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Incidence of Parasites on Frozen Alaska Pollock (*Gadus chalcogrammus,* Pallas, 1814) Fillets

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HIGHLIGHTS

- Parasitic diseases transmitted by fish are a global public health problem.
- The occurrence of parasites in Alaska pollock fish is significant.
- Control measures are needed to minimize the occurrence of parasites in fish.

Abstract: The presence of parasites in marine fish is a serious problem for the fishing industry in many countries. Some species of parasites can present a risk to consumers. Human health can be compromised by allergic reactions to parasitic antigens (hypersensitivity). The aim of the present study was to report the occurrence of parasite larvae in samples of frozen Alaskan fish fillets. A total of 108 samples of frozen Alaska pollock fish fillet were purchased from commercial establishments in the state of São Paulo - Brazil, from July 2017 to November 2019. The parasite search was carried out using the method dissection under transmitted light (candling table) according to AOAC (2005), method 985.12. Only 18 samples (17%), from four different brands, did not present any parasitic form, the others (90 samples) had at least one parasitic form, with the presence of cestodes of the order Trypanorhyncha (found in 73 samples) and nematodes of the *Anisakidae* family prevailing (found in 57 samples). It was also observed that more than half of the studied samples (76 samples) presented up to 5 parasitic forms in the kilogram rate. The results demonstrate the need for more data regarding the incidence of parasites in fish and their impact on human health, in order to alert the population and health authorities about its dangers, so that preventive and corrective measures are taken in the inspection process of these products, aiming to reduce any possibility of the occurrence of illnesses.

Keywords: Fish Products; Food control; Nematoda; Cestoda; Hypersensitivity.

INTRODUCTION

Global fish production is estimated to have reached about 179 million tonnes in 2018, with a total sale value estimated at USD 401 billion, of which 82 million tonnes, valued at USD 250 billion, came from aquaculture production. FAO data shows that consumption of fish and its products has increased considerably worldwide. China has remained a major fish producer, accounting for 35% of global fish production in 2018. In general, excluding China, a significant share of production in 2018 came from Asia (34%), followed by the Americas (14%), Europe (10%), Africa (7%) and Oceania (1%) [1].

One factor that contributed to the increase in fish consumption are the benefits provided to human health, for being a type of food that has a low content of saturated fats and good digestion due to the presence of its nutrients, high in proteins and polyunsaturated fatty acids [2].

Over the years, catches of major marine species have registered marked variations, as well as fluctuations, among the top-producing countries. Catches of anchoveta made it once again the top species at more than 7,0 million tonnes in 2018. Alaska pollock (*Gadus chalcogrammus*, Pallas, 1814) ranked second with 3,4 million tonnes, while skipjack tuna (*Katsuwonus pelamis*) was third for the ninth consecutive year at 3,2 million tonnes [1].

The presence of parasites in fish products, both freshwater and marine, is a serious problem for the fishing industry in many countries, resulting in economic losses to the producer or the industry due to decreased production or the condemnation of highly parasitized fillets. Some species of parasites may present a risk to consumers, as humans can be accidentally infected after eating improperly processed fish and seafood containing viable larvae. Human health can also be compromised by allergic reactions to parasite antigens (hypersensitivity) [3, 4].

Parasitic infections associated with the consumption of fishery products have always been a concern for public health and the economy. The main foodborne zoonoses, associated with the consumption of fishery products, are mainly attributed to *Opisthorchiidae*, *Paragonimus* spp., *Anisakidae*, *Heterophyidae* and *Diphyllobothriidae*, according to global ranking of foodborne parasites [5]. Among these parasites, anisakid nematodes are one of the most important from the sanitary point of view, as they are capable of inducing pathologies in humans, such as anisakidosis, anisakiasis and pseudoterranovosis [6, 7].

In fish and seafood, parasites are found mainly in internal organs (stomach, intestine, liver and abdominal cavity), and can be eliminated during the industrial processing of these products. However, there is the possibility of migration of larvae from these organs to the host's musculature. This migration can occur both in live fish and in caught fish, especially during storage periods in boats and warehouses. Therefore, it is recommended that the fish is gutted on board the boat [4, 8].

The document published by EFSA (2010) [9] recommends that researches should be encouraged to clarify the complete life cycle, geographical and seasonal distribution, prevalence, intensity, and anatomical location of parasites of public health importance in fishery products. Thus the objective of the present study was to determine the occurrence of parasites larvaes in 108 samples of frozen Alaska fish fillets (*Gadus chalcogrammus,* Pallas, 1814) from 30 different brands.

