Epidemiol. Infect. (2012), **140**, 192–206.
doi:10.1017/S0950268811001798

REVIEW ARTICLE
Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: a systematic review of the existing research and survey of aquaculture-allied professionals

M. WEIR[1], A. RAJIĆ[1,2]*, L. DUTIL[2], N. CERNICCHIARO[1], F. C. UHLAND[3], B. MERCIER[4] AND N. TUŠEVLJAK[1,2]

[1] *Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada*
[2] *Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada, and Saint-Hyacinthe, Québec, Canada*
[3] *Faculté de Médecine Vétérinaire, Saint-Hyacinthe, Québec, Canada*
[4] *Zoo Sauvage de Saint-Félicien, Saint-Félicien, Québec, Canada*

(*Accepted 12 August 2011; first published online 12 September 2011*)

SUMMARY

Using systematic review methodology, global research reporting the frequency of zoonotic bacterial pathogens, antimicrobial use (AMU) and antimicrobial resistance (AMR) in ornamental fish, and human illness due to exposure to ornamental fish, was examined. A survey was performed to elicit opinions of aquaculture-allied personnel on the frequency of AMU and AMR in ornamental fish. The most commonly reported sporadic human infections were associated with *Mycobacterium marinum*, while *Salmonella* Paratyphi B var. Java was implicated in all reported outbreaks. *Aeromonas* spp. were most frequently investigated ($n=10$ studies) in 25 studies surveying ornamental fish from various sources. High levels of resistance were reported to amoxicillin, penicillin, tetracycline and oxytetracycline, which was also in agreement with the survey respondents' views. Studies on AMU were not found in our review. Survey respondents reported frequent use of quinolones, followed by tetracyclines, nitrofurans, and aminoglycosides. Recommendations for future surveillance and public education efforts are presented.

Key words: Domestic pets, outbreaks, resistance to drugs, swimming pool (fish tank) granuloma, zoonoses.

INTRODUCTION

Ornamental aquatic organisms include over 1500 fish, 100 coral and 300 invertebrate species, usually kept in aquaria for display purposes. Freshwater fish species are farm-raised, and comprise 90–96 % of the overall ornamental fish trade [1]. Globally, the industry is valued at over US$900 million and US$3 billion at the wholesale and retail levels, respectively, with the USA being the largest importer of ornamental fish [1, 2]. The movement pathways of ornamental fish (Fig. 1) are very complex and dynamic as ornamental fish shipments move thousands of miles from production sources and countries of origin via holding and transhipment facilities, wholesalers and retailers for eventual display in public aquariums or hobbyists' homes around the world [1]. It is estimated that one million Canadian and 12 million US households own aquariums [3]. Ornamental fish farms are traditionally small, family-run operations with often closely guarded husbandry practices developed through years

* Author for correspondence: Dr A. Rajić, Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, 110 Stone Road West, Guelph, Ontario, N1G 3W4, Canada.
(Email: arajic@uoguelph.ca)

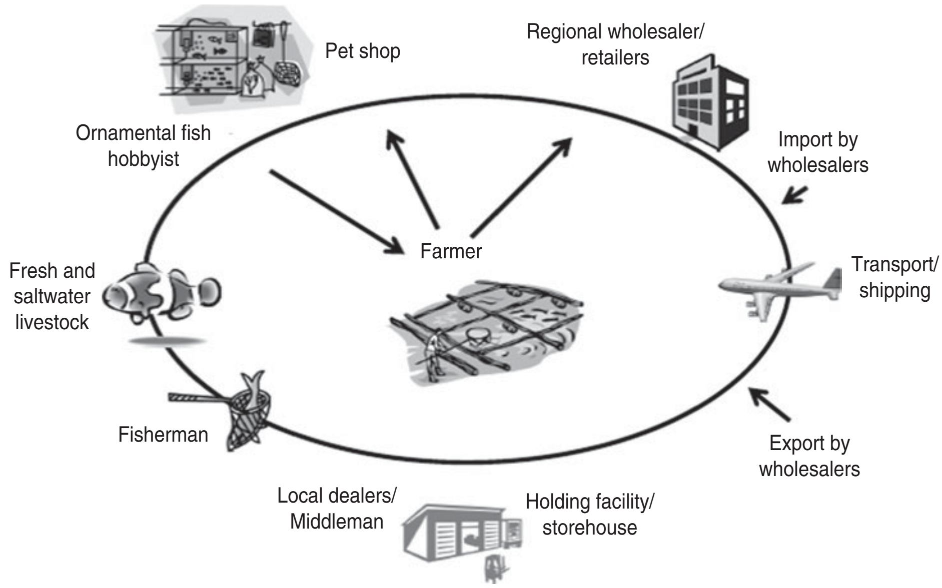

Fig. 1. Pathways of ornamental fish production, shipment and acquisition (adapted from Livengood & Chapman [1]).

of experimentation [4]. Numerous public health concerns have been linked with this industry, including zoonotic pathogens, antimicrobial use (AMU) practices and antimicrobial resistance (AMR). A lack of qualified production personnel and equipment, poor and unsustainable production and transport practices, routine or improper use of medications to suppress disease, and limited veterinary oversight of imports/exports is commonly noted in the industry [1, 5, 6]. Health and safety import regulations for ornamental fish might vary considerably among countries [7–12].

