Characteristics of bacterial isolates in Swiss farmed and ornamental fish from a retrospective study from 2000 to 2017

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Summary

Aquaculture is a rapidly growing field of food production of high economic importance. Bacterial infections are an important threat to aquaculture growth and also a common problem in ornamental fish. Some pathogenic agents and aquaculture production types are reported to be associated with increased disease. However, a detailed description of bacterial pathogens causing disease in Swiss aquaculture and ornamental pet fish is still missing. In this study we describe 1448 bacterial isolations originating from 1134 diagnostic laboratory submissions from farmed and ornamental fish in Switzerland for the period from 2000 to 2017. A strong seasonality was observed with submissions peaking in spring and summer. Bacterial isolations in fish submitted from organic farms were approximately six times more frequent than in conventional fish farms. *Flavobacteriaceae*, aeromonads and *Yersinia ruckeri* were the most common isolates from aquaculture, and motile aeromonads and *Vibrio* spp. were most often isolated from ornamental fish. The results of this study provide some interesting hypotheses, but further research is needed to better characterize risk factors for bacterial diseases in both aquaculture and aquarium fish in Switzerland.

Key words: Bacterial infections, aquaculture, production type, ornamental fish, risk factor, Switzerland

Retrospektiven Studie über den Nachweis von Bakteriellen Erreger aus Schweizer Zucht- und Zierfischen von 2000 bis 2017

Die Aquakultur ist ein schnell wachsender Bereich der Lebensmittelproduktion von hoher wirtschaftlicher Bedeutung. Bakterielle Infektionen stellen eine wichtige Bedrohung für das Wachstum der Aquakultur und auch ein Problem bei Zierfischen dar. Einige Arten von Krankheitserregern und Aquakulturanlagen sind Berichten zufolge mit einer erhöhten Krankheitswahrscheinlichkeit assoziiert. Eine umfassende Beschreibung von bakteriellen Erregern, die Krankheiten in Schweizer Aquakulturfischen und Zierfischen hervorrufen, fehlt jedoch noch. In dieser Studie beschreiben wir 1448 bakterielle Isolate, die aus 1134 diagnostischen Laboreinsendungen von Zucht- und Zierfischen in der Schweiz im Zeitraum von 2000 bis 2017 stammen. Es wurden starke saisonale Schwankungen beobachtet, mit einem Peak im Frühjahr und Sommer. Bakterielle Infektionen bei Fischen, die von Biobetrieben stammen, wurden etwa sechsmal häufiger diagnostiziert als Infektionen bei Fischen von herkömmlichen Fischfarmen. *Flavobacteriaceae, Aeromonas* spp. und *Yersinia ruckeri* waren die häufigsten Isolate in der Aquakultur. *Vibrio* spp. und bewegliche Spezies von *Aeromonas* spp. wurden am häufigsten aus Zierfischen isoliert. Die Ergebnisse dieser Studie liefern eine Grundlage für weitere Forschungen, um Risikofaktoren für bakterielle Erkrankungen sowohl in der Aquakultur als auch bei Zierfischen in der Schweiz besser zu charakterisieren.

Schlüsselwörter: Bakterielle Infektionen; Aquakultur; Produktionsart; Zierfische; Risikofaktor; Schweiz

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Introduction

The per person consumption of fish worldwide, and in Switzerland, is constantly increasing[1,2,3,4]. This increase, coupled with population growth[1,5,6], has resulted in a global increase in the demand for fish. However, overfishing is a well-known problem and global fishing yields have already reached their limits[7,8]. Therefore, it is not surprising that aquaculture is a rapidly growing field[1,9,10]. Mortality proportions are much higher in aquaculture than in land living food animals[11], which negatively impacts the economic stability of fish farms as well as the welfare of the fish[12,13]. Important causes of mortality in aquaculture are infectious diseases[14,15], including bacterial pathogens[16,17]. Risk factors for mortality caused by bacterial pathogens include overcrowding[18,19], poor water quality[18], low genetic variability in aquaculture fish populations[20,21], antimicrobial resistance[16] or failing coevolution between farmed fish and local pathogens[12].

In Switzerland, there is still room for aquaculture to grow, as it only accounts for about 1% of aquatic food consumption[22]. Aquaculture in Switzerland is focused on the production of salmonids, mainly rainbow trout. Infectious diseases are a threat to the growth of salmonid aquaculture as there are several bacterial agents known to cause high morbidity and mortality in salmonid production, including *Aeromonas* spp., *Flavobacterium* spp. or *Yersinia ruckeri*.

Motile aeromonads (e.g. *Aeromonas caviae, Aeromonas hydrophila* and *Aeromonas sobria*) can cause septicaemia with variable morbidity and mortality in several fish species[23,24,25]. There is still controversy over these pathogens being primary pathogens or ubiquitous opportunists, causing disease as a secondary infection following a primary pathogen infection or in association with stressful conditions[26,27]. Classical external symptoms are dark coloration with large hemorrhages irregularly distributed over the body surface. In advanced stages, ulcers of various sizes can develop[28,29]. Clinical disease occurs mostly at temperatures above 10 °C[27].

Aeromonas salmonicida subsp. salmonicida, the causative agent of furunculosis, is a major problem in salmonid farming[29,30,31]. In adult fish, *A. salmonicida* causes hemorrhagic septicemia with or without bulging skin lesions, as well as lytic dermal and muscle necrosis[26,29,31]. In young fish, the disease course is often peracute, presenting as high mortality without previous clinical symptoms[26,29]. Disease can occur at 6 °C, but most often occurs in spring as temperatures increase[27].

Flavobacterium psychrophilum is the causative agent of different disease syndromes including an external form with lesions on gills and/or skin, and a systemic form[26,31,32] called Rainbow Trout Fry Syndrome (RTFS), which is a serious problem in salmonid farms in Europe, mainly in young rainbow trout[31,32,33]. RTFS is characterized by hemorrhages in the muscle and intestine, splenomegaly, necrosis and granulomatous inflammation of the kidney and spleen. Mortality is typically between 5 and 10%, but can be as high as 90%[31,32]. Disease usually appears when water temperatures are between 4 and 10 °C, with maximum mortality at 15 °C[34,35].

Yersinia ruckeri, the causative agent of Enteric Redmouth Disease, can affect many freshwater and marine fish; however rainbow trout are particularly sensitive[26,27,30,31,33,36], and young fish are most often affected[26]. Typical symptoms are multiple hemorrhages and hyperemia in the mouth, on the head, fin base and on the lateral line, and petechial hemorrhages in liver, intestine and musculature[26]. The disease is often chronic and associated with low mortality rates[26]. Non-symptomatic carriers can occur[26,27]. Stressful conditions can trigger peracute disease outbreaks with high mortality without previous clinical symptoms[31,37]. The disease usually occurs when water temperatures exceed 10 °C[26].