MATERIAL AND METHODS

Equipment

To isolate the parasites, visualization was performed on an adapted candling table and its identification through a stereoscopic microscope (Model SZ-III-BR-SIT, MICRONAL®) and optical microscope (model CBA-K, Olympus®).

Sample

A total of 108 frozen samples, from 30 different brands and 70 different batches, of Alaska pollock fish fillet, were purchased from commercial establishments in the state of São Paulo - Brazil, from July 2017 to November 2019. According to the packaging, 92 samples were from China, 6 from Portugal, 5 from the United States, 1 from Brazil, and 4 samples without identification of the country of origin. All brands still use the old scientific name (*Theragra chalcogramma*) on their packaging, although the change to *Gadus chalcogrammus*, Pallas, 1814, occurred in 2013, being recognized by the *National Marine Fisheries Services Scientific Publication Office* [10].

All samples had the following characteristics: frozen, packaged in 400 g to 1000 g and described as Alaska pollock fish fillet by the manufacturer. The sample (aliquot analyzed) corresponded to 1000 g of the product, therefore, in some cases, it was necessary to add more than one package (always from the same batch informed on the package) to obtain one sample.

Isolation of parasites forms

Parasite research was carried out using the dissection method (candling table) according to AOAC [11], technique 985.12. Briefly, the fillets, in room temperature, were placed in a glass refractory under a light source and, by transparency, the parasitic forms were detected, isolated in Petri dishes containing ultrapure water, taken stereoscopic microscope and separated from muscle tissues with the assistance of a scalpel and forceps, for later verification of their structures and possible identification under an optical microscope.

Morphological identification of parasitic forms

Nematodes were identified according to Mattiucci and coauthors [12], Zhu and coauthors [13], Hartwich and coauthors [14], Felizardo and coauthors [15] and Ferrantelli and coauthors [16] and cestodes, according to Beveridge and Campbell [17]. Cestode parasites were identified up to the Order level and nematodes up to the Family level.

Analysis of results

The results were analyzed in absolute parasitic structures found in each batch of sample or average parasitic structures when more than one unit per batch was analyzed. Statistical analysis was performed using Microsoft Excel 2010 and GraphPad Software 2018.

RESULTS

The Table 1 presents the results found, according to the 30 brands and 70 batches analyzed. For samples with more than one unit analyzed per batch, the result presented is the mean and its standard deviation (X ± SD) of the parasitic forms found.

Table 1. Samples analyzed, batch and quantity (X ± SD, when applicable) of parasitic forms found.