The data on the frequency of zoonotic bacterial infections in humans due to exposure to ornamental fish is limited and prone to under-reporting due to the difficulty or failure to associate exposure with disease occurrence. Similarly, the data on the frequency of these pathogens, AMU practices and levels of AMR in ornamental fish populations are lacking or are presented in a fragmented fashion [13–17]. Published reviews on these issues are relatively uncommon [13]. Systematic reviews are frequently used to identify, critically appraise and summarize the existing knowledge and gaps on a given subject using transparent and replicable methodology [18]. Questionnaire surveys are also frequently used as a cost-effective way of eliciting opinions from targeted populations (e.g. field experts) or to identify and fill knowledge gaps, and inform future research and policy developments [19]. Our objective was to apply these two methodologies in a complementary fashion and to integrate their findings through discussion and interpretation. A systematic review (SR) was conducted to evaluate and summarize the results of published research reporting the frequency of zoonotic bacterial pathogens, AMU and AMR in ornamental fish, or human illness due to (potential) exposure through ornamental fish. A questionnaire was administered in order to elicit opinions of aquaculture allied personnel on the frequency of AMU and AMR in ornamental fish. We discuss the results of the two independent yet complementary studies, which were part of a larger initiative investigating similar aspects in seafood-related aquaculture species, in terms of existing research knowledge and gaps, surveillance opportunities, and public health education needs.

METHODS

Literature search

Multiple population (e.g. ornamental fish) and outcome (e.g. bacterial prevalence) search terms were applied in six electronic databases in October 2008, and updated in October 2010, with the search restricted to research published during or after 1990. A complete list of search terms and combinations, and electronic databases is given in the Supplementary material (Appendix 1, available online). A simple Google internet search was conducted to identify additional potentially relevant references. Search verification included a manual search of reference lists of all electronically identified articles found to be relevant through the review process and reference list of

https://doi.org/10.1017/S0950268811001798 Published online by Cambridge University Press