In Switzerland, rainbow trout are mostly kept in tanks/raceways or in ponds as a flow-through system. However, recirculation systems are becoming more popular[38]. Surface water or spring water are the most common sources of water used in all farming types. The majority of Swiss fish farms discharge their wastewater directly into surface water[38]. Organic farms are an important player in the Swiss market and the organic market has grown considerably in recent years[39,40]. Organic farms are required to adhere to production practices that differentiate them from non-organic farms[41]. The most important differences are quality of soil, which must be of natural origin (opposed to concrete floor), covered with pebbles, structural hiding elements and a restriction in density of fish/m^3 [41].

Ornamental fish are important pet animals in developed countries[42,43]. In Switzerland, about 5% of the population owns ornamental fish and fish represent the biggest group in absolute number of kept pet animals in Switzerland[44]. Koi carp is the pet fish species of greatest economic importance in Switzerland. Ornamental fish are also important in zoological gardens and public aquaria. Ornamental fish can be subject to very high mortality rates from various causes[45], including bacterial diseases[46]. There are several bacterial agents known to cause serious disease in ornamental fish.

Motile aeromonads are not only seen in farmed fish, but also in ornamental fish[26]. *Aeromonas* spp. are probably the most important bacterial pathogens of koi carp[47].

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Vibrio spp. are important pathogens of aquarium fish, mainly of marine fish[31]. *Vibrio* spp. are facultative pathogens that can also be found on healthy fish. High water temperatures are an important risk factor[26], but stressful conditions in general have also been reported to play an important role in pathology. Clinical signs may vary according to the *Vibrio* spp. involved but are usually either skin lesions or bacterial hemorrhagic septicemias[26,31].

Other important bacterial pathogens causing disease in farmed and ornamental fish which were not isolated at the Centre for Fish and Wildlife Health (CFWH), University of Bern, Bern, Switzerland are not considered in this study.

Veterinary diagnostic laboratories are often used as a source of information about diseases in the animal populations they serve. They are convenient sources of information as they often store data in databases, allowing for easy access without additional costs for sample testing or population sampling. Individual laboratories often use the same testing procedures over time allowing for comparisons between samples. However, laboratory samples are often biased and non-representative of the population, therefore it is important to be careful when making inferences about a population from these data. However, information from laboratories can be used for purposes that are not dependent on a random or representative population sample, for example to explore the breadth of variation in the different types of diseases and pathogens in samples received by a laboratory from a specific population.

This study gives a summary of bacterial pathogens in Swiss aquaculture and ornamental pet fish. The purpose of our study was to analyse collected data of bacterial pathogens found in diseased aquaculture, pond and aquarium fish in Switzerland over a period of 18 years. The study was conducted by analysing records from all

Supplementary Table 1: Variables in the dataset, included are the number of missing values and the number of distinct entries for each variable.
There is a total of 1448 values for each variable, if counting missing.

Variable	Missing values	Missing values for the category if these values were only defined for a particular category	Distinct values
Date	0	-[1]	1009
Year	0	-[1]	18
Case identification number	0	-[1]	1134
Premises	80	-[1]	374
Premise category	80	-[1]	8
Geographical location	90	-[1]	337
Species	0	-[1]	8
Intended use	0	-[1]	4
Aquarium water quality	1119	0[2]	2
Facility type	995	59[3]	10
Floor type	995	59[3]	3
Organic label status	922	54[3]	2
Water source	990	57[3]	3
Water outflow	991	57[3]	3
Production amount per year	984	75[3]	6
Age	1104	81[4]	4
Organ	425	-[1]	14
Bacteria	489	-[1]	75
Bacteria family	489	-[1]	3

[1] The category was determined for all the submissions, and not only for a special category.
[2] The category was only determined for submissions in which the intended use was aquarium fish. There are 258 different submissions of aquarium fish and 329 isolations from aquarium fish.
[3] The category was only determined for premises of submissions in which the intended use was farmed fish. There are 102 different fish farms in the database. The number of missing values indicates the number of distinct farms for which there is missing value, and not the number of isolations for which it misses the value.
[4] The category was only determined for submissions in which the species was rainbow trout. There are 392 different submissions of rainbow trout and 433 isolations from rainbow trout.

fish samples received by the CFWH in which bacteria were cultured during the period from 2000 to 2017 and where these bacteria were classified as important for disease induction. We aimed to explore a possible connection between the collected extrinsic data from each submission and the results of the bacteriological investigations. As this is a retrospective study examining existing archive data, there are some limitations regarding the comparability of the data.

Material and methods

Data sources

The data used for this study originated from the database of the CFWH. The database consisted of 1448 bacterial isolates collected from 1134 different submissions of clinically ill fish during the period from 2000 to 2017. The study was based on bacterial isolations where a resistance test was performed. Therefore, these bacterial infections were investigated as a potential cause of the disease that resulted in the fish submission to the laboratory. Submissions with no bacterial growth or where other causes were identified as main cause of disease were not included in this study. Included were submissions of various fish species kept as food or pet animals. Submissions included here consisted of animals sent alive or of swabs taken from living fish. Submissions were classified into 4 different classes based on their intended use: farmed fish (54.2% of submissions, 615 submissions), aquarium fish (22.8%, 258 submissions), pond fish (22.5%, 255 submissions) and wild fish (0.5%, 6 submissions) (Tables 1, 2). Submitted fish were grouped into 8 different groups by families or species. The largest group of aquaculture fish was rainbow trout (34.6% of all submissions, 392 submissions), followed by brown trout (9.1%, 103 submissions), other salmonids (4.5%, 51 submissions) and other farmed fish (7.3%, 83 submissions), such as perch, pike perch, sturgeon and other less common species. Rainbow trout were further grouped into the following age categories: less than one-year-old, between one and two-years-old, and more than two-years-old. The categorisation was made according to the size of the fish, and for unclear cases the time of the year was also used. Submissions that contained many fish of different ages were categorized as mixed.

Among pet fish, the largest group was aquarium fish (22.0%, 250 submissions), followed by koi carp (19.3%, 219 submissions), goldfish (1.9%, 22 submissions) and other pond fish (1.2%, 14 submissions) (Table 2). The water source for aquarium fish was either freshwater (77.1%, 199 submissions) or seawater (22.9%, 59 submissions). As the cultivation conditions were not adapted to seawater conditions, the amount of seawater isolations might be biased. Each submission consisted of

Table 1: Intended use of fish (aquarium, farm, etc) and the type of the premises from which they came.