Brand[1]	Batch[2]	Units of same batch	Cestodes of the order Trypanorhyncha[3]	Nematodes of the family *Anisakidae*[3]	Other parasitic forms	Mean (SD) of parasitic forms
01	a	2	-	-	-	0,0 ±0,0
02	a	1	8	1	-	9
	b	1	-	-	1	1
03	a	2	-	-	-	0,0 ±0,0
	b	1	1	3	-	4
	c	1	3	1		4
	d	2	3; 2	-	-	2,5 ±0,7
	e	1	2	-	-	2
	f	1	5	3	-	8
04	a	1	4	-	-	4
	b	1	5	2	-	7
	c	1	4	-	-	4
05	a	1	3	-	-	3
06	a	1	2	-	-	2
07	a	1	-	-	-	0
	b	1	-	-	-	0
08	a	2	11; 14	1; 1	0; 1	14 ±2,8
09	a	3	4; 0; 1	0; 0; 1	-	2 ±2,0
10	a	1	2	2	-	4
11	a	1	6	-	-	6
12	a	2	5; 7	1; 0	0; 1	7 ±1,4
	b	1	4	-	-	4
	c	1	2	2	-	4
13	a	1	7	-	1	8
	b	2	13; 7	0; 1	-	10,5±3,5
	c	1	1	4	-	5
	d	1	1	2	-	3
	e	1	11	-	-	11
14	a	1	1	-	-	1
15	a	1	-	2	-	2
	b	2	4; 2	3; 1	-	5 ±2,8
16	a	1	-	2	-	2
	b	2	1; 4	0; 4	-	4,5 ±4,9
	c	2	1; 0	-	-	0,5 ±0,7
17	a	2	3; 9	0; 1	-	6,5 ±4,5
	b	1	3	2	-	5
	c	3	22; 1; 2	2; 0; 0	-	9 ±13,0
18	a	1	6	-	-	6
19	a	2	-	1; 1	-	1 ±0,0
	b	2	6; 10	-	-	8 ±2,8
20	a	2	-	-	-	0,0 ±0,0
21	a	1	-	-	-	0
22	a	2	5; 9	1; 5	1; 0	10,5 ±4,9
	b	2	10; 9	6; 2	-	13,5 ±3,5
	c	2	4;3	3; 6	-	8 ±1,4
23	a	2	4; 2	1; 0	-	3,5 ±2,1
	b	3	0; 1; 2	2; 3; 0	-	2,7 ±1,2
24	a	3	0; 1; 2	1; 3; 10	-	5,7 ±5,7
	b	1	-	4	-	4
25	a	1	2	-	-	2
	b	1	1	-	-	1
	c	1	5	3	-	8
26	a	2	5; 5	-	-	5 ±0,0
27	a	2	0; 4	1; 0	-	2,5 ±2,1
	b	1	-	1	-	1
	c	2	8; 8	1; 2	0; 1	10 ±1,4
	d	2	3; 7	0; 1	-	5,5 ±3,5
	e	2	10; 0	2; 0	-	6 ±8,5
28	a	1	2	0	1	3
	b	2	3; 0	1; 0	-	2 ±2,8
	c	2	0; 1	1; 1	-	1,5 ±0,7
29	a	1	1	1	-	2
	b	1	-	-	-	0
	c	3	2; 0; 0	2; 0; 3	-	2,3 ±2,1
	d	2	-	15; 1	-	8 ±9,9
	e	2	-	1; 0	-	0,5 ±0,7
	f	1	-	-	-	0
	g	1	-	-	-	0
30	a	1	1	1	-	2
	b	3	1; 0; 0	1; 1; 1	0; 1; 0	1,7 ±0,6
TOTAL	**70**	**108**	**334**	**132**	**8**	**-**

[1]01 to 30 = brands (different numbers, different brands); [2]a to g = batches (different letters, different batches for the same brand); [3]number of parasites found per sample separated by ";" (2; 1).

Four brands studied (01, 07, 20 and 21), did not presented any parasites in the analyzed fillets, which corresponds to 13% of the samples. The results of brands 01, 20 and 21 were from only one batch, as well as brands 05, 06, 08, 09, 10, 11, 14, 18 and 26 that only one batch was analyzed. As we can see in Table 1, twenty six brands had two samples analyzed from the same batch and four had three samples from the same batch.

In Table 1, we can see that in some cases (09a; 16b; 17c; 24a; 27e; 28b; 29d) the standard deviation is greater than the average, demonstrating a lack of homogeneity between the samples. These results are due to the fact that the number of parasitic forms found in the samples of the same batch is discrepant. For a better visualization of these results, Figure 1 presents the results for brands with more than one sample per batch. Sample 03a are not added to Figure 1 because no parasitic forms were found during the analysis.