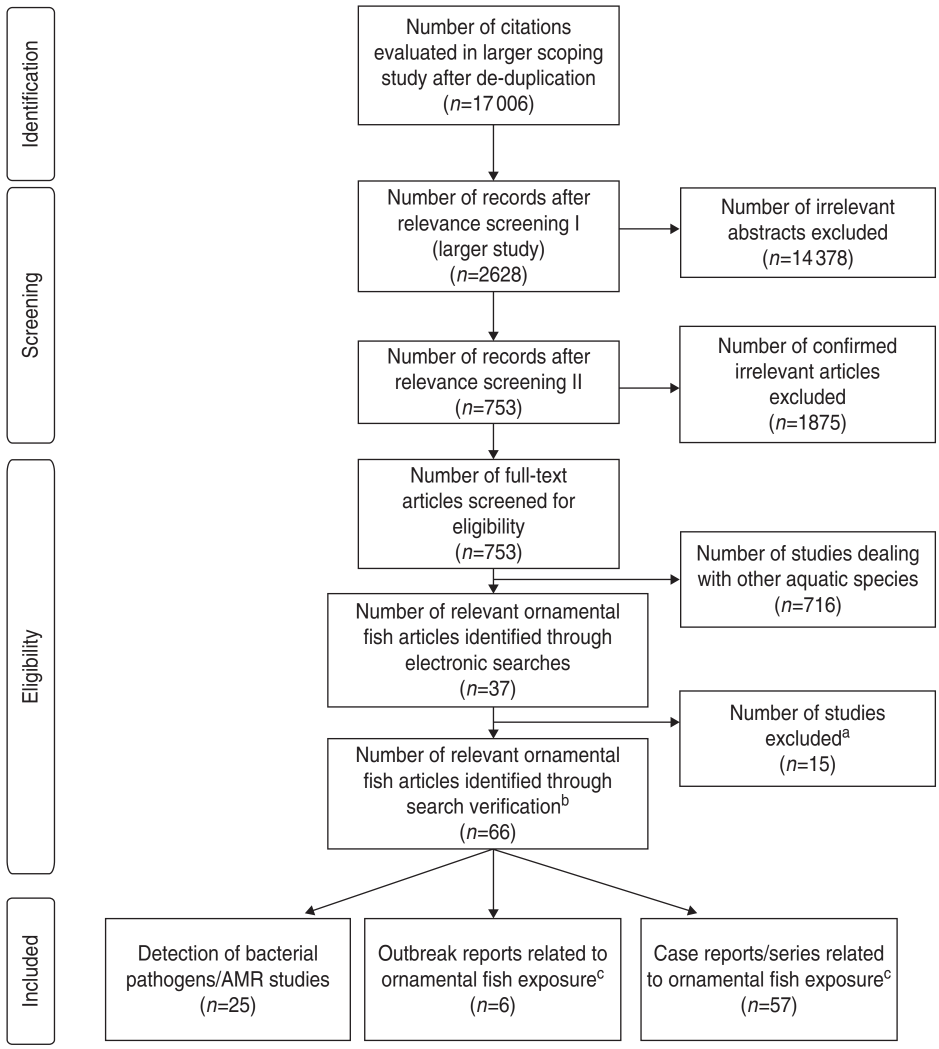

Fig. 2. Flow of studies that entered the larger scoping study on the same topics in aquaculture species intended for human consumption as pertaining to selection of studies entering the ornamental zoonoses review. [a] Excluded due to: lack of raw/unadjusted data and/or measures of association/effect ($n=5$); irrelevant study to review question ($n=9$); Slavic language, unable to translate ($n=1$). [b] Initial electronic database searches did not include *Mycobacterium marinum* as a search term, resulting in an additional 44 *M. marinum* case reports/series; reference list searches and simple Google searches identified an additional 22 bacterial, pathogen and outbreak reports not captured otherwise. [c] In-depth quality assessment precluded due to study design (case report/series, outbreak reports).

our pilot study report (B. Mercier, unpublished results) developed previously to estimate the approximate amount of research in this area and guide our review. All electronic citations were downloaded and de-duplicated in a bibliographical management program Procite 5.0 (Thomson ResearchSoft, USA), followed by a manual de-duplication.

Relevance screening, methodological assessment, data extraction and summarization

A flow of the SR process used in the larger initiative and as it pertains to this study is shown in Figure 2. Initial, abstract-level relevance screening (RS) was conducted to identify primary research, published in English, French and Spanish (the first language of various project team members), investigating bacterial zoonoses, reported AMU and/or AMR in ornamental fish or human illness due to any of these exposures. All potentially relevant abstracts were procured as full articles, confirmed for relevance, type of study design and evaluated for methodological soundness and reporting (MS). An *a priori* decision was made to restrict MS assessment to very basic evaluation and to conduct it simultaneously with data extraction (DE). A traditional MS assessment

https://doi.org/10.1017/S0950268811001798 Published online by Cambridge University Press

approach was not feasible because our pilot study indicated that all relevant research was of descriptive nature, precluding such assessment. Instead, full articles were evaluated for two general (exclusion) criteria; reporting of minimum sufficient raw or adjusted data, and replicable method protocols (e.g. laboratory methods). The former did not apply to case or case-series articles. The studies that met these criteria proceeded to DE, along with any case or case-series studies, and were included in the review. Study-specific data, for example study location(s), population(s), outcome(s), sample type and point, laboratory testing (including AMR), and reported results were extracted. RS and simplified MS assessment were completed by two independent reviewers using previously designed and pre-tested forms. DE was conducted by two independent reviewers (M.W., N.C., L.D.) on the first 20 articles, using *a priori* developed DE forms; however, the remaining 68 articles were extracted by a single reviewer due to a good agreement observed for the initial 20 articles. All forms used in this SR are given in the Supplementary material (Appendix 2, online). RS and MS steps were conducted using a web-based SR format (Distiller®, Evidence Partners Inc., Canada), while DE was conducted in Excel (Microsoft, USA) spreadsheet format. The extracted data were cleaned, summarized and reported by study design and bacterial species (outcome). Reported resistance to antimicrobials was grouped by antibiotic class to enhance consistency and interpretation across studies.

Questionnaire

Database of aquaculture-allied professionals

Thirteen aquaculture-allied professionals, indicated by our team aquaculture experts, were contacted by email and asked to provide names and/or contacts of other colleagues and professionals with expertise in aquaculture, zoonotic bacteria and AMU/AMR. Additional participants were recruited via a blog on Aquavetmed E-news. All contact information received was entered into our 'target respondents' database (Microsoft Excel).