Type of premise	Aquarium fish	Farmed fish	Wild fish	Pond fish	Total
Commercial farms	0	460	0	0	460
Fishery societies	0	37	0	0	37
Cantonal fisheries	0	70	3	0	73
Fish stores	17	0	0	34	51
Private pet owners	121	28	0	178	327
Zoos	86	0	0	2	88
Fish researcher	26	7	0	3	36
CFWH institute	1	8	3	0	12
Unknown premise	7	5	0	38	50
Total	251	610	6	217	1084

Table 2: Intended use of fish (aquarium, farm, etc.) and the species or group of fish.

Family	Aquarium fish	Farmed fish	Wild fish	Pond fish	Total
Rainbow trout	0	392	0	0	392
Brown trout	0	102	1	0	103
Other salmonidae	1	45	4	1	51
Other farm fish	2	76	1	4	83
Aquarium fish	248	0	0	2	250
Koi	0	0	0	219	219
Goldfish	7	0	0	15	22
Other pond fish	0	0	0	14	14
Total	258	615	6	255	1134

one to several hundred fish. In general, if more than one fish was included in the submission, bacteriology was performed on three clinically affected fish specimens individually. Bacteriology was performed immediately after euthanasia to exclude overgrowth by non-pathogenic bacteria. Samples were routinely taken from liver, spleen and head kidney, and additional organs that were considered diagnostically important. For diagnosis of *Flavobacteria* spp., skin, gills and spleen were routinely sampled. However, when individual live fish were examined (these were mainly koi carps), sampling was performed non-lethally (most often with skin or mouth swabs) and the swabs were submitted to the laboratory, creating a sampling bias. Farmed fish are almost exclusively treated on a population level, where sacrificing a few animals for post-mortem examination is a common practice. Organ samples were cultivated on blood agar plates (bioMérieux, Switzerland) for 48 hours at 22 °C. In salmonid samples, an additional bromthymol-blue lactose agar plate (Merck, Germany) supplemented with 0.5% sucrose was used to differentiate *Aeromonas* spp. and *Yersinia* spp. In salmonids, samples of gills, skin and spleen were cultured on special agar plates that favour growth of *Flavobacteria* spp.[48] for 5 days at 15 °C. In warm water fish species, *Flavobacteria* spp. were grown at 22 °C for 5 days. Bacterial growth was recorded every day. Colony morphology was evaluated, *Flavobacteria* spp. were identified under the light microscope based on the typical bacteria morphology[27]. Bacterial colonies were further differentiated to the species level by the CFWH using the API identification system (API20E or API20NE) (bioMérieux, Germany) or were sent to the Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland for identification by MALDI-TOF. Prior to identification by API identification system, a catalase and oxidase fermentation reaction were performed[27]. For catalase and oxidase positive bacteria, API20E was used. In case, the API system failed to identify the bacteria species, colonies were sent to the Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland for identification. Most of *Flavobacteria* spp. were not further identified to species level.

Important bacterial species in farm fish, e.g. *Renibacterium salmoninarum*, and in pet fish, e.g. *Mycobacterium* spp., which were usually diagnosed by different techniques beside culture, were not included in this study. These bacterial infections were diagnosed by the CFWH by histology, immunohistochemistry and / or PCR.

The dataset included the owner name, postal code of owner, fish species, length of fish, organ affected, and final diagnosis for each bacterial culture. Data about aquaculture facilities, such as floor type, water source, outflow of the water and production volume were added from a previously reported study[38]. Organic farms were identified from the official list of farms accredited by Bio Suisse, the federation of Swiss organic farmers. Bio Suisse is the main organization of organic agriculture in Switzerland which includes 32 organic farmers' associations among its members.

Data and Analyses

The database contained 1448 rows (one row for each bacterial isolation). There were 19 variables. Data were not available for all variables for all bacterial isolates, as some of the variable values did not apply to all submissions, which explains the missing values for some variables. Supplementary table 1 contains all variable names as well as numbers of missing and distinct values for each variable.

The data were collected in a Microsoft Excel 2007 table. Analyses were performed in an R Notebook with R Studio 1.1.383 and R 3.5.0 „Joy in Playing".

Results

Technical data

Submissions

The data contained 1134 different submissions originating from 374 different premises, except for 50 submissions in which the premise was missing. There were 263 different private pet fish owners (70.3% of premises), 42 commercial fish farms (11.2%), 19 fish stores (5.1%), 18 fishery societies (4.8%), 15 cantonal fish farms (4.0%), 10 research facilities (2.7%), 6 zoological gardens (1.6%), and the CFWH (0.3%).

The mean number of submissions per premise was 2.90 (median = 1, SD = 7.23, range: 1 - 95). The largest number of submissions came from commercial fish farms

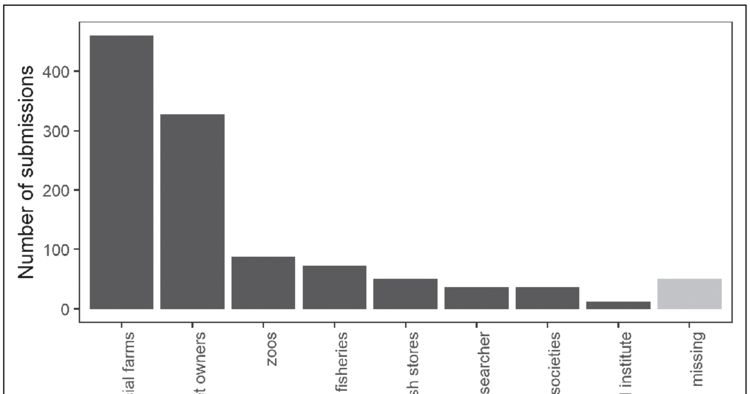

Figure 1: Bar chart of the number of fish submissions from each submitter type to the Centre for Fish and Wildlife Health (CFWH), University of Bern.

(40.6%, 460 submissions) and private pet fish owners (28.8%, 327 submissions), with smaller number of submissions coming from the other premise types (Figure 1).

Seasonality

There was considerable variation in the number of submissions per month, with an overall slight decrease in the number of submissions over the study period (Figure 2). However, there was an increase in the number of submissions of farmed fish, and a decrease in the number of ornamental fish and koi carp submissions. A strong seasonality was observed in the number of submissions with two peaks in the summer (June and August), and a decrease during the winter months (Figures 2, 3). The two summer peaks were mainly due to farmed fish submissions. Koi carp were mostly submitted between April and August, with a clear peak in May-June. In contrast, there was no clear seasonality for aquarium fish submissions (Figures 3).

Geographical location

Fish submissions originated mainly from Switzerland, with some exceptions for fish that originated just on the other side of the Swiss border. Submissions from outside of Switzerland were excluded from the geographic analysis. Three hundred thirty-six different locations were defined by postal code and the mean number of submissions per postal code was 3.18 (median = 1, SD = 7.92, range: 1–95). Most of the submissions originated from the Swiss plateau (Figure 4).

