

Zoonotic and Qualitative Aspects of Raw Meat-Based Diets for Dogs in The Netherlands: A Follow-Up Study

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Abstract: Background: The Dutch branch organization for pet products promised the public that it will improve the quality of raw meat-based diets (RMBDs) for dogs after several diagnoses of tuberculosis, brucellosis, and hyperthyroidism in dogs fed RMBDs. Objective: The objective of this study was to re-evaluate the risk factors of commercially available raw meat diets for dogs in The Netherlands. Methods: Seven commercial brands of RMBDs that were previously investigated were re-tested, as well as a newly introduced high-pressure processing (HPP) product. Raw beef sausage for humans was included for comparison. In total, 40 animal RMBDs (five batches per product) were tested for the presence of colony-forming units (CFUs), *Salmonella* spp., and *Escherichia coli* directly after defrosting and 4 h later, as well as thyroid hormone. Results: Exceeded EU standards for CFUs and *Salmonella* bacteria were present in several samples. In the HPP product, bacteria were still present; however, the counts were lower. There were no differences in CFUs directly after defrosting and 4 h later. The human raw meat product was negative for bacteria. Thyroid hormone could be detected in 20 out of 37 samples. In seven of these samples, the levels were >0.75 µg/g, which have been associated with hyperthyroidism. Conclusions: The hygiene (including the use of HPP production) and accurate removal of thyroid tissue during the production of RMBDs still need attention to prevent the presence of zoonotic bacteria, high CFUs, and diet-induced hyperthyroidism.



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1. Introduction

Raw meat diets have gained popularity among dog owners in The Netherlands [1]. These raw meat-based diets (RMBDs) are associated with several microbiological risks for dogs and dog owners [2,3]. A previous study evaluated the microbial and parasitological quality of seven commercially available RMBDs in The Netherlands and concluded that most products were contaminated with zoonotic bacteria and parasites [4]. Bacteria found in this study were *E. coli* serotype O157:H7, extended-spectrum beta-lactamase-producing *E. coli*, *Listeria* spp., and *Salmonella* spp. [4]. Other significant zoonotic infections related to raw meat in The Netherlands are *Mycobacterium bovis* [5] and *Brucella suis* [6]. The Dutch findings are similar to reports from Belgium [7], Canada [8], Finland [9], Germany [10], Italy [11], Sweden [12], the UK [13], and the USA [14]. There may be health implications for pets and pet owners if zoonotic pathogens are introduced into the household by RMBDs.

The hygiene quality of 39 RMBDs for dogs in Sweden has been studied, specifically for the presence of *E. coli* with transferable resistance to extended-spectrum cephalosporin (ESC). The results showed that raw food diets could be a source of ESC-resistant *E. coli* for their owners, and they underlined the importance of maintaining good hygiene when handling these products to prevent infection of humans [12]. These hazards are confirmed by a worldwide internet survey of owners that fed RMBDs to their animals, where the zoonotic potential of RMBDs was investigated. Around 39 participants reported proven illnesses of themselves caused by RMBDs [9]. Of course, RMBDs are not intended for human consumption but cross-contamination cannot be excluded since RMBDs are likely to be handled in the kitchen. Kitchen hygiene and the importance of handwashing after handling any potentially contaminated material should be communicated to pet owners feeding their pets RMBDs [4,14]. For hygiene and environmental reasons, all dog feces should be removed regardless of diet type; however, feces of dogs that are fed RMBDs are more likely to be contaminated with pathogenic bacteria compared to feces from dogs that are fed a dry kibble diet [15].

Outbreaks of RMBD-related hyperthyroidism in dogs have also been described [16–20]. The clinical signs and plasma thyroid hormone (T4) levels recovered to normal after changing the diet to a commercial dry food. Analysis of the RMBDs revealed high levels of T4, which could be due to the presence of thyroid tissue in these RMBDs [20].

In The Netherlands, a lot of media attention on safety issues regarding RMBDs for dogs has stimulated the pet food industry to use techniques from the human food industry, such as high-pressure processing (HPP), to reduce microbiological contamination. This also triggered the Dutch branch organization for pet products (Dierbenodigdheden en voeding (Dibevo)) to promise the public that it will improve the quality of RMBDs for dogs. This is in line with current developments in the pet food industry, as producers of RMBDs are becoming active members within FEDIAF (The European Pet Food Industry) and the PFMA (Pet Food Manufacturers Association), to improve the quality and safety of these RMBDs. Therefore, this study aimed to re-evaluate the risk factors of commercially available raw meat diets for dogs in The Netherlands.