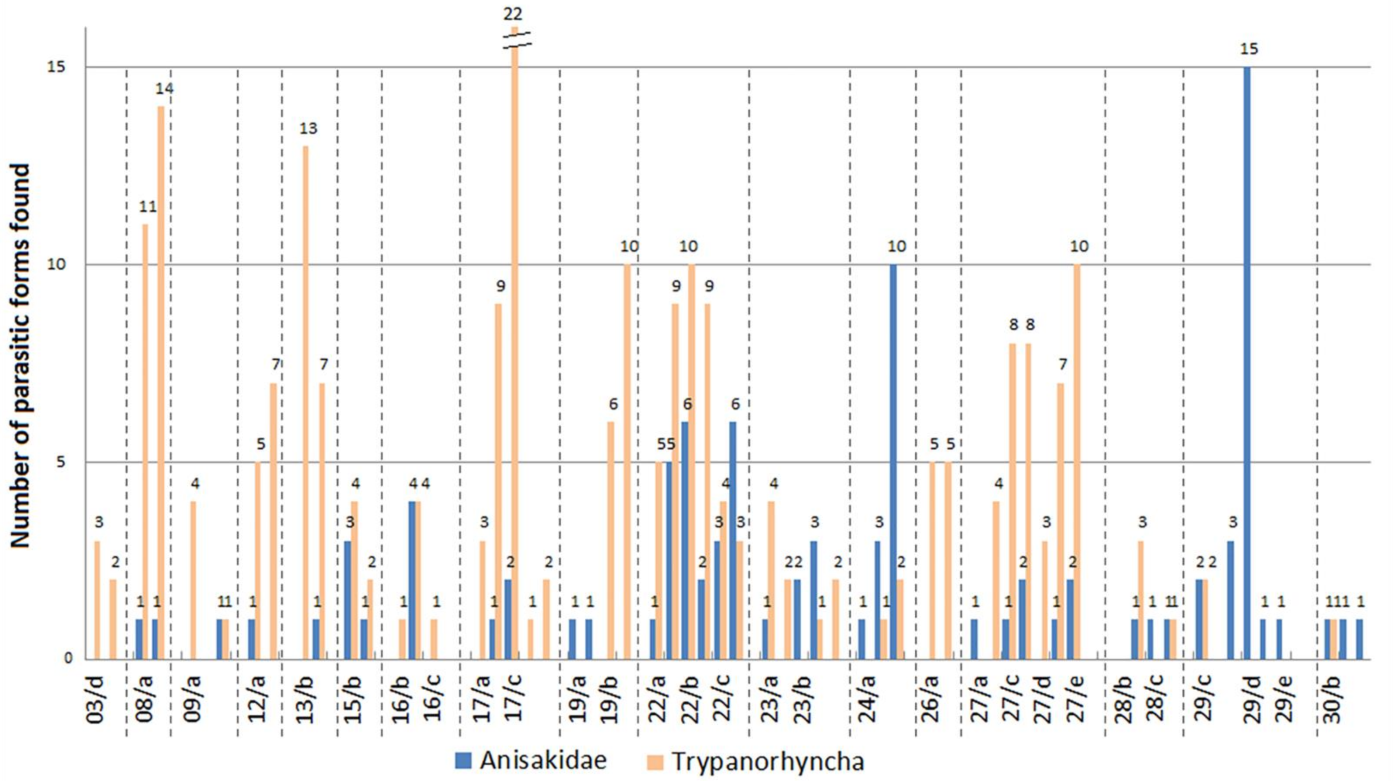

Figure 1. Comparison between fish fillet samples from the same batch. 03 to 30 = brands (different numbers, different brands); a, b, c, d, e = different batches (different letters, different batches).

Observing Figure 1, it is possible to notice that sample 17 stands out from the others due to the number of parasites. For brand 17, three samples were analyzed from batch c (17c1, 17c2 and 17c3). The sample 17c1 was the one with the largest number of parasitic forms in the same sample, presenting 22 cestodes of the order Trypanorhyncha and 2 nematodes of the family *Anisakidae* (totaling 24 parasitic forms), the results of 17c1 were different from the others (17c2 and 17c3) that presented only 1 and 2 cestodes parasites of the order Trypanorhyncha, respectively.

The sample 08a2 presented 1 nematode of the family *Anisakidae*, 14 cestodes of the order Trypanorhyncha and 1 unidentified parasite (totaling 16 parasitic forms). The unidentified parasite does not show the expected characteristics of nematodes or cestodes, and in the isolation process it was damaged, making its identification impossible. It is interesting to note that the two samples of the brand from the same batch, 08a1 and 08a2, presented 1 nematode of the family *Anisakidae* each, 11 and 14 cestodes of the order Trypanorhyncha, respectively, a very coherent number between both samples.

The same fact was observed among samples 22b, where sample 22b1, the second sample with the largest number of parasites, presented 6 nematodes of the *Anisakidae* family and 10 cestodes of the order Trypanorhyncha (totaling 16 parasitic forms), where the second sample analyzed, 22b2, presented 2 nematodes of the family *Anisakidae* and 9 cestodes of the order Trypanorhyncha (totaling 11 parasitic forms). Figures 2 and 3 show some morphological characteristics of the parasites prevalently found.