Questionnaire description, administration and analysis

The questionnaire included five sections, and was pre-tested by five professionals with expertise in veterinary medicine and/or aquaculture and/or microbiology and epidemiology. From 26 questions pertaining to various aquatic species, four closed questions (using a five-point scale, e.g. 'never' to 'always' and one multiple-choice question) related to ornamental fish. These included: (1) frequency of AMU in ornamental fish by antimicrobial drug classes, (2) frequency of AMU in ornamental fish by production phase, (3) purpose of AMU in ornamental fish and (4) frequency of resistance observed in ornamental fish to various antimicrobial drug classes. The respondents were given the option to skip 'ornamental fish' questions if they felt that they did not have sufficient expertise in this field. A Spanish version, translated from English into Spanish by a bilingual doctoral student, was developed for administration in Spanish-speaking regions (South and Central America and the Caribbean). The questionnaire was administered using Survey Monkey®, a web-based application (Survey Monkey, USA). Two weeks prior to initial administration a letter was sent by email to all individuals listed in the above-mentioned database inviting their participation. Each participant was provided with a unique link and had a choice to refuse and opt-out of further communication. In addition, a brief questionnaire was designed for non-responders to assess non-response bias. An email was sent to 75 (randomly selected) English and all 17 Spanish non-respondents. Spanish non-respondents were also contacted up to five times via telephone to elicit additional responses. A full copy of both questionnaires is available in the Supplementary material (Appendix 3, online). Ethical approval for the surveys was received from the University of Guelph Review Ethics Board (protocol no. 09MY010).

The data from both questionnaires were exported separately to spreadsheets (Microsoft Excel), cleaned and imported into Stata 10 (Stata Corporation, USA) for frequency tabulations. A Fisher's exact test ($P<0{\cdot}05$) was used to evaluate potential differences in proportions between respondents and non-respondents.

RESULTS

Systematic review

General characteristics of the studies included in the review

Eighty-eight articles were included in the review (Fig. 2). The 'human illness' articles included: 41 case studies reporting single occurrence of human illness, 16 case-series studies reporting two or more occurrences of human illness, and six outbreak studies reporting clusters of human illness due to various bacterial zoonotic infections and suspected or confirmed exposure to ornamental fish. In addition, 25 studies of small to modest size surveys (2–533 fish-level samples) of ornamental fish populations originating from various sources and measuring prevalence of various bacterial pathogens and/or AMR were also included in the review. No studies were identified reporting AMU in ornamental fish, although in one study levels of antibiotic residues in ornamental fish were reported [20]. AMR was mainly reported in studies surveying ornamental fish from imported and/or domestic sources (see Supplementary material, Appendix 4, online). The most frequent country importation sources of ornamental fish were from Asian and South American regions. In seven, 13 and two studies, only samples of healthy, sick, or both healthy and sick, ornamental fish were collected and tested, respectively, and in six studies fish transport or production water was also examined. Farm-level sample collection of ornamental fish was conducted in only six studies. Only one relevant article (published in Czech language) was excluded due to language. A complete list of articles included in the review is given in Appendix 4 (online).

Reported bacterial illness in humans due to (potential) exposure to ornamental fish

Case reports. Globally, *Mycobacterium marinum* ($n=32$ case reports, $n=16$ case series) was the most frequently reported zoonotic pathogen linking human cases to ornamental fish exposure (individual study data are not shown for reasons of brevity) [21–67]. Besides frequently reported cutaneous lesions, osteomyelitis, tendinitis, septic arthritis and synovitis were also reported. Antibiotic treatment was reported in all cases, and resistance profiles of isolates recovered from humans in 11 studies. Deaths were reported in three immunocompromised patients of various ages. *Mycobacterium szulgai* was reported in four cases and *Aeromonas hydrophila*, *Comomonas* spp., *Edwardsiella tarda*, *Erysipelothrix rhusiopathiae*, *Vibrio cholera* and *Salmonella* Paratyphi B var. Java (now renamed *Salmonella* Paratyphi B var. L-tartrate+) were each reported as a single case (Appendix 4, online).

Outbreak reports. All reported outbreaks linked to ornamental fish exposure involved *Salmonella* serovars [68–73]. In Australia, clusters of cases associated with *S*. Paratyphi B var. Java [70, 71] were linked to home aquaria as identical isolates were recovered from human case and tank samples. Similarly, officials in New Zealand reported a cluster of *S*. Paratyphi B var. Java cases ($n=14$ cases) [72] and identical isolates were also confirmed in the fish-tank water and humans in 6/10 cases [73]. *S*. Paratyphi B var. Java was also reported in two children in the UK, where this pathogen was isolated from the children's family home aquaria and a tank of the wholesaler that had supplied fish to the children's families retailers [72]. Two outbreaks of *S*. Paratyphi B var. Java were reported in the province of Québec, Canada [68, 69], initially indicating [69] that 3/6 aquaria owned by cases were positive for several *Salmonella* serovars, including Paratyphi B var. Java, Matopeni, and Typhimurium phage-type 104. Through epidemiological traceback, 3/7 retail fish tanks were found positive for *S*. Blockley, *S*. Matopeni, *S*. Agona, *S*. Stanley, *S*. Hadar and *S*. Kallo and 1/18 wholesaler tanks supplying retail shops was positive for *S*. Blockley and *S*. Wandsworth [69]. In an additional investigation (2000–2003), *S*. Paratyphi B var. Java was detected in 18/31 (58%) of home aquaria and 8/34 (23·5%) of retail fish tanks, and in a follow-up survey (2003–2004) of two fish importers from 19·7% of samples. The former were mostly collected from consignments imported from Malaysia and Thailand, and 28 different serovars were confirmed, with *S*. Schwarzendgrund being the most common (23%) (C. Vincent, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, unpublished results).