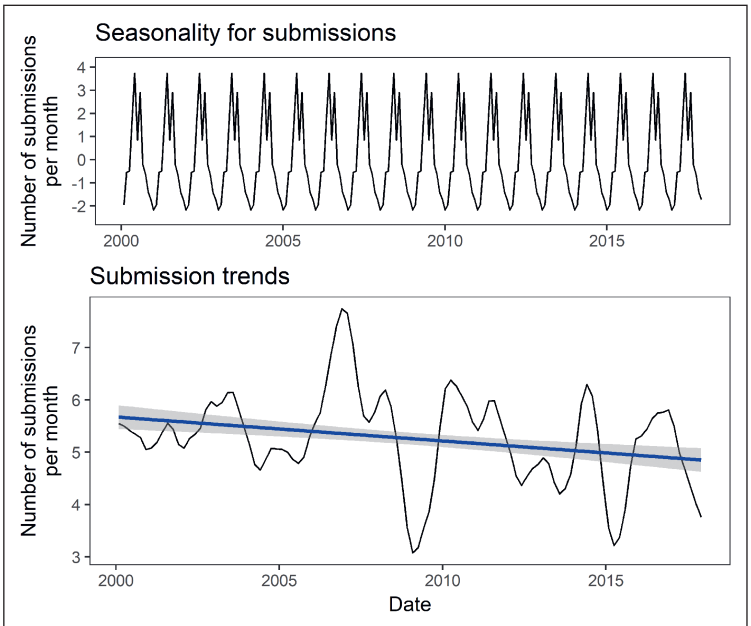

Figure 2: Time series decomposition using non-parametric regression (Loess smoothing) of the total number of fish submission per month to the Centre for Fish and Wildlife Health (CFWH), University of Bern for the study period from 2000 to 2017. a. model of the yearly seasonality of monthly fish submissions. The model is normalized so that the mean is zero, and since it is a regression model, the model is the same for each year. b. the long term trend. The fluctuating line is a smoothed average that shows the yearly trend and the straight line is the regression that best fits all of the data points (with accompanying 95% confidence interval shaded in grey.

Fish farm types and characteristics

The type of production system for farmed fish included farms that owned tanks and/or raceways in a flow-through system (77.3%, 34 farms), farms that had ponds (50.0%, 22 farms) and farms with recirculating systems (11.4%, 5 farms). Many fish farms had more than one production system type. Only 9.1% (4 farms) of all fish farms had quarantine stations for incoming and/or sick fish. Two fish farms (4.6%) had other facility types in addition to the ones mentioned above.

There were 19 farms (43.2%) with exclusively concrete floors, 7 farms (15.9%) with exclusively natural environment, and 18 farms (40.9%) with both flooring types. The majority (89.8%, 44 farms) of the fish farms in this study were conventional farms, from which 56.8% (256 submissions) of all submissions originated (mean of 5.8 submissions per farm, median = 2, SD = 8.5, range: 1-41). The number of organic farms submitting samples was smaller (10.2%, 5 farms). There were 195 (43.2%) submissions from organic farms. The number of submissions per farm was higher for organic farms (mean of 39.0 submissions per farm, median = 30, SD = 35.7, range: 2-95) compared to conventional farms; however, 95 submissions originated from one organic farm.

Fish farms obtained their water from either surface water (43.5% of farms, 20 farms), spring or tap water (37.0%, 17 farms) or both (19.6%, 9 farms). Water outflow from fish farms was reported to be directly into surface water (87.0% of farms, 40 farms), sewage (4.3%, 2 farms) or both (8.7%, 4 farms). The size of the farms submitting samples varied considerably. There were 4 farms producing less than 1,000kg of fish per year (14.3%), 6 farms (21.4%) producing between 1,000 and 5,000kg, 3 farms (10.7%) producing between 5,000 and 20,000kg, 8 farms (28.6%) producing between 20,000 and 80,000kg, 4 farms (14.3%) producing between 80,000 and 150,000kg and 3 farms (10.7%) producing more than 150,000kg per year. The mean number of submissions per farm was calculated for each production group. Farms producing between 80,000 and 150,000kg of fish per year submitted the greatest number of fish to the laboratory (Figure 5).

Age groups

The age of fish submitted was only available for rainbow trout. Age classes were categorized as follows: less than one year old (59.9%, 187 submissions), 1 to 2 years old (24.7%, 77 submissions), over 2 years old (12.8%, 40 submissions) and 2.6% of all submissions (8 submissions) consisted of fish of multiple age classes.

Analysis of the isolated bacteria

There were 1448 bacteria isolates from the 1134 submissions. The mean number of different bacteria isolated per submission was 1.28 (median = 1, standard deviation (SD) = 0.57, range: 1–6).

Seventy-five different bacteria species were identified from 959 isolates and 489 isolates (33.8% of the isolates) could not be identified at the species level. The most common bacteria family was *Flavobacteriaceae* (40.6% of identified isolates, 389 isolations), comprising the genera *Flavobacterium* with *Flavobacterium* spp. (96.4% of *Flavobacteriaceae* isolations, 375 isolations), *F. psychrophilum* (0.8%, 3 isolations), *F. aquidurense/piscis* (0.3%, 1 isolation), and *F. tructae* (0.3%, 1 isolation) and *Chryseobacterium* with *C. indologenens* (2.1%, 8 isolations) and *C. joostei* (0.3%, 1 isolation). Most of the *Flavobacteriaceae* were diagnosed based on morphological features under the light microscope, therefore an identification up to species level was not possible.

Aeromonadaceae were the second most common bacteria family (33.8% of identified isolations, 324 isolations), comprising *A. sobria* (29.9% of *Aeromonas* spp. isolations, 97 isolations), *A. hydrophila/caviae* (22.8%, 74 isolations), *A. salmonicida* subsp. *salmonicida* (22.2%, 72 isolations), *A. hydrophila* (18.5%, 60 isolations), *Aeromonas* spp. (3.1%, 10 isolations), *A. veronii* (2.5%, 8 isolations), *A. ichtiosoma* (0.6%, 2 isolations) and *A. jandaei* (0.3%, 1 isolation). Two hundred forty-six isolates (25.7% of all identified isolations) were other bacterial pathogens, the most important being *Y. ruckeri* (22.0%, 54 isolates), *Vibrio* spp. (16.3%, 40 isolates) and *Lactococcus* spp. (7.3%, 18 isolates). However, these proportions should be interpreted very carefully because 33.8% (489 isolates) of the isolated bacteria were not identified. *Flavobacteriaceae* are easy to recognise with a light microscope and since all the submitted fish were examined under light microscope, we could surmise that few *Flavobacteriacea* were unidentified, which means that the true proportion of *Flavobacteriaceae* in our sample could be as low as 26.9%. Similarly, aeromonads could have a true proportion between 22.4% (324 isolates) (if there were no *Aeromonas* spp. among unidentified isolates) and 56.1% (813 isolates) (if all unidentified isolates were *Aeromonas* spp.). If we considered that the unidentified bacteria are in the same proportions as the identified ones (except for those identified as *Flavobacteriaceae*), aeromonads would represent 41.6% (602 isolates) of the isolations.

