Comparative efficacy of candling and glass plate compression for detection of diphyllobothriosis in rainbow trout (*Oncorhynchus mykiss*) musculature

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Summary

The efficiency of the direct candling technique on fillets (candling 1) was compared with examination of cuts 4 mm thick or less (candling 2) and glass plate compression for the detection of plerocercoids of *Diphyllobothrium* spp. in muscles of rainbow trout, *Oncorhynchus mykiss*. Application of the three procedures gave the following results (percentage of infected fish/percentage of isolated plerocercoids): candling 1: 40.9/22, candling 2: 29.5/18.8, glass plate compression: 29.5/59.2, and combination of candling 1 and 2: 70.5/40.8. The combination of the three techniques yielded 100% sensitivity: 44 infected fish were detected of 77 trout examined. When different regions of the musculature were compared using the three techniques, a high density of plerocercoids and the highest percentage of infection (90.9%; 40 infected trout) were detected in the ventral musculature. Candling 1, candling 2 and glass plate compression on the ventral musculature gave the following case numbers and percentages, respectively, for the total of 44 cases: 9 (20.5%), 9 (20.5%), and 22 (50%).

Keywords

Candling – *Diphyllobothrium* – Glass plate compression – Muscle – Rainbow trout.

Introduction

Chile is an important exporter of salmonids, especially to the United States and Japan in the northern hemisphere and Brazil in South America. In 2002, the first case of diphyllobothriosis was recorded in a rainbow trout, *Oncorhynchus mykiss*, reared in netpens in southern Chile (17). Salmon farming in lakes can be hazardous because infections originating in wild fish can infect the farmed fish. For animal health reasons, this practice is avoided in countries such as Norway, Ireland, Scotland, the United States and Canada (1). Some cases of human diphyllobothriosis have been associated with salmon imports in several countries, such as Spain (2), France (22) and Switzerland (20). Between 2004 and 2005, outbreaks of human diphyllobothriosis appeared in several Brazilian cities in which human infection had never been detected previously. The possible origin of the infection was proposed to be either Chilean salmon imports or wild fish from Brazil (3, 9, 10, 12). The former hypothesis led to the temporary suspension of salmon exports to Brazil, with economic loss to the Chilean industry.

Diphyllobothriosis, caused by *Diphyllobothrium latum*, is endemic to southern Chile between 39º S and 41º S (13). Its principal definitive hosts are humans and domestic dogs (16). The species was apparently introduced by immigrants or tourists from the northern hemisphere. It is spread through faeces containing parasite eggs and is favoured by poor hygiene conditions and the emptying of sewage waters into rivers and lakes (4, 7). Eggs of the parasite

develop into coracidium larvae in the water. Coracidia infect the first intermediate hosts, copepods such as *Diaptomus diabolicus* or *Boeckella gracilis*, forming procercoid larvae. Once a planktivorous fish has ingested an infected copepod, the procercoids develop into plerocercoids. Next, ichthyophagous fish become infected when they ingest planktivorous fish (14). Subsequently, the ichthyophagous fish hosting the plerocercoid stage of the parasite infect the definitive hosts. Infection by *D. dendriticum* is also recorded in freshwater ecosystems; its principal definitive hosts are the seagulls *Larus dominicanus* and *L. maculipennis* (16). Both *Diphyllobothrium* species can be transmitted to humans by the consumption of raw, smoked or undercooked fish (13). In 2006, the Chilean National Fishing Service (11), through the Department of Fishing Safety, established a procedure to verify the absence of the parasite in fish exports from salmon farms or commercial fishing. The procedure consists of the visual examination under a direct light source of 200-g samples of salmon muscle tissue, chosen randomly. From each sample, ten fillets, 4 mm in maximum thickness, are observed by candling. The efficiency of the above procedures has not been evaluated for *Diphyllobothrium* spp. in introduced salmonids in Chile.

The objective of this study was to compare the efficiency of candling and plate compression techniques in the inspection for diphyllobothriosis in muscle tissues of rainbow trout. A further goal was to propose procedures to improve the detection of *Diphyllobothrium* spp. plerocercoids in muscles.

Materials and methods

Fish

Seventy-seven specimens of rainbow trout were collected in Lake Panguipulli (39° 43´ S, 72° 13´ W), using 20-, 30- and 40-mm mesh-nets. Fish were transported to the laboratory at 4°C and stored until examination about 6 h after capture. The weight and standard length were recorded for each fish.

