for 24 h±3 h.

Number of coliforms

The test was performed according to the PN-ISO 4832:2007 standard. Pre-suspension of 1 mL obtained after mixing 10 g of the sample with 90 mL of the dilution fluid was transferred onto two sterile Petri dishes. Then about 15 mL of Violet Red Bile with Lactose (VRBL) agar medium (BioMaxima, Lublin, Poland) was added to the plate. After complete solidification, a top layer of about 5 mL of the same medium was added to obtain relatively anaerobic growth conditions. The plates were incubated at 37°C for 24 h±2 h.

Number of coagulase-positive staphylococci (CoPS), *Staphylococcus aureus*, and other species

The test was performed according to the PN-EN ISO 6888-1:2001 standard. A surface culture of 1 mL of the pre-suspension on Baird-Parker agar medium (Scharlab, Barcelona, Spain) was performed. To obtain 1 mL of the test sample, 0.33 mL of the initial suspension was inoculated onto the surface of three small agar plates (90 mm). The plates were incubated at 37°C for 24 h±2 h and then the incubation was prolonged for another 24 h±2 h. The colonies obtained were checked by coagulase test.

Determination of yeasts and moulds counts

Dry cat food is characterized by low water activity; therefore, the PN-ISO 21527-2:2009 standard for products with water activity lower or equal to 0.95 was applied to determine the number of yeasts and moulds. Pre-suspension of 0.1 mL (10 g of the product was mixed with 90 mL of 0.1% peptone water) was inoculated onto the surface of a DG-18 agar plate (Graso, Starogard Gdański, Poland). The plates were incubated at 25°C±1°C for 5 to 7 days.

Enterobacteriaceae count

The test was performed according to PN-EN ISO 21528-2:2017-08. Pre-suspension of 1 mL obtained after mixing 10 g of the sample with 90 mL of dilution fluid was applied to a sterile Petri dish. Then about 15 mL of Violet Red Bile with Glucose (VRBG) agar medium (BioMaxima, Lublin, Poland) was added to the plate. After complete solidification, a top layer of about 5 mL of the same medium was added to obtain relatively anaerobic growth conditions. The sample was performed in duplicate, according to PN-EN ISO 7218:2008/A1:2013-10. The plates were incubated at 37°C for 24h±2h.

Determination of total aerobic microbial count (TAMC)

The test was performed by depth culture according to the PN-EN ISO 4833-1:2013-12 standard. Pre-suspension of 1 mL was transferred to two sterile Petri dishes each and then about 15 mL of Plate Count Agar medium (BioMaxima, Lublin, Poland) was added. Observations were made after 72 hours of incubation at 30°C, under conditions that ensure the growth and multiplication of aerobic bacteria.

Listeria monocytogenes

Detection of *Listeria monocytogenes*

The test was performed according to PN-EN ISO 11290-1:2017-07. 225 mL of Semi-Fraser Broth (Graso, Starogard Gdański, Poland) was added to 25 g of product. The resulting stock suspension was incubated at 30°C for 25 h±1 h. Subsequently, 0.1 mL of the obtained culture was transferred to 10 mL of Fraser medium (Graso, Starogard Gdański, Poland) and a scratch culture was performed on two selective media ALOA (BioMaxima, Lublin, Poland) and Oxford (BioMaxima, Lublin, Poland). The inoculated Fraser medium was incubated at 37°C for 24 h ±2 h, while the inoculated ALOA and Oxford media were incubated at 37°C for 24 h±2h–48 h±2 h. The material obtained from the culture on Fraser broth was streaked onto two selective media ALOA (BioMaxima, Lublin, Poland) and Oxford (BioMaxima, Lublin, Poland), and then incubated at 37°C for 24 h±2 h–48 h±2 h.

Number of *Listeria monocytogenes*

The test was performed in accordance with PN-EN ISO 11290-2:2017-07. A surface culture of 1 mL of the initial suspension including 10 g of sample and 90 mL of half-Fraser (Graso, Starogard Gdański, Poland) broth on ALOA (BioMaxima, Lublin, Poland) agar medium was performed. The seeded plates were incubated at 37°C for 24 h±2 h–48 h±2 h.