See Table 3 for a description of the bacteria isolates grouped by the intended use of the fish.

Bacteria were isolated from 14 different organs, most frequently from spleen (40.7% of isolates, 416 isolates), skin (28.0%, 286 isolates), liver (11.6%, 119 isolates) and kidney (11.6%, 119 isolates). However, these data were biased as these organs were most often sampled for routine diagnostics. Additionally, as the database for this study comprised bacterial isolations where a resistance test was performed, infections of skin and gills are underrepresented as these are usually not treated with an-

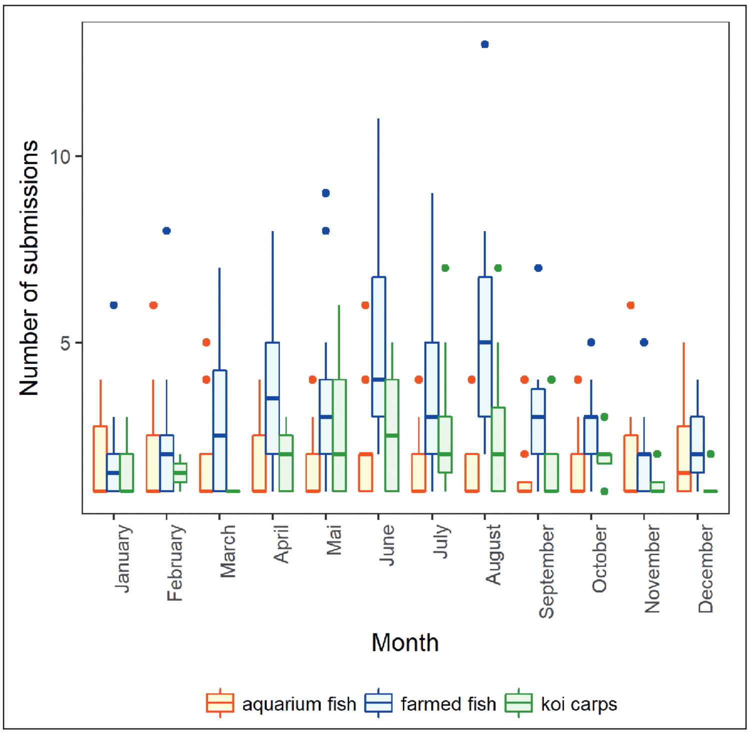

Figure 3: Boxplot of the number of submissions per month for farmed fish, aquarium fish and koi carp, red bars represent number of submissions of aquarium fish, blue bars represent number of submissions of farmed fish and green bars represent number of submissions of koi carp.
The bold line in the box represents the median (second quartile). The lower and upper extremities of the box represent the first (25%) respectively the third quartile (75%). The whiskers represent values that are between the value of the median plus or minus 1.5 interquartile range (IQR), ending at the value of the last value fitting in the range, according to Tukey, 1977[67]. Outliers were plotted individually with dots.

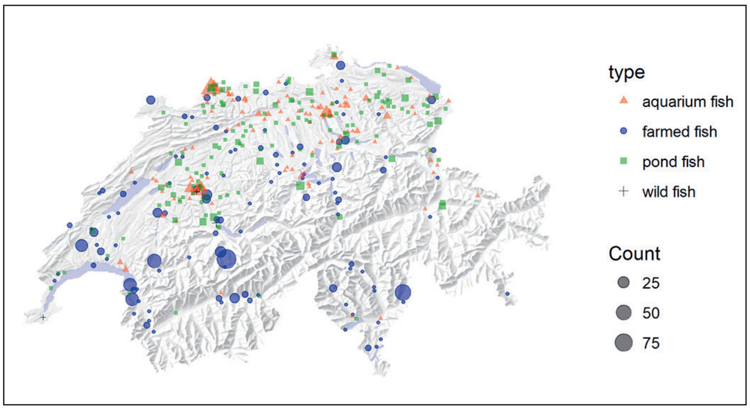

Figure 4: Geographic location premises of submitted fish to the Centre for Fish and Wildlife Health (CFWH), University of Bern and the number of submissions per premise grouped by intended use of the submitted fish.

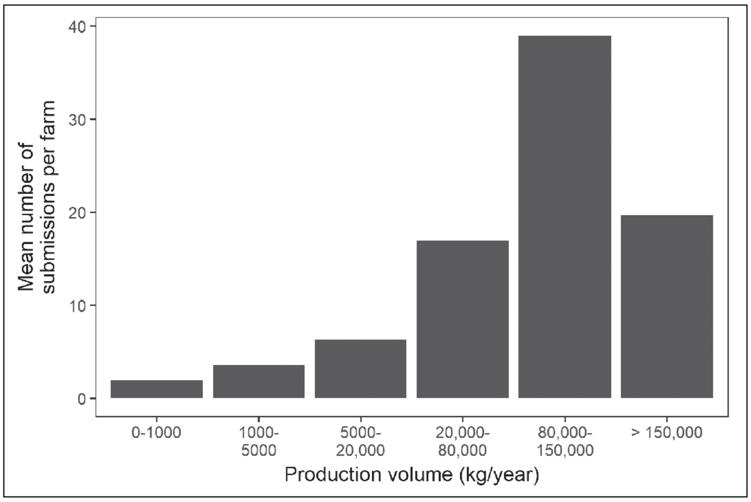

Figure 5: Mean number of fish submissions per farm for each category of production volume to the Centre for Fish and Wildlife Health (CFWH), University of Bern during the study period from 2000 to 2017.

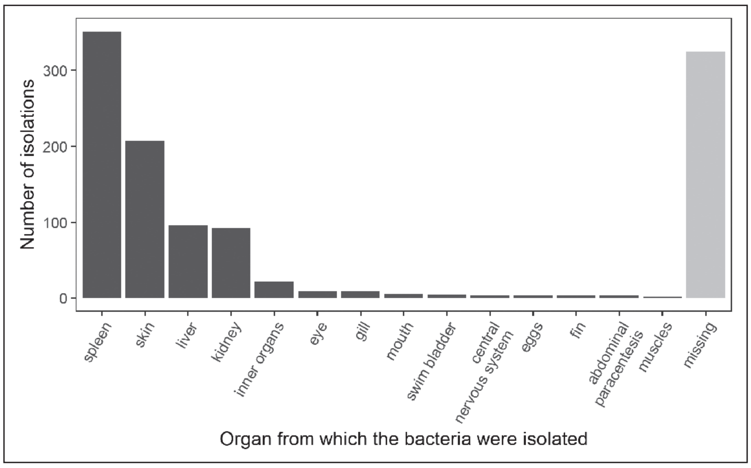

Figure 6: Fish organs from which the bacteria were isolated.

tibiotics. All other organs did not exceed 3% of isolations (27 isolates) (Figure 6).