Examination

Ventral (epiaxial) and dorsal (hypaxial) musculature were separated into four sectors each: right anterior ventral (RAV), right posterior ventral (RPV), left anterior ventral (LAV), left posterior ventral (LPV), right anterior dorsal (RAD), right posterior dorsal (RPD), left anterior dorsal (LAD) and left posterior dorsal (LPD). Three procedures were used to examine each section. A candling table with a 30 × 60 cm glass top was equipped with three 25-watt white light bulbs located 20 cm below the glass surface (8). Each sector was weighed and examined over the candling table (candling 1), isolating the observed plerocercoids. Afterwards, each sector was sliced into cuts 4 mm thick or less and re-examined by candling (candling 2), isolating the plerocercoids. Finally, the slices were pressed by hand between two glass plates (18 × 10 × 0.8 cm) and observed under a stereomicroscope (14). In addition, the mesenteries, spleen, liver, heart, gonads, gallbladder and swim bladder were scraped into a dish containing 0.15 M NaCl and examined under a stereomicroscope. The stomach and intestine were opened longitudinally in saline solution; their external and internal surfaces as well as the mucous scrapings were examined for plerocercoids using the stereomicroscope.

Parasite identification

Plerocercoids of *Diphyllobothrium* spp. were fixed in formol-saline (4% in 0.15 M NaCl) and identified. *Diphyllobothrium latum* was differentiated from *D. dendriticum* on the basis of morphological characteristics (15, 18).

Parasite infection

The percentage of fish infected with *Diphyllobothrium* spp. in each anatomical location was determined out of the total number of fish examined (n = 77). The percentage of fish infected by *Diphyllobothrium* spp. in the different muscular regions was determined out of the total number of fish infected (n = 44) in the musculature.

The sensitivity corresponds to the percentage of infected fish detected with each procedure, or combination of procedures, in examination of the muscles, divided by the total number of fish infected in this location. The mean intensity corresponds to the average number of plerocercoids detected in all infected fish. The mean abundance corresponds to the average number of plerocercoids in all the fish examined, as well as in the different muscular regions. The mean density corresponds to the average number of plerocercoids in each 50 g of muscle in each region, considering all the examined fish. For the analyses of the mean number of parasites and fish weight, the Mann–Whitney U-test and Student's t-test were used, respectively. Comparison of the density in different muscular regions was carried out using Wilcoxon's signed rank test; Spearman's range test was applied for correlation analysis. Significant differences are indicated where $p < 0.05$.

Results

Seventy-two trout (93.5%) of the 77 examined were found to be infected by *Diphyllobothrium* spp. in different

anatomical locations, using all the procedures indicated for muscles, viscera and mesenteries. A total of 2,612 plerocercoids were isolated: 2,342 of *D. latum*, 217 of *D. dendriticum*, and 53 were not identified because they had suffered alterations. The mean intensity and mean abundance were 33 and 30.4 plerocercoids of *D. latum* per host infected or examined, respectively. Seventy-one trout (92.2%) presented *D. latum* plerocercoids, which were concentrated more often in the mesenteries, stomach, intestine and muscles (Table I). *Diphyllobothrium dendriticum* was identified in only 48.1% of trout. It presented a higher frequency and concentration in the mesenteries, intestines, stomach and liver (Table I). The trout examined measured 36.9 ± 8.1 cm (17-51 cm) standard length and weighed 881.8 ± 500.4 g (75-2,130 g). The abundance (rs = 0.68, $n = 77$, $p < 0.05$) and density of muscular infection (rs = 0.43, $n = 77$, $p < 0.05$) showed significant correlation with the standard length of the fish. The combined use of the candling techniques (1 and 2) and the glass plate compression technique revealed infection by *Diphyllobothrium* spp. in the musculature of 44 (57.1%) of the 77 fish examined. The musculature presented *D. latum* plerocercoids in 55.8% of trout (Table I), with intensity and mean abundance of 7.1 and 4.1 plerocercoids per infected or examined host, respectively. Infection of the muscles by *D. dendriticum* was found in seven (9.1%) trout, and in six cases coexisted with plerocercoids of *D. latum* (Table I). The intensity and mean abundance of *D. dendriticum* reached 1.4 and 0.1 parasites per host infected or examined, respectively. The musculature of infected trout contained, on average, 300 plerocercoids of *D. latum*, 10 of *D. dendriticum* and 4 of unidentified *Diphyllobothrium* spp.