2. Materials and Methods

2.1. Sample Selection and Processing

Eight brands of RMBDs were tested: seven from a previous study [4] as well as a new product produced with HPP. For each brand, five packages of a single type of product of different batches with different production dates were purchased. After purchase, the products were transported with icepacks and immediately stored in the freezer at $-20\text{ }^{\circ}\text{C}$ in our lab. Raw beef sausage intended for human consumption was purchased and stored in the fridge at $4\text{ }^{\circ}\text{C}$. The compositions of the products were as follows (based on package and website information, not further specified/quantified): brand 1: beef, chicken, beef organs, tripe, salmon oil, vitamins, and minerals; brand 2: chicken, tripe, beef (26% minimum beef), beef liver, beef kidney, beef spleen, vitamins, minerals, and sunflower oil; brand 3: beef, chicken, vegetables, rice, cold-pressed vegetable oil, vitamins, and minerals; brand 4: beef (63%), beef tripe, beef heart (7%), sheep fat, rice, cold-pressed sunflower oil, cold-pressed linseed oil, vitamins, minerals, and trace elements; brand 5: beef (79%) (beef tripe (26%), beef lung, beef fat, beef meat, beef collagen), carrots, spinach, vitamins, minerals, pea fibers, and linseed oil; brand 6: beef 37% (muscle meat, kidney, lung, liver), chicken 35% (muscle meat, carcass), rice, zucchini, broccoli, spinach, peas, pumpkin, chicory pulp (dried), rapeseed oil, fish oil, beet pulp (dried), vitamins, and minerals, produced with HPP; brand 7: chicken 52% (back, skin, muscle meat), beef 36% (tripe, lung, kidney, liver, fat), corn, beet pulp (dried), salmon oil, and sunflower oil; brand 8: beef 56% (muscle, tripe,

liver, heart, lung, kidney, spleen), chicken 18% (back, muscle meat), flakes, sunflower oil, vitamins, and minerals.

2.2. Bacterial and T4 Examination

The overall microbiological quality was determined by the culturing of aerobic bacteria and the presence of coliforms, as described previously [4]. A detailed description can be found in the Supplementary File 1. In short, before analysis, the frozen products, all packaged in vacuum-sealed plastic, were thawed under running tap water at room temperature. All products were processed while still cold (0–4 °C) to prevent substantial bacterial growth for one sample (direct defrosting), and the other sample was thawed for 4 h at ambient temperature (delayed defrosting) to resemble feeding practices used when feeding raw pet foods at home. This distinction was made to enable investigating whether the level of contamination could be influenced by the method of defrosting.

The samples were tested for *Salmonella* spp. according to the Horizontal method for the detection of *Salmonella* species (ISO 6579:2002 +A1:2007) [21]. A detailed description can be found in the Supplementary File 2. When three of the five *Salmonella* indicators were present (LDC test, indole test, β-Galactosidase reagent, TSI, and urease), we tested for *Salmonella* through serology.

The samples were tested for *E. coli* according to the Horizontal method for the detection of *E. coli* (ISO 16654:2001) [22]. A detailed description can be found in the Supplementary File 3. The total colony count of *E. coli* was determined after 24–28 h.

Thyroid hormone analysis of the samples was performed after homogenizing a 5 g sample. A total of 2 mL of ultrapure water was added and mixed by vortex. A total of 15 mL of acetonitrile and 1 mL of concentrated ammonia (25%) solution were added and mixed by vortex. Then, the mixture was centrifuged for 10 min at 4000 × g. The supernatant was transferred into 3 mL tubes and dried overnight in a SpeedVac for 12 h. The pellet was then dissolved in 25 µL of ethanol 100%, followed by 225 µL of T4-free plasma, and centrifuged for 10 min at 4000 × g. The T4 concentration of the supernatant was determined with the use of an Immulite immunoassay. With a 100% recovery, the minimum level of detection is 0.1 µg T4/g tissue. T4-free plasma with ethanol was used as the negative control. A detailed description can be found in the Supplementary File 4. Previous samples that have resulted in clinical signs in dogs (>0.75 µg/g) were used as a positive control [20].

2.3. Statistical Analysis

The results of the colony-forming units (CFUs), *Salmonella* spp., *E. coli*, and T4 counts were compared to the tolerance levels in EU legislation. For CFUs, the maximum tolerance is 5.00×10^6 per gram (Commission Regulation EU142/2011 Annex XIII, 'Petfood and certain other derived products') [23], so all samples exceeding this level are significant. For the comparison of CFUs between direct defrosting and delayed defrosting, a Wilcoxon signed-rank test was performed, as the Shapiro–Wilk test demonstrated that the data were not normally distributed. For *Salmonella* spp., there is a zero-tolerance policy, so all positive samples are significant. For *E. coli*, the maximum tolerance is 5.00×10^4 per gram, so all samples exceeding this level are significant. T4 levels should be below 0.75 µg/g [20].