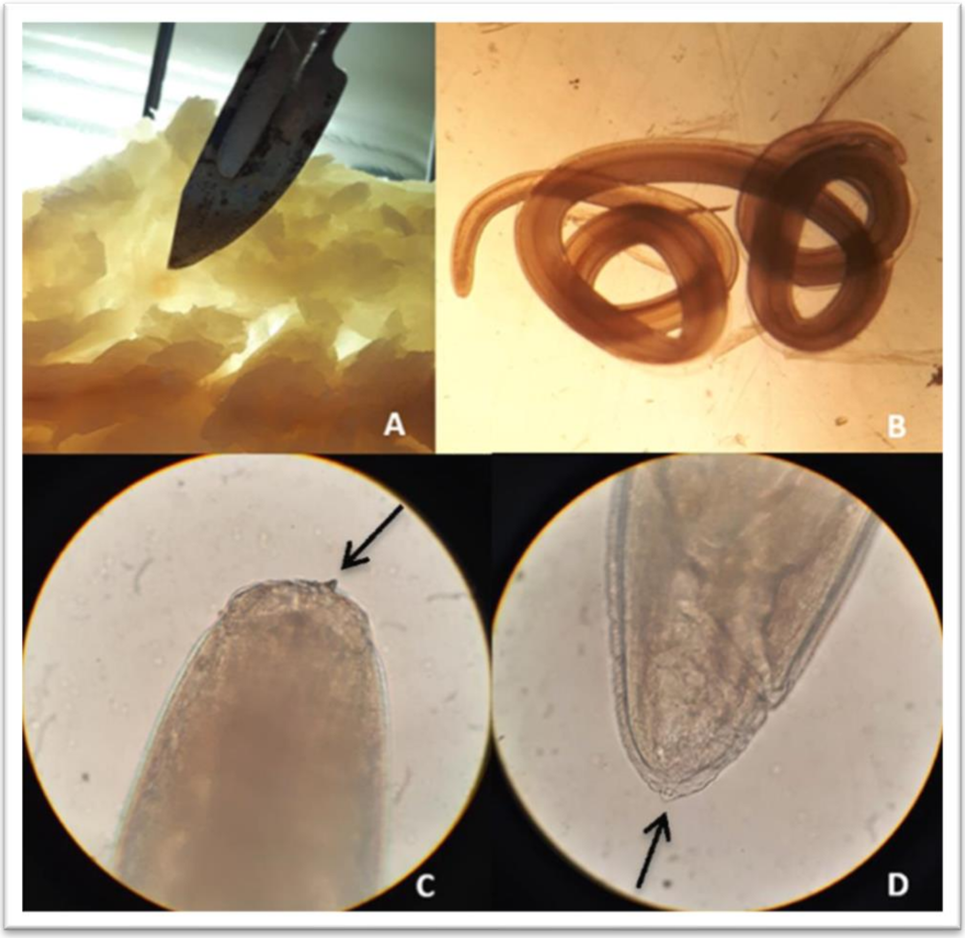

Figure 2. Nematodes of the order Ascaridida, family *Anisakidae*, isolated from Gadus chalcogrammus. (A) Parasitic structure visualized by the candling table technique; (B) Nematode of the *Anisakidae* family under a stereomicroscope, 4x magnification; (C) Presence of a larval tooth in the anterior portion, characteristic of a nematode of the *Anisakidae* family, under an optical microscope, 10x magnification; (D) Presence of a mucron in the posterior portion, characteristic of the nematode of the *Anisakidae* family, under an optical microscope, 10x magnification.