Zoonotic bacterial pathogens and AMR in various ornamental fish populations

Mycobacterium, Aeromonas and *Salmonella* genera were the most frequently reported pathogens (Appendix 4). AMR was tested and reported mainly for *Aeromonas* spp. with resistance to the tetracycline, sulfonamide and quinolone classes frequently reported with wide ranges of resistance of 24–96%, 2·9–88%, and 6–91%, respectively (Table 1). Generally, isolates recovered from healthy warm-water and cold-water ornamental fish had considerable differences in frequency of AMR [5, 20, 74]. Five studies also reported multi-drug resistance and the presence of resistance genes/plasmids [74–78]. For brevity, data are shown only for studies reporting AMR on $\geqslant 20$ bacterial isolates (Table 1).

Table 1. *Summary of studies (n ≥ 20 isolates) reporting antimicrobial resistance (AMR) in ornamental fish*

Reference (year)	Pathogen	Number of isolates/species/serovar distribution (no. isolates)	AMR by pharmaceutical class (% resistance or range of % resistant isolates when more than one antimicrobials in class)
Domestic sources, cold-water fish species			
[78] (2010)	*Aeromonas* (selected for)*	n = 72 isolates (includes *A. sobria, A. hydrophila, A. veronii* subsp. *sobria*)	Amphenicol (6·9), chloramphenicol (6·9), quinolones (25·0), tetracyclines (50·0)
[76] (2004)	*Aeromonas* *Vibrio*	n = 75 isolates (mixed for AMR testing); included *A. hydrophila, V. fluvialis, V. furnissii*)	Aminoglycosides (16·0–85·3), cephems (29·3–100·0), chloramphenicol (34·7), nitrofurans (65·3), penicillins (61·3–94·7), quinolones (9·3), sulphonamides (14·7), tetracyclines (88·0)
[79] (1993)	*Aeromonas* (selected for)*	n = 60 isolates; *A. sobria* (41), *A. hydrophila* (15), *Aeromonas* spp. (4)	Aminoglycosides (5·0), macrolides (10·0), nitrofurans (17·0), penicillins (100·0), quinolones (1·7–13·0), sulphonamides (12·0–32·0), tetracyclines (60·0)
Domestic sources, warm-water fish species			
[80] (2007)	Choramphenicol-resistant heterophils† (numerous species)	n = 46 (farm 1) and n = 37 (farm 2); includes samples from fish intestines, pond water, pond sediment‡ (not fully described)	Chloramphenicol (100 %)†
[77] (2003)	*Pseudomonas* spp.	n = 60 isolates; *P. cepaciae* (12), *P. diminuta* (8), *P. fluroescens* (14), *P. putida* (12), *P. stutzeri* (7), *P. vesicularis* (7)	Aminoglycosides (100·0), penicillins (100·0), polypeptides (100·0), sulphonamides (100·0)
[75] (1992)	*Aeromonas* (selected for)*	n = 34 isolates; *A. hydrophila*	Aminoglycosides (23·5–38·2), chloramphenicol (2·9), macrolides (58·8), penicillins (100·0), rifampicin (0·0), sulphonamides (8·0–91·1), tetracyclines (44·0)
Imported sources, cold-water fish species			
[74] (2009)	*Aeromonas* (selected for)*	n = 33 isolates, some from transport water n.r.‡	Aminoglycosides (3·0–6·1), amphenicol (0·0), carbapenems (0·0), cephems (0·0–52·0), chloramphenicol (6·0), macrolides (33·0), monobactam (6·0), nitrofurans (0·0–12·0), penicillins (3·0–100·0), quinolones (2·9–33·0), sulphonamides (6·1), tetracyclines (24·0–36·0)
Imported sources, warm-water fish species			
[74] (2009)	*Aeromonas* (selected for)*	n = 94 isolates, some from transport water n.r.‡	Aminoglycosides (5·3–61·0), amphenicol (19·1), carbapenems (0·0–2·1), cephems (0·0–83·0), chloramphenicol (56·0), macrolides (77·0), monobactam (1·1), nitrofurans (1·1–66·0), penicillins (7·4–100·0), quinolones (55·0–85·0), sulphonamides (67·0), tetracyclines (85·0–91·0)
[81] (2002)	*Aeromonas*§ (selected for)*	n = 58 isolates, some from transport water n.r.‡	Aminoglycosides (8·5–54·2), cephems (83·1), chloramphenicol (52·5), nitrofurans (32·2), penicillins (88·1), quinolones (11·9–88·1), sulphonamides (57·6–88·1), tetracyclines (76·3)
[82] (1990)	*Aeromonas* (selected for)*	n = 70 isolates; *A. caviae* (20), *A. sobria* (20), *A. hydrophila* (30)	Aminoglycosides (26·0), macrolides (64·0), nitrofurans (51·0), penicillins (100·0), quinolones (8·5–30·0), sulphonamides (41·0–80·0), tetracyclines (96·0)
Unreported sources, fish species not reported			
[83] (2009)	*Aeromonas*	n = 27 isolates; *A. hydrophila*	Aminoglycosides (50·0–80·9), chloramphenicol (31·8), penicillins (83·3–100·0), polypeptides (88·9), quinolones (51·8–80·0), sulphonamides (41·7), tetracyclines (81·8–84·6)