3. Results

3.1. Quantitative Scores for Aerobic Bacteria (CFUs)

The number of CFUs per brand per batch per gram either after direct defrosting or after delayed defrosting is shown in Table 1. In most cases, the differences between direct defrosting and delayed defrosting were limited, and none of them were significant (Wilcoxon signed-rank test). Noteworthy were the large differences in the number of

CFUs per brand. Furthermore, brands 2, 3, and 4 each had one or more batches exceeding 5.00×10^6 CFUs per gram that thus do not meet the EU standards as stated in Annex XIII, Petfood and certain other derived products, of Commission Regulation (EU) No. 142/2011 [23].

Table 1. Total aerobic bacterial count and *E. coli* directly and 4 h after defrosting, *Salmonella* spp. presence, and thyroid hormone levels of commercially available raw meat-based diets for dogs ¹.

Brand and Batch Code	CFU Direct	CFU After 4 h	<i>E. coli</i> Direct	<i>E. coli</i> After 4 h	T4 (µg/g)
1.1	6.70×10^5	6.23×10^5	6.23×10^2	6.50×10^2	<0.1
1.2	7.20×10^5	7.30×10^5	2.00×10^3	2.75×10^3	<0.1
1.1	6.70×10^5	6.23×10^5	2.00×10^1	5.00×10^1	<0.1
1.2	7.20×10^5	7.30×10^5	1.00×10^1	1.30×10^2	<0.1
1.3	1.70×10^6	8.21×10^5	0.00×10^0	0.00×10^0	<0.1
1.4	5.25×10^4	3.99×10^4	4.60×10^3	4.62×10^3	<0.1
1.5	8.25×10^3	3.30×10^4	1.00×10^2	8.00×10^1	<0.1
2.1	2.76×10^6	2.84×10^6	1.27×10^4	1.14×10^4	ND
2.2	4.80×10^4	6.25×10^4	5.40×10^4	5.30×10^4	5.1
2.3	1.40×10^6	1.73×10^6	7.00×10^3	8.40×10^3	0.2
2.4	6.32×10^6	6.64×10^6	8.00×10^2	9.03×10^2	0.2
2.5	3.20×10^5	4.10×10^5	8.40×10^4	1.43×10^5	<0.1
3.1	2.35×10^5	2.47×10^5	4.50×10^4	7.60×10^4	<0.1
3.2	4.00×10^6	3.68×10^6	4.15×10^3	1.02×10^4	<0.1
3.3	5.28×10^6	5.28×10^6	3.90×10^4	2.20×10^4	<0.1
3.4	4.72×10^6	7.00×10^6	2.90×10^3	2.84×10^3	<0.1
3.5	1.03×10^6	7.30×10^5	7.40×10^4	2.90×10^4	<0.1
4.1	5.00×10^5	4.90×10^5	2.00×10^4	4.30×10^4	ND
4.2	5.00×10^6	5.00×10^6	9.30×10^4	9.10×10^4	0.5
4.3	2.19×10^6	2.69×10^6	1.36×10^5	1.38×10^5	0.3
4.4	3.84×10^6	3.31×10^6	2.00×10^2	2.21×10^2	0.5
4.5	5.24×10^6	4.00×10^6	1.70×10^2	1.35×10^2	0.4
5.1	1.08×10^5	1.12×10^5	3.00×10^4	2.30×10^4	0.0
5.2	3.20×10^5	1.24×10^5	0.00×10^0	0.00×10^0	2.6
5.3	1.96×10^6	8.10×10^5	1.00×10^1	1.00×10^2	1.4
5.4	4.20×10^6	3.40×10^6	1.10×10^2	1.16×10^2	1.9
5.5	2.15×10^4	9.98×10^3	5.00×10^1	6.00×10^1	0.1
6.1	3.85×10^4	3.92×10^4	0.00×10^0	0.00×10^0	<0.1
6.2	6.75×10^4	5.35×10^4	4.00×10^1	7.50×10^1	<0.1
6.3	4.35×10^3	6.55×10^3	1.00×10^1	1.00×10^2	<0.1
6.4	4.07×10^4	2.55×10^4	1.80×10^3	2.10×10^3	0.3
6.5	2.15×10^4	9.98×10^3	4.45×10^2	4.15×10^2	<0.1
7.1	8.75×10^4	9.02×10^4	9.00×10^1	2.10×10^2	ND
7.2	5.80×10^4	7.05×10^4	2.00×10^1	4.00×10^1	0.1
7.3	2.25×10^4	3.00×10^4	9.20×10^2	1.00×10^3	0.2
7.4	3.50×10^4	2.80×10^4	7.23×10^2	7.50×10^2	0.2
7.5	9.06×10^3	1.52×10^4	1.70×10^3	1.10×10^3	0.3
8.1	4.90×10^5	5.23×10^5	4.20×10^2	2.65×10^2	0.1
8.2	5.90×10^5	6.60×10^5	1.70×10^3	1.10×10^3	2.2
8.3	5.90×10^4	6.70×10^4	4.20×10^2	2.65×10^2	1.8
8.4	6.85×10^4	3.99×10^4	0.00×10^0	2.00×10^1	0.2
8.5	3.90×10^5	7.30×10^5	1.60×10^3	2.45×10^3	2.1