Table I
Results of the examination of 77 rainbow trout from Lake Panguipulli, Chile: number of infected fish and number (and location) of *Diphyllobothrium* spp. plerocercoids

Location	*D. latum*				*D. dendriticum*			
	Infected trout		Number of plerocercoids		Infected trout		Number of plerocercoids	
Stomach	59	(76.6%)	1,045	(44.6%)	12	(15.6%)	57	(26.3%)
Intestine	46	(59.7%)	302	(12.9%)	13	(16.9%)	23	(10.6%)
Liver	33	(42.9%)	134	(5.7%)	8	(10.4%)	53	(24.4%)
Spleen	7	(9.1%)	7	(0.3%)	1	(1.3%)	2	(0.9%)
Gonads	18	(23.4%)	93	(4.0%)	6	(7.8%)	11	(5.1%)
Heart	1	(1.3%)	1	(0.04%)	0	(0%)	0	(0%)
Kidney	1	(1.3%)	2	(0.09%)	0	(0%)	0	(0%)
Mesenteries	54	(70.1%)	458	(19.6%)	24	(31.2%)	61	(28.1%)
Muscles	43	(55.8%)	300	(12.8%)	7	(9.1%)	10	(4.6%)
Total	**71**	**(92.2%)**	**2,342**	**(100%)**	**37**	**(48.1%)**	**217**	**(100%)**

When applied to the 44 fish in which the muscles were infected, candling 1 detected 40.9% of the fish with muscle infection and 22% of the plerocercoids. With candling 2, the percentage of trout detected to be infected decreased to 29.5% and the number of plerocercoids to 18.8%. With glass plate compression the percentage was again 29.5% and the number of plerocercoids increased to 59.2% (Table II). Candling 1 gave positive results in infected trout with a mean of 11.3 ± 15.1 (1–69) plerocercoids, whereas in trout where infection was not detected by candling 1 the fish presented a mean of 3.6 ± 3.6 (1–17) plerocercoids. Therefore, the means showed significant differences ($z = 3.23$, $p < 0.05$): candling 2 and principally the glass plate compression technique detected more fish with light infections.

Table II
The efficacy of different procedures in detecting *Diphyllobothrium* infection in 44 rainbow trout with infected musculature: number of infected fish and number of plerocercoids detected by each method

Procedure	Infected fish		Plerocercoids [a]	
	Number	Percentage	Number	Percentage
Candling 1[b]	18	40.9	69	22.0
Candling 2[c]	13	29.5	59	18.8
Glass plate compression	13	29.5	186	59.2
Candling 1 and 2	31	70.5	128	40.8
Candling 1, 2 and glass plate compression	44	100	314	100

a) Includes 300 *D. latum*, 10 *D. dendriticum* and 4 unidentified *Diphyllobothrium* spp.
b) Observation of different locations of musculature independent of their thickness
c) Observation of different locations of musculature in slices of ≤ 4 mm thick

The density of infection with *Diphyllobothrium* spp. was similar for anterior and posterior musculature (T statistic of Wilcoxon test = 386, $p > 0.05$) as well as for the right and left sides (T = 348.5, $p > 0.05$) (Table III). The density in the ventral musculature was significantly higher than that in the dorsal musculature (T = 143.5, $p < 0.05$), with higher parasite numbers per 50 g of tissue. Moreover, 40 (90.9%) of the 44 cases of muscle diphyllobothriosis were found in the ventral musculature. When the three procedures were applied, examination of the total ventral musculature gave the following case numbers in relation to the 44 cases with muscle infection: 9 (20.5%), 9 (20.5%), and 22 (50%) for candling 1, candling 2, and glass plate compression, respectively. The mean weight of the musculature was compared for ventral and dorsal regions (Student's $t = 1.86$, $p > 0.05$), anterior and posterior ($t = 2.84$, $p < 0.05$), and left and right ($t = 0.245$, $p > 0.05$); the differences were significant only between anterior and posterior muscles (Table III).