Calculation of results

The results were calculated and presented according to PN-EN ISO 7218:2008/A1:2013-10, using the formula:

$$N = \frac{\sum C}{V \times [n1 + (0{,}1 \times n2)] \times d}$$

where:

∑C – total colonies on two selected plates from two successive dilutions, of which at least one contains a minimum of 10 colonies; V – the volume of inoculum applied on each plate, in mL; n1 – number of plates obtained from the first dilution; n2 – number of plates obtained from the second dilution;d – the dilution index corresponding to the first dilution obtained.

If less than 10 colonies were obtained per plate, but the set of two plates contained at least 4 colonies, the result was calculated using the formula:

$$N = \frac{\sum C}{V \, n \, d}$$

where:

∑C – the sum of colonies counted on two plates; V – the volume of inoculum applied on each plate, in mL; n – number of plates; d – the dilution index corresponding to the first dilution obtained.

Table 1. Microbiological analysis of the tested commercial cat foods was performed after opening.
The table shows the mean obtained from 5 replicates of the analysis of one batch of feed.

No.	*Salmonella* spp.	Coliforms	CoPS	Yeasts and moulds	Enterobacteriaceae	TAMC	L. monocytogenes
	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)
1a (n=5)	ND	ND	ND	ND	ND	$8.3x10^2$	ND
1b (n=5)	ND	ND	ND	ND	ND	$3.0x10^1$	ND
2a (n=5)	ND	ND	ND	ND	ND	$6.2x10^2$	ND
2b (n=5)	ND	ND	ND	ND	ND	$1.2x10^3$	ND
3a (n=5)	ND	ND	ND	ND	ND	$1.3x10^3$	ND
3b (n=5)	ND	ND	ND	ND	ND	$1.0x10^3$	ND
4a (n=5)	ND	ND	ND	ND	ND	$3.8x10^3$	ND
4b (n=5)	ND	ND	ND	ND	ND	$8.1x10^3$	ND
5a (n=5)	ND	ND	ND	ND	ND	ND	ND
5b (n=5)	ND	ND	ND	ND	ND	$5.7x10^1$	ND
6a (n=5)	ND	ND	ND	ND	ND	ND	ND
6b (n=5)	ND	ND	ND	ND	ND	$9.7x10^2$	ND

TAMC – total aerobic microbial count; a – food in original packaging; b – food purchased by weight; ND – not detected.

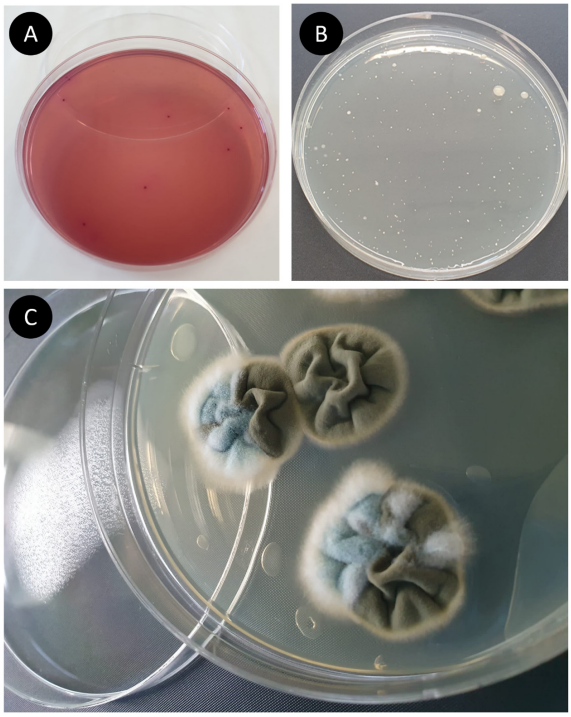

Figure 1. Example photos of culture plates showing: (A) Enterobacteriaceae, (B) TAMC, (C) yeasts and molds.

Statistical analysis

The statistical analysis was performed using Tibco Statistica 13.3 (StatSoft, Palo Alto, CA, USA). The values of the parameters were presented as arithmetic means. The normality of variable distributions was verified by the Shapiro–Wilk Test. Data with a normal distribution were analyzed using the Student's *t*-test and the Mann-Whitney U test was used for data with a non-normal distribution.