¹ Total aerobic bacterial count and *E. coli* in colony-forming units (CFUs) directly and 4 h after de-frosting. *Salmonella* spp. presence has the brand and batch code marked in bold in the first column. Thyroid hormone (T4) in µg/g tissue; values that exceed 0.75 µg/g are marked in bold. ND = not determined.

3.2. *Salmonella* spp.

For brand 3, *Salmonella* spp. bacteria were isolated in two out of five batches. For brands 2, 7, and 8, *Salmonella* spp. were found in one out of five batches (all of these diets contained chicken). In four of the eight brands (brands 1, 4, 5, and 6), *Salmonella* spp. were not present.

3.3. *Escherichia coli*

The *E. coli* count per brand per batch is shown in Table 1. For the third brand, there was a remarkable difference between direct and delayed defrosting, but this was not apparent in the other products. Brands 2, 3, and 4 exceeded the maximum amount as stated in the EU standards for human consumption (500 CFUsg *E. coli*) in two out of five batches [24].

3.4. Thyroid Hormone

Thyroid hormone concentrations are shown in Table 1. Brands 2, 5, and 8 had batches with levels exceeding the maximum limit of 0.75 µg/g. Because of cost limitations, not all batches were analyzed.

4. Discussion

This study aimed to re-evaluate the risk factors of commercially available raw meat diets for dogs in The Netherlands. We hypothesized that the risks would be lower as the branch organization has promised improvements. However, multiple batches of several brands still had CFUs, *Salmonella*, and/or *E. coli* exceeding the maximum tolerance levels; only some brands stayed within the limits for all the parameters tested. Additionally, we tested the effect of two defrosting methods. Delayed defrosting (after 4 h at ambient temperature) does not seem to affect bacterial counts much when compared to direct defrosting. The variations seen in the *E. coli* counts in brand 3 between direct and delayed (after 4 h) defrosting can be explained by the differences between samples within one batch, stressing the importance of homogenization and taking multiple samples within a batch of raw pet food. The safety of products seems to be related to specific brands, so some may have better implemented the hazard analysis critical control points (HACCPs) or were stricter in their control of ingredients and/or end products compared to others. However, due to the small number of observations per brand and the high variance in results, it is impossible to generalize the results. Nonetheless, the indications given by the results are alarming for several of the brands tested.

Whereas most of the RMBDs did not meet EU standards, and all of them had several batches that at least had some CFUs, the raw beef sausage intended for human consumption was not contaminated. This underlines that current techniques enable us to produce completely uncontaminated food. The RMBD produced using HPP (brand 6) scored the lowest on all parameters tested but was still contaminated.

Compared to a previous study [4], the levels of *E. coli* (17% vs. 80%) and *Salmonella* spp. (14% vs. 20%) in the seven diets were lower, showing the effect of closer monitoring of these indicator pathogens, but there is still room for improvement.

The high presence of T4 in the 40 samples of RMBDs (at least 54%) is concerning, with 19% of the tested RMBD batches exceeding the maximum limit [20]. Usually, dogs do not consume large amounts of only one batch, so there could be a dilution effect of higher T4 levels from one batch to another batch with negligible amounts of T4, as an explanation for the relatively low numbers of clinical cases reported so far. This dilution effect can also be explained by the consumption of other foods and treats aside from the RMBD. It could also be that dogs remain asymptomatic, and they may have subclinical disease. Thyroid tissue

is usually present in the neck area; however, none of the brands tested mentioned using necks in the list of ingredients.

Although the methods used for this study are proven at different levels, the reliability of the results should be interpreted within the limits of this research. We tested eight brands with a verification sample of five for each brand. Each sample was tested once, which influences the confidence interval and limits the possibility of extrapolating the results to other brands or general conclusions. Further research should mainly focus on expanding the study by testing more samples per brand, where conclusions can be generalized.

5. Conclusions

It can be concluded that the hygiene and accurate removal of thyroid tissue during the production of RMBDs still need more attention to prevent the presence of *Salmonella* bacteria, high CFUs, and diet-induced hyperthyroidism. The use of HPP techniques in the production of RMBDs may decrease the number of bacteria but is not a guarantee of microbiological safety.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pets2010004/s1>, Detailed description of materials and methods. Working protocols for colony forming units (CFUs) (1), *Salmonella* spp. (2), *E. coli* (3), and thyroid hormone (T4) (4).

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