Table 1 (*cont.*)

Reference (year)	Pathogen	Number of isolates/species/serovar distribution (no. isolates)	AMR by pharmaceutical class (% resistance or range of % resistant isolates when more than one antimicrobials in class)
Unreported sources, warm-water fish species			
[84] (1992)	*Aeromonas* (selected for)*	$n=42$ isolates; *A. sobria* (24), *A. hydrophila* (12), *Aermonas* spp. (4)	Aminoglycosides (9·5), cephems (0·0), macrolides (19·0), nitrofurans (19·0), penicillins (100·0), quinolones (12·0–26·0), sulphonamides (9·5–26·0), tetracyclines (71·0)
Unreported sources, marine fish species			
[85] (2009)	*Vibrio* spp.*	$n=106$ isolates; some from display aquaria water, fish intestines	Aminoglycosides (7·9), cephems (1·9), nitrofurans (1·9), penicillins (0·9–29·2), quinolones (0·9–2·8), sulphonamides (0·9–5·7), tetracyclines (1·9)

n.r., Not reported.
* Isolated through the use of selective bacteriological culture media.
† Isolates selected for chloramphenicol-resistance.
‡ Serovars not fully described.
§ Motile *Aeromonas*.

Questionnaire

Demographic characteristics of survey participants self-rating their level of experience with ornamental fish as 'medium to high' or 'high' ($n=113$, 56·8% of respondents) is shown in Table 2. Almost 70% of these performed primarily clinical or field work and 78·6% said they had experience with AMU; however, not all these respondents provided responses to all questions (Tables 3 and 4).

Quinolones were the most frequently reported antimicrobial drug class used, followed by tetracyclines, nitrofurans, and aminoglycosides (Table 3). The highest proportion of fish treated was reported for the brood-stock production phase (31·3% of respondents stating '71–100% of fish were treated with antimicrobial drugs', Table 4). Sixty-five percent of respondents indicated between 71% and 100% of antimicrobials were used for therapeutic use, while use as preventive treatment and growth promotion was less frequent (Table 4). Three factors, 'inappropriate duration of treatment' (74·5%), 'absence of accurate diagnosis' (73·5%) and 'use of antimicrobials in place of improving husbandry' (73·5%) were selected as the main contributors to the development of AMR. Resistance to tetracyclines was most frequently reported, followed by penicillins and potentiated sulphonamides (Table 5).

Twenty-three (32·9% of forms sent) English and no Spanish non-respondents answered the non-response form. From Fisher's exact test, no differences ($P>0·05$) were observed between responders and non-responders. Most non-responders selected 'I don't believe I can contribute as it is not relevant to my professional experience' as a reason for refusal in participation (60·9%, 14/23) followed by 'I don't have time' (21·7%, 5/23).

DISCUSSION

Systematic review

While the percentage of the population having contact with aquaria through hobby or work is largely unknown, reports indicate that individuals exposed to ornamental fish might be at greater risk for zoonotic infections, particularly for *M. marinum* and *Salmonella* serovars. This has prompted clinicians to advise caution around handling ornamental fish and aquaria for vulnerable populations [58, 71, 86]. It is important to note that in the majority of the case

Table 2. *Demographic characteristics of 113 survey respondents self-rating their professional experience with ornamental fish at medium-high to high*