The sampled organ in relation to the intended use of the fish is presented in Table 4. In case of farmed fish, bacteria were mainly isolated from inner organs. For ornamental fish, particularly for koi carp, samples were mainly taken from skin or mouth swabs.

Seasonality

There were seasonal patterns in the bacteria species isolated from farmed fish. During the summer peak in farmed fish submissions, the most common bacteria isolated were Flavobacteriaceae. This is in contrast to isolations of Aeromonas spp. from farmed fish which peaked in April and had a small but consistent number of isolations for all other months. Other bacteria species from farmed fish submissions were most commonly isolated between March and August (Figure 7).

Fish farm characteristics

Among the submissions from classic farms, 54.9% (157 isolates, mean of 3.6 isolations per farm, median = 1, SD = 7.7, range: 0-39) of the identified bacteria were *Flavobacteriaceae*, 28.0% (80 isolates, mean of 1.8 isolations per farm, median = 1, SD = 3.0, range: 0-14) were aeromonads (from which 39 isolates were *A. salmonicida*) and 17% (49 isolates, mean of 1.1 isolations per farm, median = 0, SD = 3.1, range: 0-16) were other bacteria while 9.8% (31 isolates) of the isolated bacteria were not further identified. Among the submissions from organic farms, 70.3% (135 isolates, mean of 27.0 isolations per farm, median = 29, SD = 19.3, range: 1-52) of the identified bacteria were *Flavobacteriaceae*, 5.7% (11 isolates, mean of 2.2 isolations per farm, median = 0, SD = 3.9,

Table 3: Some bacteria of interest and the intended use of the fish they were isolated from.

Bacteria	Farmed fish	Aquarium fish	Pond fish
Aeromonas spp.			
A. salmonicida	65	1	4
A. sobria	15	42	38
A. hydrophila/caviae	62	19	53
Flavobacteriaceae			
Flavobacterium spp.	372	0	2
Other bacteria			
Y. ruckeri	54	0	0
Lactococcus spp. (*L. garviae*)	18 (14)	0 (0)	0 (0)
P. shigelloides	1	12	0
Vibrio spp.	2	34	4
V. alginolyticus	1	4	1
V. fluvialis	0	0	3
V. vulnificus	1	13	0
Total isolations	741	329	368
Total submissions	615	258	255

range: 0-9) were aeromonads (from which 2 isolates were *A. salmonicida*) and 24.0% (46 isolates, mean of 9.2 isolations per farm, median = 0, SD = 16.5, range: 0-38) were other bacteria. 8.1% (17 isolates) of the isolated bacteria were not further identified.

In this study, 40.9% of the farms used both types of flooring (natural and concrete floor) in parallel. There were no differences in the mean numbers of isolations of aeromonads and other bacteria based on floor type. However, *Flavobacteriaceae* were isolated on 212 occasions from 25 farms with natural soil (86 isolations from 7 farms using exclusively natural soil) versus 164 isolations from 36 farms with concrete floor (38 isolations from 19 farms using exclusively concrete floor).

All bacteria were more commonly isolated from farms using exclusively tap or spring water compared to farms using exclusively surface water. Two hundred seventy-four isolations (141 *Flavobacteriaceae*, 51 aeromonads and 82 other bacteria isolations, from which 44 isolations of *Y. ruckeri*, 35 originating from a single farm, and 12 of *Lactococcus* spp.) originated from farms using exclusively spring or tap water as a source, as compared to 97 isolations (80, 13 and 4 isolations, respectively) from farms using exclusively surface water.

Age group

Flavobacteriaceae were mainly isolated from submissions of rainbow trout less than one year old (73.5% of isolations, 169 isolations), while they were only isolated 39 times (17%) from submissions of trout between 1 and 2 years old, 19 times (8.3%) from submission of fish more than two year old and 3 times (1.3%) from submissions with rainbow trout of various age categories. Aeromon-

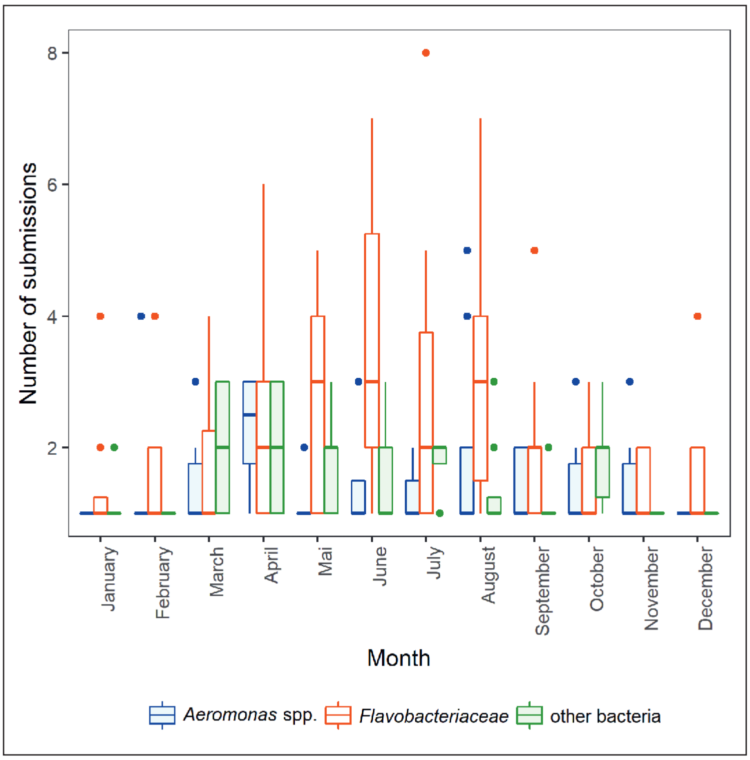

Figure 7: Boxplot of the number of submissions of farmed fish per month for *Aeromonas* spp., *Flavobacteriaceae* and other bacteria during the study period from 2000 to 2017. Blue bars represent submission numbers of *Aeromonas* spp., orange bars of *Flavobacteriaceae* and green bars represent numbers of submissions of other bacteria.
The bold line in the box represents the median (second quartile). The lower and upper extremities of the box represent the first (25%) respectively the third quartile (75%). The whiskers represent values that are between the value of the median plus or minus 1.5 interquartile range (IQR), ending at the value of the last value fitting in the range, according to Tukey, 1977[67]. Outliers were plotted individually with dots.