Table III
Weight of different *Oncorhynchus mykiss* muscles and percentage, abundance and density of *Diphyllobothrium* spp. infection detected by application of candling (1 and 2) and glass plate compression

Muscle location	Muscle weight (grams) mean ± SD (range)	Fish infected/examined (percentage infected)	Abundance [a] mean ± SD (range)	Density [b] mean ± SD (range)	Percentage of cases detected [c]
Ventral	190.8 ± 112.9	40/77	2.5 ± 6.2	0.6 ± 1.29	90.9
	(16–847.4)	(51.9)	(0–49)	(0–9.6)	
Dorsal	228.3 ± 136.1	30/77	1.4 ± 3	0.3 ± 0.9	68.2
	(14–1,067.4)	(39.0)	(0–20)	(0–2.7)	
Anterior	238.3 ± 141.9	35/77	2.7 ± 6.7	0.7 ± 2.4	79.5
	(16–1,102.1)	(45.5)	(0–52)	(0–19.4)	
Posterior	180.8 ± 106.3	32/77	1.2 ± 2.5	0.3 ± 0.6	72.7
	(14–812.7)	(41.6)	(0–17)	(0–2.9)	
Left	207.1 ± 160.5	34/77	2.1 ± 5.3	0.4 ± 0.9	77.3
	(16–1,036.1)	(44.2)	(0–43)	(0–6.8)	
Right	212.0 ± 117.2	35/77	1.8 ± 3.7	0.4 ± 0.7	79.5
	(14–878)	(45.5)	(0–26)	(0–4.1)	

a) Mean number of parasites in the muscles of all trout examined
b) Number of plerocercoids/50 g of muscle from all trout examined
c) From a total of 44 cases of muscle infection
SD: standard deviation

Discussion

The highest parasite abundance was recorded in the intestinal tracts of the trout, as has been noted in previous research (13, 16). The abundance and density of infection at the musculature level were found to be higher in larger fish. This situation can be attributed to several causes, such as the longevity of plerocercoids, their recurrent transmission and accumulation over time, and the feeding habits of their hosts, as seen in earlier studies on the abundance of parasites in farmed and wild salmonids (5, 6, 21). When fish are small, infection occurs mostly by ingestion of copepods. However, in larger trout, ingestion of other fish that act as intermediate hosts and consumption of larger prey favour infection (14). The examination of small fish should be more meticulous because of the low infection density. Given that candling methods have such low sensitivity for the detection of plerocercoid infections in fish (detecting only 70.5% of infections even when candling 1 and 2 were applied in combination), their application seems to allow only partial control of the transmission of infection to the consumer. Use of both candling methods in combination isolated 40.8% of the plerocercoids. Salmonids originating from regions where diphyllobothriosis is endemic, such as Chile, should undergo certification controls to declare the absence of plerocercoids of *Diphyllobothrium* in the ventral musculature. When the three procedures used in this study were applied in combination to the ventral muscles, 90.9% of muscle infection was detected. The procedures included the examination of sections of muscle tissue that weighed between approximately 16 g and 847 g each; in order to detect 90.9% of the infected fish, examination of up to 18 compression glass plates was necessary, which was quite labour intensive. However, this method could be applied to the monitoring and surveillance programmes used on fish farms to detect infections in salmonids.

High parasite density and abundance in the ventral muscles may be associated with ingestion of procercoids or plerocercoids by copepod or planktivorous fish, respectively. Subsequently, the parasites move from the digestive tract, where the highest concentration of plerocercoids was found in this study, towards the ventral musculature, which is in direct contact with it. Wootten and Smith (21) observed that infection with *D. dendriticum* affected only the ventral musculature of farmed rainbow trout, and they found higher quantities of plerocercoids in the anterior viscera. The present study had similar results. Countries with endemic infections must increase the effectiveness of their food control mechanisms and monitoring in farming centres. Although this will increase their costs, the costs would diminish once the infection in the region was under control. This is especially true of the freshwater ecosystems in southern Chile, where the prevalence of infection in wild rainbow trout from lakes in the endemic region was previously found to fluctuate between 3.7% and 78% for *D. latum* and between 3% and 75% for *D. dendriticum* (13). The present study found that in Lake Panguipulli the maximal percentage of fish infected with *D. latum* was 92.2%.