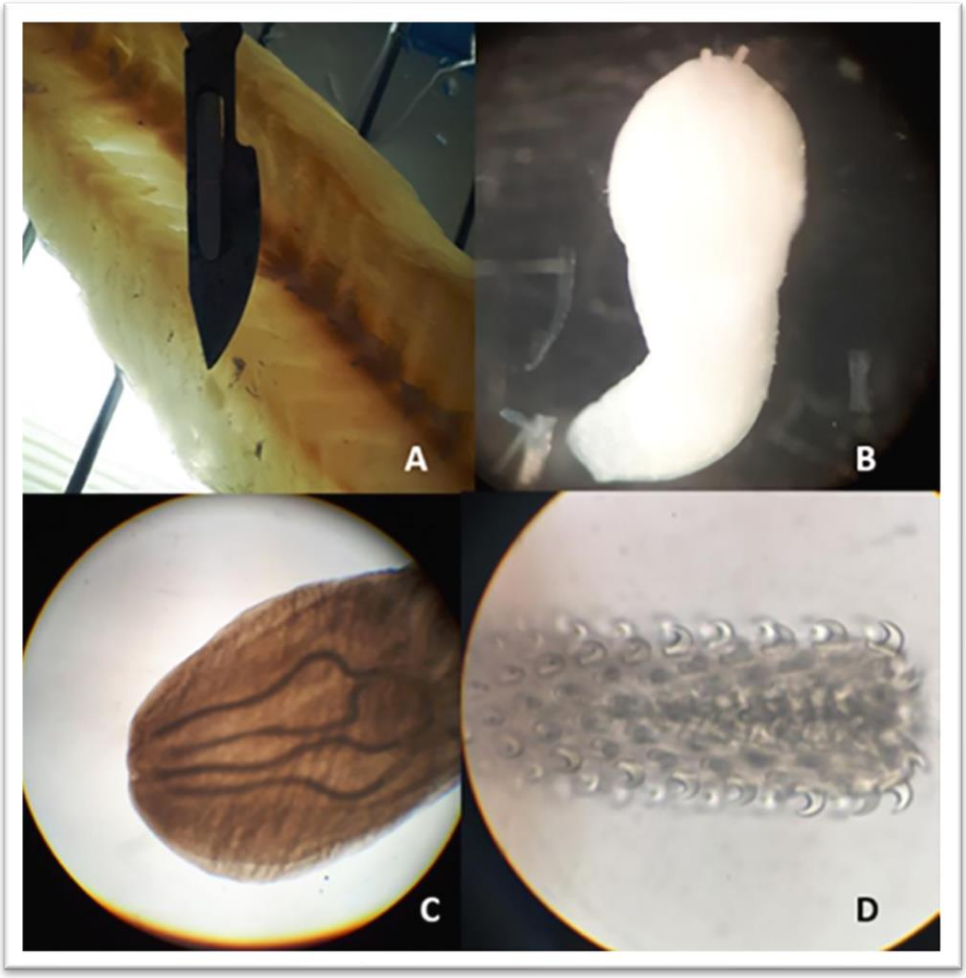

Figure 3. Cestodes of the Trypanorhyncha Order isolated from Gadus chalcogrammus. (A) Parasitic structure visualized by the candling table technique; (B) Cestode of the order Trypanorhyncha under a stereomicroscope, 4x magnification; (C) Cestode of the order Trypanorhyncha under optical microscope, 10x magnification, with detail for the presence of 4 characteristic tentacles; (D) Morphological characteristic of the hooks of the tentacle under an optical microscope, 100x magnification.

Briefly, Table 2 shows the results of 108 samples by number of parasites present. Only 18 samples (17%) did not present any parasitic form, the others (90 samples) presented at least one parasitic form. It was also observed that 70% of the studied samples (76 samples) presented up to 5 parasitic forms per kilogram of sample. In total, three samples showed more than 15 parasitic forms per kilogram.

Table 2. Summary of the number of parasites present and results of the search in order of parasites research in frozen Alaska pollack fish fillets.

Number of parasites present	Cestodes of the order Trypanorhyncha	Nematodes of the family *Anisakidae*	Other parasitic forms	Number of sample in the range
not found (<1)	35 (32%)	51 (47%)	100 (93%)	18 (17%)
from 1 to 5	52 (48%)	53 (49%)	8 (7%)	58 (54%)
from 6 to 10	16 (15%)	3 (3%)	0	20 (19%)
from 11 to 15	4 (4%)	1 (1%)	0	9 (8%)
> ou = 16	1 (1%)	0	0	3 (3%)

DISCUSSION

The presence of parasites in fish can be assessed using different methodologies: visual inspection and transillumination, known as candling table (regulated), destructive examination, pressing and observation with ultraviolet light [18], spectrophotometry [19], artificial digestion [20] and molecular techniques (PCR-RFLP, ITS-1 region) [21]. Studies such as the one by Fraulo and coauthors [22] and Cammilleri and coauthors [20] demonstrate that the candling table technique is not the most suitable for the detection of parasites in fish, which digestion techniques followed by identification of molecules are high sensitivity. But this technique is the official method of the European Union [23] and the one recommended by the official Brazilian legislation [24].

The candling table technique was effective for this study because it is a sample of fish fillet of uniform thickness, without skin and because it is a fish with a light colored muscle. This technique also has the advantage of not fragmenting the parasites. For thicker fillets, a step of opening them was added manually to ensure the observation of parasitic forms in the entire sample. It should be noted that samples from different countries were analyzed in this study and there was no difference in results regarding the origin of the fish.