Table 4: Organs from which bacteria were isolated and the intended use of the fish.

Organ	Aquarium fish	Farmed fish	Wild fish	Pond fish	Total
Abdominal paracentesis	0	2	0	2	4
Central nervous system	0	5	0	0	5
Eggs	0	4	0	0	4
Eye	1	11	0	1	13
Fin	1	4	0	0	5
Gill	0	10	0	1	11
Inner organs	11	15	0	1	27
Kidney	35	67	0	17	119
Liver	58	38	0	23	119
Mouth	1	2	0	3	6
Muscles	0	3	0	0	3
Skin	43	73	1	169	286
Spleen	38	348	1	29	416
Swim bladder	4	0	0	1	5
Total	192	582	2	247	1023

ad isolations were also more often isolated from submissions of fish less than one year old (42.3% of isolations, 11 isolations) than from those of older fish (age classes from one to two year old and more than two years old both 23.1%, 6 isolations) or of mixed age category (11.5%, 3 isolations). Other bacteria were isolated more often from submissions of trout from one to two year old (52.5% of isolations, 31 isolations) than from submissions of fish more than two years old (24.6%, 15 isolations) or of fish less than one year old (18%, 11 isolations). Other bacteria were only isolated thrice (4.9%) from submissions of trout with variable age category.

From submissions from fish less one year old, *Flavobacteriaceae* was also the bacteria most frequently isolated (169 isolates, 88.5% of isolations), preceding both aeromonads and other bacteria with each 11 isolations (5.8%). *Flavobacteriaceae* were also the most common isolates from submissions of fish between one and two years old (39 isolations, 50.6%), shortly followed by other bacteria (32 isolations, 41.6%), while aeromonads represented only 7.8% (6 isolates). This was also the case for submissions from trout more than two years old (*Flavobacteriaceae*: 47.5% of isolates, 19 isolates; other bacteria: 37.5%, 15 isolates; aeromonads: 15%, 6 isolates). All three bacterial groups were equally isolated from submissions of fish of various age classes, with each 3 isolations (33.3%).

Discussion

There were far fewer commercial fish farms (42) than private pet fish owners (263) recorded as premises in our database. However, 40.6% of the submissions originated from commercial fish farms versus 28.8% from private pet fish owners, which implies a much higher number of submissions per premise for fish farms. Commercial fish farms have a higher density of fish and a higher total number of fish than private owners. However, the hypothesis that increased fish density is associated with higher numbers of bacterial infections is still being discussed in the literature and is considered controversial[49,50]. It could be hypothesized, for several reasons, that farmed fish experience more bacterial infections compared to other fish. For example, commercial fish farms have a higher fish turnover and therefore a higher probability of introduction of infectious agents, which could influence the occurrence of disease experienced by commercial farms. In addition, the commercial farm production environment is more changeable compared to aquaria and ponds which may be stressful for farmed fish, predisposing them to bacterial infections. On the other hand, it may be that commercial fish farmers are more concerned about losses due to disease or place a higher value on the information from post-mortem diagnostics and therefore are more likely to submit dead fish to laboratories. For pet fish, costs for diagnostics often exceeds far the value of the animals, even for professional fish traders. Additionally, treatments for ornamental fish are easily available for professionals, therefore, diagnostic is less often used preceding the treatment.

Over the study period, the average number of submissions per month fluctuated greatly, with a slight overall long-term downward trend. This decreasing trend was due to a fairly pronounced decrease in number of aquarium and koi carp submissions, which was larger than the significant increase in the number of farmed fish submissions. We did not have sufficient data to determine whether these changes were related to the number of fish held in each category in Switzerland during this period. A possible explanation could be the increased specialization of some fish veterinarians in the past few years, who may have taken some of the diagnostic work as part of their clinical work (Schmidt-Posthaus, pers. comm.).

There were also significant seasonal variations, with two peaks in June and August, due mainly to the seasonality in farmed fish submissions. One reason for this strong seasonality may be the production cycle of farmed fish. The majority of farmed fish are rainbow trout, which are hatched between the end of November and January[51], making the age distribution of the farmed fish population relatively uniform. Fish are poikilothermic animals, and therefore their immune system is temperature dependent[52,53]. Each fish species has a temperature optimum, and variations below or above this optimum compromise the effectiveness of their immune system[54]. Pathogens cause disease when optimal conditions for the pathogens are met[55], and since these conditions are fairly uniform in terms of the host (i.e. age specific), these infections are likely to occur at the same time of the year if the fish population is the same age.

In our study, *Flavobacteriaceae* were mainly isolated from farmed fish in May, June and August. Decostere, D'Haese, et al. (2001) reported that *Flavobacteriaceae* affect juveniles most often[56], particularly before the 20th week of their life[57]. The majority of submitted rainbow trout in our study from which we isolated *Flavobacteriaceae* were less than 1 year old. However, given the seasonality of hatching (December and January), the fish in our study were likely older than 20 weeks when the peaks were observed in our data. Only systemic infections with *Flavobacteriaceae* were included in this study. As mainly young rainbow trout were affected and the bacteria were cultured at low temperature, we concluded that most of the *Flavobacteriaceae* belonged to *F. psychrophilum*. Marcos-López, Gale, et al. (2010) reported that *F. psychrophilum* is a winter disease in the UK, with optimum temperatures below 10 °C[55], which is in agreement with Holt, Rohovec, et al. (1993)[35]. Holt, Amandi, et al. (1989) however, reported that the maximum morbidity occurred between 12 and 15 °C[34]. Our peaks occurred later in the year than the ones reported and could be explained by increased stress caused by changing temperatures in spring[26,29,47]. The water source used for a large proportion of Swiss fish farms is spring water. Spring water temperature increases slowly in spring, probably reaching the optimal temperature for *F. psychrophilum* later in the year compared to other countries such as the UK.

In pet fish, no *Flavobacteria* spp. were further analysed by a resistance test. This could also have technical reasons as cultivation of flavobacteria at higher temperature conditions which are necessary for warm water species is difficult and bacterial cultures were usually overgrown by other bacteria. Therefore, these infections were not included in the dataset presented in this analysis.

Aeromonas salmonicida subsp. *salmonicida* appears to primarily cause losses in young fish[31], with elevation in temperature being a known stress factor[26,29,47]. Fish farms also often transfer juvenile fish from tanks to raceways in April (Schmidt-Posthaus, Diserens, own observations), which is another stressor that could be associated with increased mortality. The clear peak in April in our study is in agreement with reports in the literature[29].