RESULTS

The results are presented in Table 1. For quantitative methods (coliforms, CoPS, yeasts and moulds, *Enterobacteriaceae*, TAMC, *Listeria monocytogenes*), the notation "not detected" was used for standardization when a result $<1.0\times10^1$ cfu/g was obtained. For the qualitative method in *Salmonella* spp. and *Listeria monocytogenes* the result was "not detection in 25g" with the laboratory's limit of detection (LOD) obtained at 5 cfu/g for *Salmonella* spp. and 7 cfu/g, for *Listeria monocytogenes*. Therefore, no growth of coliforms, *Salmonella* spp., or CoPS, yeasts and moulds, *Enterobacteriaceae* and *Listeria monocytogenes* were observed. Typical growth of aerobic mesophilic microorganisms was obtained in 10 out of 12 samples (83%).

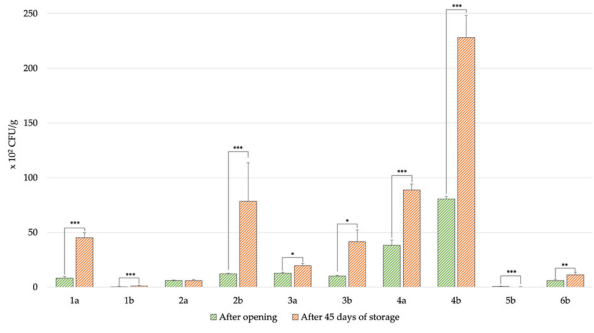

Figure 2. Comparison of total aerobic microbial count in the tested feeds. *$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.0001$

After 45 days of storage, tests were carried out again. No growth of *Salmonella* spp., coliforms, or CoPS was detected. Growth of yeast and moulds was observed in 3 out of 12 samples (25%, Fig. 1C). Growth of *Enterobacteriaceae* was observed in 1 out of 12 samples (8%, Fig. 1A). No growth of *Listeria monocytogenes* was observed. Detailed results are presented in Table 2. Statistical analysis was carried out to compare results from open and stored feeds. The differentiators at which changes were observed were compared: yeasts and moulds, Enterobacteriaceae, and TAMC. Statistical significance was then verified. For yeasts and moulds and Enterobacteriaceae, the samples reached a statistical difference. In the comparison of TAMC in the two groups, 9 out of 12 trials achieved a statistical difference (Fig. 2).

DISCUSSION

Although the food is processed at high temperatures that destroy microorganisms, still spore forms may be retained, and the contamination itself may occur after the production, for example during storage of the food in a retail shop or at home (FEDIAF, 2018). FEDIAF in its list of hazards during the production of dry pet food in the biological nature of hazards mentions *Aeromonas, Campylobacter, Clostridium botulinum, Clostridium perfringens, Enterobacteriaceae, Escherichia coli, Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus,* and moulds and yeasts including mycotoxins (FEDIAF, 2018). However, not all of them concern the finished dry food product, but also the raw materials used in its production. For this reason, the organization suggests monitoring the microbiological status by testing environmental samples obtained from surfaces not in and in contact with the product and samples of finished feeds (FEDIAF, 2018). The exact requirements for the production facility are set out in the hazard analysis and critical control points (HACCP) procedures. The procedures based on HACCP principles and other regulations stem from Regulation (EC) No 1069/2009 of 21 October 2009 and Commission Regulation (EU) No 142/2011 of 25 February 2011 (Osinski *et al.*, 2014). These include many areas related to pet food production. In terms of microbiological safety. The regulation sets out the following standards that a product must meet after production: the product must be packaged in packaging which is protected against the intrusion of microorganisms, and 5 samples of the product tested for *Salmonella* spp. in 25 g must not show the presence of these bacteria (n=5, c=0, m=0, M=0). The result for *Enterobacteriaceae* is considered satisfactory if the number of bacteria in all samples does not exceed 10 cfu/g (m) or in two samples the result is between 10 and 300 cfu/g (c) if, in the remaining samples, the value obtained does not exceed 10 cfu/g. The result is considered abnormal if the number of bacteria in one or more samples equals or is greater than 300 cfu/g (M) – (n=5, c=2; m=10; M=300 in 1 g) (European Commission, 2011).

Particularly after 2012, when there were two major outbreaks of human salmonellosis in the US caused by contaminated pet food products, attention was directed toward this pathogen (Chen *et al.*, 2019). Actually, in most previous studies conducted on dry commercial pet

Table 2. Microbiological analysis of the tested commercial cat foods was performed after 45 days of storage.
The table shows the mean obtained from 5 replicates of the analysis of one batch of feed.