Question asked (N = total number respondents per question)	Respondents (%)	Total respondents per response category (n)
Country profile of respondents to English questionnaire (N = 113)		
North America (USA and Canada)	81·4	92
Europe	7·1	8
Australia	4·4	5
Asia	5·3	6
Israel	1·8	2
Respondent's area of expertise (N = 113)		
Fish health/clinical medicine	80·5	91
Microbiology	17·7	20
Outbreak investigation	13·3	15
Epidemiology	11·5	13
Food safety	10·6	12
Molecular biology/genetics	8·0	9
Other	23·9	27
Primary work activities (N = 113)		
Clinical/field	69·0	78
Research	34·5	39
Laboratory	33·6	38
Administration	23·0	26
Animal health surveillance	28·3	32
Public health surveillance	5·3	6
Other	20·4	23
Aquaculture/seafood/ornamental fish as proportion of daily activities (N = 113)		
< 25 %	40·7	46
26–50 %	14·1	16
51–75 %	12·4	14
76–100 %	32·7	37
Experience with antimicrobial use (N = 112)		
Yes	78·6	88
No	21·4	24

reports analysed, clinicians hypothesized causation due to direct topical contact of cases with fish or aquarium water (other than *Salmonella* cases, which are caused by ingestion), but this was rarely confirmed by concurrent isolation of the suspected pathogen from the fish exposure source. In only 10 studies was the linkage confirmed through detection of a phenotypic match from the patient and their fish and/or aquarium water [35, 68–73, 87–89], while a genotypic match was seldom confirmed [70, 73]. Lack of research reporting contributions of other zoonotic pathogens to human illness due to exposure to ornamental fish may be due to under-reporting. Infection may cause only relatively mild gastrointestinal disease, chiefly diarrhoea [13, 15], and physicians might therefore only seldom see such cases [90, 91]. Many pathogens of aquatic origin have fastidious culture requirements, making diagnosis difficult if not suspected and specifically requested for isolation by clinicians. The human case reports incriminating *M. marinum* in 'fish handler's disease' or 'fish tank granuloma' [13] are probably over-represented in the literature due to external, easily visible (and photographable) lesions suitable for publication.

Epidemiological and bacterial links between cases and posited source-exposures were more frequently investigated in outbreaks of salmonellosis, probably as result of coordinated (and perhaps regulatory) response within respective jurisdictions [68–73, 88]. The detection of the 'fish tank strain' *S.* Paratyphi B var. Java in cattle, genetically different only in the presence of plasmids coding for sulphonamide and trimethoprim resistance [92], and *S.* Javiana, the predominant strain in ornamental fish in Trinidad also found in cases of human gastroenteritis, pet animals, cattle, and wildlife [93], indicate possible 'cross-over' into

Table 3. *Reported frequency of usage of selected antimicrobial drugs in ornamental fish*

Antimicrobial drug class	Frequency of antimicrobial use* (% of respondents) Never to rarely used†	Occasionally used	Frequently to almost always used†	Total respondents (*n*)
Aminoglycosides	43·4	30·2	26·4	53
Macrolides	68·8	22·9	8·3	48
Nitrofurans	41·2	25·5	33·3	51
Penicillins	61·7	29·8	8·5	47
Phenicols	56·7	23·5	19·6	51
Potentiated sulphonamides	47·9	35·4	16·7	48
Quinolones	11·9	33·9	54·3	59
Sulphonamides	70·0	16·0	14·0	50
Tetracyclines	28·8	28·8	42·3	52
Other	42·8	35·7	21·4	14

* Categories for frequency of antimicrobial use were based on a five-point ordinal scale: 1, never used; 2, rarely used, 3, occasionally used; 4, frequently used; 5, always used.
† Categories 'never used' (1) and 'rarely used' (2) and categories 'frequently used' (4) and 'always used' (5) were collapsed.

Table 4. *Reported usage of antimicrobial drugs in ornamental fish by production phase and purpose for using antimicrobial drugs*

Phase*	Proportion of fish treated (% of respondents) 0–30 %‡	31–70 %§	71–100 %¶	Total respondents (*n*)
Hatchery (eggs/larvae)	78·9	21·0	0·0	19
Fingerlings	69·6	17·4	13·0	23
Brood stock	43·8	25·0	31·3	32
Purpose†	**Proportion of the total volume of antimicrobial drugs used for each purpose (% of respondents) 0–30 %‡**	**31–70 %§**	**71–100 %¶**	
Growth promotion	94·4	5·6	0·0	18
Preventive treatment	62·1	24·1	13·8	29
Therapeutic treatment	15·2	19·6	65·2	46

* Estimate of what proportion of ornamental fish production was treated with antimicrobial drugs in each production phase.
† Estimate of the proportion of the total volume of antimicrobial drugs used in ornamental fish culture for the purposes listed.
‡ Collapsed to include 0–10 %, 11–20 % and 21–30 %.
§ Collapsed to include 31–40 %, 41–50 %, 51–60 % and 61–70 %.
¶ Collapsed to include 71–80 %, 81–90 % and 91–100 %.