A large part of the studies that demonstrate the presence of parasites, including cestodes of the order Trypanorhyncha and nematodes of the order Ascaridida, family *Anisakidae*, in Alaska pollock, were carried out in Japan, Belgium, Alaska and British locations [25-35]. This occurs maybe because anisakiasis is endemic in eastern Asian countries and regions, including Japan, Korea, Mainland China and Taiwan.

Several studies reported percentages above 80% for the presence of *Anisakis* sp in Alaska pollock samples, some even reaching 100% positivity [29, 31, 33, 34]. In this study, *Anisakis* sp was found in 53% of the samples. The Figure 2 shows how the parasite appears in the sample and its identification. Gomes and coauthors [35] analyzed several marine hosts from different locations around Japan to characterize the parasitism of *Anisakis* larvae. Chum salmon (*Oncorhynchus keta*) and Alaska pollock (*Gadus chalcogrammus*) showed the highest overall prevalence (100%).

The study by Cipriani and coauthors [36] shows that only 3 out of 70 (4.3%) squids hosted *A. pegreffii* larvae in the mantle. Larvae infecting viscera were coiled and mainly attached to outer surface of visceral organs. Mantle-infecting larvae were situated in the posterior half. Thus, these results suggest that – although low - the risk of acquiring anisakiasis from consumption of raw, marinated and/or undercooked short-finned squid products still exists.

In Brazil, the study by Pauli-Yamada and coauthors [37] with Alaskan pollock samples showed the occurrence of Trypanorhyncha cestodes in greater frequency (88%), followed by nematodes in the group of anisakids (*Anisakidae* / *Raphidascarididae*) (11%). Results according to the data obtained from the present study, where the Trypanorhyncha cestodes were the most found parasitic form, present in 68% of the samples. The Figure 3 shows how the parasite appears in the sample and its identification. The percentage of 52% for the presence of Trypanorhycha cestodes in samples of the same type of fish was found in the study of Moles and Heintz (2007) [31]. Cestodes of the order Trypanorhyncha and nematodes of the order Ascaridida, family *Anisakidae* occur in other species of fish, some of them in percentages similar to those found in the present study [38-43].

Obviously, other parasites infect the Alaskan pollock. Studies carried out in the 1980s showed that Alaskan stocks were infected with a complex and often abundant parasitic fauna, such as *Pleistophora* sp., *Pseudophyllidean plerocercoids* and *Phocanema decipiens* in fish from Europe; as *Khawia sinesis*, *Eulacistorhynchus chiloscyllius*, *Tentacularia coryphaenea*, *Pseudonybelinia odontacantha* and *Nybelinia lingualis* in Korea; and Trematodes in the Gulf of Alaska [25-28]. In 2003, the Alaska walleye pollock industry reported product quality issues attributed to an unspecified parasite in fish muscles. Using molecular and histological methods, White and coauthors [44] identified the parasite in Bering Sea pollock as *Ichthyophonus*.

In Europe, the most important fish parasites causing illness in humans are from the *Anisakidae* family, with 24 genera, although the species most commonly associated with human infection is *Anisakis simplex*, followed by *Pseudoterranova decipiens* [5]. The document Multicriteria-based ranking for risk management of food-borne parasites, from the FAO/WHO (2014) [5] classified the importance of different foodborne parasites in a global perspective, using epidemiological, clinical and socioeconomic criteria. Using this strategy, in a list of 24 parasites with potential for food transmission, *Anisakidae* occupies the 17th place, indicating the importance of investigating this parasite in food.

Abou-Rahma and coauthors [42], published the first report of anisakid larvae from European hake in the Egyptian water. According to the results, twenty-two (36,66%) out of sixty examined fish specimens were found to be naturally infected with *Anisakis* type I larvae, mostly found as encapsulated larvae in visceral organs.

In general, each country has its own legislation for assessing the quality of samples of fishery products. The CODEX STAN 190-1995 suggests that: a sample unit shall be considered unfit for consumption when there are presence of two or more parasites per kg of the sample unit with a capsular diameter greater than 3 mm or non-encapsulated parasite and a length greater than 10 mm [45]. According to the Brazilian legislation, fish that presents massive muscular infestation by parasites, spongy aspect, are in a state of poor conservation or that presents perforations in the casings of sausages by parasites are considered inappropriate for human consumption [46]. However, the concept of massive infestation is not clear and its interpretation is subjective.