Koi carp were most commonly submitted between April and August, with peaks in June. Koi carp go into a rest period during the winter months with reduced metabolic activity[58]. Both pathogens and the fish immune system are temperature dependent[52,53]. In spring there is most likely a discrepancy between increasing pathogen virulence and reduced function of the fish immune system. The discrepancy could be accentuated by the increased stress caused by occasional temperature changes[26,29,47].

Aquarium fish submissions did not follow a clear trend, being relatively similar during all months of the year. The lack of seasonality may be due to the uniform conditions that aquaria fish are held at throughout the year.

There are some important bacterial species missing in our study, like *Renibacterium salmoninarum* in farm animals, mainly trout, or *Mycobacterium* spp. in pet fish. This is due to technical reasons, as these bacteria were usually diagnosed by different techniques, like histology, immunohistochemistry or PCR[59].

Organic farms brought approximately six times more fish submissions per farm than conventional farms. 195 submissions originated from five organic fish farms, compared to 156 submissions originating from 44 conventional farms. All organic farms and the majority of conventional farms keep rainbow trout as the main fish species. These submissions were distributed over the whole examination period; single outbreaks in the organic farms can be excluded. One possible explanation for the increase in submissions from organic farms could be enrichment of the organic farm environment with structures, e.g. natural floor covered with pebbles or hiding structures. These could impact tank hygiene (e.g. by higher accumulation of food particles and faeces)[60] favouring the accumulation of pathogenic agents in the tank and resulting in increased risk for bacterial infections[26,61]. This could potentially explain the isolations of *Flavobacteriaceae* in our study. However, 56.8% of all the farms included in our study, both conventional and organic farms, use natural floor. Of these, 72.0% also had concrete floors and 28.0% had exclusively natural floor. In general, there was no observed difference in the number of submissions between the farms possessing natural floor and the ones possessing only concrete floor. However, farms possessing natural floor seemed to have more *Flavobacteriaceae* isolations.

The higher level of bacterial isolations from farms using spring or tap water was surprising. The risk of spring water contamination should be lower, and water quality should be higher than other water sources[62], resulting in reduced stress and susceptibility to infections.

In this study, 87.0% of the food producing fish farms released their outflow water into surface water. Bacterial infections are often treated with antibiotics and in aquaculture antibiotic are mostly administered orally[26]. Since water is mostly released into the surface water, it is likely that antimicrobial residues are also released into surface water. This should be considered a potential risk for the development of antibiotic resistances and should be investigated in future studies. An additional risk is the release of bacterial pathogens into surface water which could pose a risk for infection to native wild fish.

Flavobacterium spp., *A. salmonicida, Y. ruckeri* and *Lactococcus garviae* were mostly isolated from farmed fish, which is in agreement with reports from the literature[26,31]. Motile aeromonads were isolated from farmed fish, but most often from pet fish. *Flavobacterium* spp., motile aeromonads, *A. salmonicida* and *Y. ruckeri*, the most relevant aquaculture bacterial pathogens in our study, have all been reported in freshwater wild fish[47,63,64,65]. Additionally, *Flavobacterium* spp. and *A. salmonicida* were isolated from wild fish in our study. Aquaculture should therefore be considered to be a risk for pathogen transmission for these common diseases to wild fish[66].

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Caractéristiques des isolats bactériens chez des poissons d'élevage et des poissons d'ornement suisses issus d'une étude rétrospective de 2000 à 2017

L'aquaculture est un secteur de la production alimentaire en pleine croissance et d'une grande importance économique. Les infections bactériennes constituent une menace importante pour la croissance de l'aquaculture mais également un problème courant chez les poissons d'ornement. Certains agents pathogènes et types de production aquacole seraient associés à une plus forte incidence de certaines maladies. Une description complète des agents pathogènes bactériens responsables de maladies chez les poissons d'élevage et d'ornement en Suisse fait cependant défaut. Nous décrivons dans cette étude 1448 isolats bactériens provenant de 1134 soumissions de poissons d'élevage et de poissons d'ornement en Suisse à un laboratoire de diagnostic entre 2000 et 2017. Une forte saisonnalité a été observée au printemps et en été. Les infections bactériennes chez les poissons de fermes biologiques étaient environ six fois plus fréquentes que dans les exploitations conventionnelles. *Flavobacteriaceae*, *Aeromonas* spp. et *Yersinia ruckeri* sont les isolats qui ont été les plus communément isolés des soumissions des piscicultures. *Vibrio* spp. ainsi que les espèces motiles d'*Aeromonas* spp. ont été les principaux isolats mis en évidence chez les poissons d'ornement. Les résultats de cette étude fournissent des hypothèses intéressantes, mais des recherches supplémentaires sont nécessaires pour mieux caractériser les facteurs de risque des maladies bactériennes chez les poissons d'aquaculture et d'aquarium en Suisse.

Mots clés: Infection bactérienne, aquaculture, type de production, poisson d'ornement, facteur de risque, Suisse

Studio retrospettivo dal 2000 al 2017 sulle caratteristiche degli isolati batterici nei pesci di allevamento e ornamentali in Svizzera

L'acquacoltura è un settore di elevata importanza economica in rapido sviluppo per la produzione alimentare. Le infezioni batteriche sono un'importante minaccia per la crescita dell'acquacoltura e un problema comune nei pesci ornamentali. Alcuni agenti patogeni e tipi di produzione dell'acquacoltura sarebbero associati ad un incremento delle malattie. Tuttavia, manca ancora una descrizione dettagliata dei patogeni batterici causanti le malattie nell'acquacoltura svizzera e nei pesci ornamentali. In questo studio descriviamo 1448 isolati batterici provenienti da 1134 studi di laboratorio di diagnostica riguardanti i pesci di allevamento e ornamentali in Svizzera durante il periodo tra il 2000 e il 2017. Si è osservata una forte stagionalità con picchi durante la primavera e l'estate. Gli isolati batterici estratti dai pesci provenienti da allevamenti biologici erano approssimativamente sei volte più frequenti rispetto agliisolati degli allevamenti convenzionali. *Flavobacteriaceae*, aeromonadi e *Yersinia ruckeri* sono gli isolati più comuni rilevati nell'acquacoltura mentre gli aeromonadi mobili e i *Vibrio* spp. quelli maggiormente presenti nei pesci ornamentali. I risultati di questo studio permettono di formulare varie ipotesi interessanti, ciò nonostante sono necessarie ulteriori ricerche per meglio caratterizzare i fattori di rischio delle malattie batteriche sia nell'acquacoltura che nei pesci di acquario in Svizzera.

Parole chiavi: Infezioni batteriche, acquacoltura, tipo di produzione, pesci ornamentali, fattore di rischio, Svizzera

Characteristics of bacterial isolates in Swiss farmed and ornamental fish from a retrospective study from 2000 to 2017

G. Delalay et al.

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