To conclude, until other less invasive testing procedures become available to detect the presence of diphyllobothriosis in the musculature of rainbow trout, examination of the ventral region should become a priority because it is the region in which the highest density and abundance of parasites are found. When results are negative for candling 1, candling 2 should produce an improvement in detection. A microscopic examination with glass plate compression of ventral musculature should increase the percentage of cases detected further. As a consequence, the laboratory performing the certification analysis should receive the whole fish to inspect, not just a 200-g sample. Examination of the ventral musculature using the glass compression technique should be given top priority. The United States Food and Drug Administration (19) recommends methods of microscopic inspection of thin slices or digestion of muscles for the detection of *Diphyllobothrium* plerocercoids. Digestion methods, however, are more expensive in terms of reagents than candling and glass compression techniques.

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Comparaison de l'efficacité respective du mirage et de la compression entre lames de verre pour détecter la présence de *Diphyllobothrium* dans la chair de truites arc-en-ciel (*Oncorhynchus mykiss*)

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Résumé

Les auteurs présentent les résultats d'une étude visant à comparer l'efficacité respective du mirage direct des filets (mirage 1), de l'examen de coupes de 4 mm d'épaisseur maximum (mirage 2) et de la compression entre lames de verre pour détecter la présence des plérocercoïdes de *Diphyllobothrium* spp. dans la chair de truites arc-en-ciel (*Oncorhynchus mykiss*).

Les résultats obtenus par les trois procédures ont été les suivants (pourcentage de poissons parasités / pourcentage de plérocercoïdes isolés) : mirage 1 : 40,9/22 ; mirage 2 : 29,5/18,8 ; compression entre lames de verre : 29,5/59,2 ; utilisation combinée du mirage 1 et du mirage 2 : 70,5/40,8. L'utilisation associée des trois techniques a permis d'obtenir une sensibilité de 100 % puisque les 44 truites parasitées parmi les 77 examinées ont été détectées.

Lors de la comparaison des différentes régions de la masse musculaire des poissons en utilisant les trois techniques, il est apparu que la région ventrale présentait la plus forte densité de plérocercoïdes et le taux le plus élevé d'infestations (90,9 % ; 40 truites parasitées). Les résultats obtenus respectivement par les techniques du mirage 1, du mirage 2 et de la compression entre lames de verre du muscle ventral ont été les suivants, sur un total de 44 cas : 9 cas détectés (20,5 %), 9 cas détectés (20,5 %) et 22 cas détectés (50%).

Mots-clés

Compression entre lames de verre – *Diphyllobothrium* – Mirage – Muscle de poissons – Truite arc-en-ciel.

Comparación del grado de eficacia de la inspección visual al trasluz y de la compresión en placas para detectar difilobotriosis en el tejido muscular de la trucha arco iris (*Oncorhynchus mykiss*)

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Resumen

Los autores describen un estudio destinado a comparar la eficacia de tres técnicas: inspección directa de filetes al trasluz (trasluz 1); examen de cortes de 4 mm de espesor máximo (trasluz 2); y compresión en placas de vidrio, para detectar plerocercoides de *Diphyllobothrium* spp. en el tejido muscular de la trucha arco iris (*Oncorhynchus mykiss*). La aplicación de esos tres procedimientos arrojó los siguientes resultados (porcentaje de peces infestados/porcentaje de plerocercoides aislados): trasluz 1: 40,9/22; trasluz 2: 29,5/18,8; compresión en placa: 29,5/59,2; y uso combinado de trasluz 1 y 2: 70,5/40,8. La combinación de las tres técnicas arrojó un 100% de sensibilidad: de las 77 truchas examinadas se detectaron las 44 que estaban infestadas. Al comparar distintas regiones de la masa muscular utilizando las tres técnicas se observó que la musculatura ventral presentaba una elevada densidad de plerocercoides y el mayor porcentaje de infestación (90,9%; 40 truchas). Las técnicas de trasluz 1, trasluz 2 y compresión en placas aplicadas a la musculatura ventral arrojaron respectivamente las siguientes cifras de casos detectados (sobre un total de 44): 9 (20,5%), 9 (20,5%), y 22 (50%).

Palabras clave

Compresión en placas de vidrio – *Diphyllobothrium* – Inspección visual a trasluz – Tejido muscular – Trucha arco iris.

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