No.	*Salmonella* sp.	Coliforms	CoPS	Yeasts and moulds	Enterobacteriaceae	TAMC	L. monocytogenes
	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)	(*CFU/g*)
1a (n=5)	ND	ND	ND	$2.2x10^3$	ND	$4.5x10^3$	ND
1b (n=5)	ND	ND	ND	$1.4x10^2$	ND	$1.1x10^2$	ND
2a (n=5)	ND	ND	ND	ND	ND	$6.0x10^2$	ND
2b (n=5)	ND	ND	ND	ND	ND	$1.4x10^4$	ND
3a (n=5)	ND	ND	ND	ND	ND	$2.0x10^3$	ND
3b (n=5)	ND	ND	ND	ND	ND	$4.2x10^3$	ND
4a (n=5)	ND	ND	ND	ND	ND	$8.9x10^3$	ND
4b (n=5)	ND	ND	ND	ND	$6.5x10^3$	$2.3x10^4$	ND
5a (n=5)	ND	ND	ND	ND	ND	ND	ND
5b (n=5)	ND	ND	ND	ND	ND	ND	ND
6a (n=5)	ND	ND	ND	ND	ND	ND	ND
6b (n=5)	ND	ND	ND	$2.3x10^3$	ND	$1.1x10^3$	ND

TAMC – total aerobic microbial count; a – food in original packaging; b – food purchased by weight; ND – not detected.

foods, no *Salmonella* sp. was reported, which is consistent with the results of our study (Hołda *et al*., 2017; Kazimierska *et al*., 2021; Leiva *et al*., 2019; Nemser *et al*., 2014; Taymaz *et al*., 2022; Yang *et al*., 2016). As the pathogen causes a dangerous disease, salmonellosis, this is good news. In 2020, 5470 cases of *Salmonella* spp. infection was reported in Poland. The chance of infection occurs through human-to-human transmission, animal-to-human transmission, also through contaminated food or drinking water (Milczarek *et al*., 2022). Cases of the spread of *Salmonella* spp. from cats to other animals and humans have been described. In cats, salmonellosis is mainly manifested by acute enteritis (diarrhea, vomiting, fever, inappetence, abdominal pain, dehydration, lethargy) that may progress to septicemia. Conjunctivitis, neutropenia, excessive salivation, fever without diarrhea, and uterine infections may also be among the usual clinical signs of the disease. Uterine infections are particularly important for pregnant cats because they can cause, stillbirths, weak offspring or abortions, which can be caused, for example, by *S. typhimurium* and *S. enteritidis* causing acute inflammation of the stomach and intestines, which as it progresses affects the development of the fetus in pregnant animals (Kuria, 2023). In addition to the possibility of *Salmonella* spp. infection through consumption of contaminated food, outdoor cats are particularly at risk, as they may become infected by preying on birds weakened by salmonellosis (Söderlund *et al*., 2019) or drinking water from sources that are not controlled, such as puddles (Kozak *et al*., 2003).

In our study, no growth of coliforms was noted in any sample. This is consistent with previous studies on dry pet food (Hołda *et al*., 2017; Kazimierska *et al*., 2021; Leiva *et al*., 2019; Nemser *et al*., 2014). This is important for animal foods because the consumption of food with pathogenic coliforms by a cat can cause food poisoning. In the case of coliforms, the bacteria can also be transmitted from the cat to humans. It is most commonly transmitted by direct contact with the animal or its excreta, especially in the case of a cut or open wound. In humans, symptoms of infection with pathogenic coliforms include food poisoning, flu-like symptoms, fever, abdominal cramps, and diarrhea (Srikullabutr *et al*., 2021).

In our study, no growth of CoPS was noted in any sample. However, they are known to cause disease in livestock and pets, such as methicillin-resistant strains of *S. aureus* (MRSA). In addition, it has been determined that MRSA can contaminate food in food processing plants and slaughterhouses, with its major presence occurring in meat products, the stuff from which pet food is made. The infection can spread from animal to human. MRSA causes infections in humans such as acne, food poisoning, ear infections and septicemia. In cats, MRSA infection leads to food poisoning and pustular dermatitis (Algammal *et al*., 2020). Domesticated animals, cats and dogs, are defined as environmental sources of CoPS (Velázquez-Guadarrama *et al*., 2017).