The presence of parasites of the *Anisakidae* family is worrying since the larva, even if not viable, has allergens resistant to cooking, freezing and partially digestion, as shown by some studies [47-50]. A systematic review by Rahmati and coauthors [51] about the worldwide prevalence of *Anisakis* larvae in fish and its relationship to human allergic anisakiasis revealed that hot spot areas for allergic anisakiasis were north and northeast of the Atlantic Ocean, southwest of the USA, west of Mexico, south of Chile, east of Argentina, Norway, UK and west of Iceland (confidence 99%). The highest rate of allergic anisakiasis was in Portugal and Norway with the prevalence rate of 18.45 – 22.50%. Therefore, allergologists should consider allergic anisakiasis as a public health issue particularly in high-risk countries, where high prevalences in fish have been demonstrated.

Brusca and coauthors [52] documented for the first time a difference in the prevalence of sensitivity in favor of *A. pegreffii* than *A. simplex* s.s. that could be related to a higher consumption of fish from the Mediterranean Sea, where this species of parasite is the most represented. The authors also presented data that may be useful for the development of clinical guidelines and to address future studies to reduce the health risk related to exposure to *Anisakis*, as there are no clear guidelines on dietary restrictions for patients with *Anisakis* hypersensitivity.

The order Trypanorhyncha usually parasites fish and marine invertebrates, however, although parasitosis is more common in fish, there are reports of accidental infections in humans by eating raw fish containing larvae [53]. Research also demonstrates the possibility of an allergic reaction through toxins produced by the Trypanorhyncha cestodes, which can remain in the fish's muscles and affect the consumers [41, 48].

When it comes to analyzing the dangers that parasites offer, the following should be considered: the species of the fish, the way it is presented, the processing, the conservation method and the intention of consuming the fish, to define the critical point of control and the stage where analytical control will be applied [24].

The environment of wild fish such as Poles cannot be controlled, as well as their feeding, being impossible to prevent them from acquiring parasites throughout life, however, their presence in the musculature is an aggravating factor, as it increases the possibility of their ingestion. In this sense, good practice measures are needed in the production chain at a stage after capture, for the reduction and/or elimination of parasitic contamination in the product that reaches the consumer, such as fillets [37].

In addition to cooking, freezing remains the most widely accepted treatment for killing larvae and reducing the risk of infection [9]. In many parts of the world, fishery products intended for raw consumption must undergo a freezing treatment. One of the first countries in Europe to apply freezing as a preventive treatment for anisakiasis was the Netherlands in 1968, with the so-called “Green Herring Laws”, which determined that fresh herring should be frozen in order to reach a temperature of at least -20°C in 12 hours and stored for a period of 24 hours before being released to the public [54]. Today, European Union countries must subject these products to at least -20°C for at least 24 hours, or -35°C for at least 15 hours, by law [23].

The USA also establishes guidelines for the freezing and storage of fish [55]. In Japan, competent authorities regularly issue information on food safety and recommend freezing as an effective preventive measure against anisakiasis [56]. However, they must be used as guidelines and not as legal requirements and are therefore not imposed by law. The effectiveness of freezing fish products depends on many factors, for example, fish species (fatty or lean), type of meat cuts (fillet or whole fish), mass and volume of the fish product and power of the freezing unit [3].

The Alaska pollock samples analyzed underwent industrial evisceration, filleting, freezing and were subsequently selected for packaging. No information was found regarding the measures used to eliminate parasitic contamination in the analyzed units, but the results of the present study indicate that the measures adopted were not sufficient to eliminate it completely, given the high percentage of positive samples.

CONCLUSION

As a result, 83% of the samples presented at least one parasitic form, with the presence of Cestodes of the order Trypanorhyncha and Nematodes of the *Anisakidae* family prevailing, it was also observed that 58% of the studied samples presented from 1 to 5 parasitic forms per kilogram of sample.

The results demonstrated the need for more data on the incidence of parasites in fish and their impact on human health, in order to alert the population about its dangers and health authorities so that preventive and corrective measures are taken in the inspection process of these products, aiming to reduce any possibility of the occurrence of illnesses. It is also necessary that the laws are clearer and more precise, in order to establish a consensus on massive infestation and acceptable limits for the presence of parasites in fish, so that the consumer's health is taken into account, as well as the industries to market their products safely and without economic losses.

Finally, health inspectors should be alert to the presence of parasites due to the disgusting aspect they transfer to the fish, leading to consumer rejection or the condemnation of fillets in fish industries.

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Conflicts of Interest: The authors declare no conflict of interest.

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