It was assumed that the number of yeasts and moulds exceeding 10^4 cfu/g indicates the poor microbiological quality of food and levels exceeding the recommended limits to ensure hygienic quality (Kazimierska *et al*., 2021). No yeast or mould growth was observed in the feeds tested after opening. However, they developed after 45 days of storage in 3 of the 12 samples (25%). The highest value was 1.32×10^3 cfu/g, which is within the suggested microbiological quality value. The presence of yeast and mould in pet food has been reported previously (Kazimierska *et al*., 2021; Leiva *et al*., 2019). In

the case of yeast and moulds, the greatest risk is posed by mycotoxins. However, the presence of moulds does not always clearly indicate product contamination with mycotoxins. Specific conditions are needed for the production of toxins (Janik *et al*., 2020). Mycotoxins may contaminate the product both during production (if the food contains cereals) and outside this stage, e.g. during improper storage of the product. However, there is no legislation in Poland setting a maximum level for these in cat food (Błajet-Kosicka *et al*., 2014). Mycotoxins can cause adverse effects, in the worst cases even cancer. While analyzing pet food with a cereal content of 6% or more, data were obtained showing a very high presence of mycotoxins in dry food. and cat food was more contaminated than dog food. Among mycotoxins, cats may be more sensitive to the effects of trichothecenes and fumonisins (Macías-Montes *et al*., 2020). Symptoms of trichothecenes poisoning include immune system disorders loss of appetite, vomiting, diarrhea, ataxia, and gastrointestinal bleeding. Fumonisins, on the other hand, can be responsible for lack of appetite, and blindness. depression, ataxia, and even liver and kidney cancer (Błajet-Kosicka *et al*., 2014).

The number of microorganisms able to grow and form colonies in the solid medium after incubation under aerobic conditions at 30°C in the samples we tested is shown in Fig. 1B. Compared to other studies conducted on dry animal foods (Holda *et al*., 2017; Kazimierska *et al*., 2021), this is not an outlier. TAMB standards for dry pet food are not specified in the guides for pet food manufacturers in Europe and the US (FEDIAF, 2018; Food and Drug Administration, 2022). Thus in animal feeds this value should not exceed 10^6 cfu/g (Kukier *et al*., 2012), because an increase in the total number of mesophilic aerobic microorganisms may increase the probability of pathogenic microorganisms and their toxic metabolites in the product (Kazimierska *et al*., 2021). Our testing for mesophilic aerobic microorganisms resulted in an acceptable level of 10^4 cfu/g.

As in our study, no *Listeria monocytogenes* were found in dry pet food in earlier studies (Bilung *et al*., 2018; Kazimierska *et al*., 2021; Nemser *et al*., 2014). In cats, the disease caused by *L. monocytogenes*, listeriosis, is rare. When infected, it can involve the whole organism, skin wounds, encephalomyelitis, and lymphadenitis (Elbert & Rissi, 2021).

The tested feeds were distinguished by a high level of microbiological safety. Despite the demonstrated increase in the number of microorganisms in tests conducted after 45 days of storage, it is worth emphasizing that this number still remained within acceptable limits in accordance with applicable standards. Carrying out analyzes on feed stored in a manner not recommended by the manufacturer has a significant potential to obtain additional data. Such activities may provide more detailed information on the impact of non-recommended storage on the microbiological quality of feed and possible health and food safety consequences.

Declarations

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Conflict of Interest. The authors declare that they have no competing interests.

Code or data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions. Conceptualization, J.Z.-W. and P.N.-R..; methodology, O.S., J.Z.-W. and P.N.-R..; software, D.B., R.H., F.L.; validation, O.S., J.Z.-W., C.T., A.P.-B., D.B., R.H., and P.N.-R.; formal analysis, J.Z.-W., and P.N.-R.; investigation, O.S., J.Z.-W., C.T., and P.N.-R.; resources, O.S., J.Z.-W., A.P.-N., D.B., R.H. and P.N.-R; data curation, O.S., J.Z.-W., A.P.-N., D.B., R.H., F.L. and P.N.-R; writing – original draft preparation, O.S., J.Z.-W.; writing – review and editing, A.P.-B., D.B., R.H., F.L. and P.N.-R.; visualization, A.P.-B., and R.H.; supervision, P.N.-R.; project administration, P.N.-R.; funding acquisition, P.N.-R. All authors have read and agreed to the published version of the manuscript.

Animal Ethics. The study did not require ethics approval.

Consent to participate. Not applicable.

Consent for publication. Not applicable.

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