terrestrial agriculture. Surveillance programmes for *Salmonella* in food and other animals should also include strains commonly found in ornamental fish populations as this source for the next epidemic strain of this particular genus must not be discounted. Future efforts to ascertain potential associations between exposure to ornamental fish and illness in humans should include well-designed and executed case-control studies along with genotyping of strains found in humans, and fish or the fish environment. Currently, the extent of population exposure to ornamental fish through hobby or work is largely unknown. Better understanding of this aspect is necessary before any larger surveillance initiative is considered. Recommendations for public promotion of good hygiene practices around ornamental fish, the institution of importation practices designed to curtail the entry of ornamental fish with antimicrobial resistant bacteria

https://doi.org/10.1017/S0950268811001798 Published online by Cambridge University Press

Table 5. *Frequency of observation of antimicrobial resistance (AMR) by drug class in ornamental fish*

	Frequency of AMR* (% of respondents)			
Antimicrobial drug class	Never to rarely†	Occasionally	Frequently to almost always†	Total respondents (*n*)
Aminoglycosides	71·4	21·4	7·1	28
Macrolides	45·8	41·7	12·5	24
Nitrofurans	52·2	21·7	26·1	23
Penicillins	32·1	25·0	42·9	28
Phenicols	60·7	32·1	7·1	28
Potentiated sulphonamides	33·3	43·3	23·3	30
Quinolones	55·9	26·5	17·6	34
Sulphonamides	40·9	45·5	13·6	22
Tetracyclines	12·1	27·3	60·6	33
Other‡	100·0	0·0	0·0	1

* Categories for frequency of observation of AMR by drug class were based on a five-point ordinal scale: 1, never; 2, rarely; 3, occasionally; 4, frequently, 5, almost always.

† Categories 'never' (1) and 'rarely' (2) and categories 'frequently' (4) and 'almost always used' (5) were collapsed.

‡ Responses under other are incomplete as no antimicrobials were described.

into the country [71, 73] and screening programmes aimed at ornamental fish imported facilities [69] were all called for by public health offices.

Aeromonas spp. were the most-frequently reported zoonotic pathogen in bacterial surveys of ornamental fish populations [5, 74–76, 79, 81–84, 94], with purposive selection of this pathogen noted [74, 75, 79, 81, 84]. This finding did not correlate with the number of case reports ($n=1$) [86] linking disease in humans to this pathogen, probably because this is primarily a fish health pathogen [13]. The second most commonly investigated pathogen was *Mycobacterium* spp. [95, 96–98], particularly *M. fortuitum* [95, 96–98] and *M. marinum* [97]. Prearo *et al.* [96] advocated that *Mycobacterium* spp. be listed in European Union (EU) ornamental fish importation guidelines as one of the pathogens required for 'disease free' certification, both from animal health and public health perspectives. The Centers for Disease Control (CDC) has already listed *M. marinum* as an emerging zoonotic pathogen [99].

When examined by antimicrobial pharmaceutical class a wide range of resistance was observed in most classes, particularly for warm-water fish species; however, the former also represented a greater number of fish species examined. AMR levels reported in research publications were largely in agreement with the survey respondents' perception of the level of AMR in ornamental fish populations, including high levels of tetracycline resistance (Tables 1 and 5). Several researchers in our review posited the potential for transfer of AMR via ornamental fish to human populations [74, 80]. In this context, resistance in ornamental fish zosonotic pathogens is somewhat concerning, particularly in the case of resistance to fluoroquinolones or other antimicrobial drugs of critical importance in human medicine [100, 101].

We were unable to identify, through SR, any study reporting AMU by ornamental fish producers. Due to lack of any published information on AMU in ornamental fish, data generated through our questionnaire fill an important gap in knowledge and provide initial baseline semi-quantitative information on AMU in ornamental fish. Comparisons between respondents and non-respondents did not indicate strong biases in our respondent population. Nevertheless, additional quantitative data measured through more robust and precise instruments are needed.

Over 65% of respondents linked development of AMR due to an emphasis on AMU in place of improvements in husbandry practices. While the country-level regulations regarding labelled AMU (along with withdrawal times) are generally in place for food-intended aquaculture production, such regulations are generally non-existent, or not enforced, for ornamental fish. Given the stressful shipping conditions facing ornamental fish (e.g. periods of hypoxia, long air flights), antibiotics are often routinely added to shipping water [6, 20] to prevent disease. The end result of this practice may be the development of AMR. The Australian government has recommended that the use of medications on fish in quarantine (at importers' facilities) be limited [7]. Efforts to ensure the health of ornamental fish in exporting countries will require a great deal of trust. For example, Singapore exporters ship an average of 50 consignments of tropical fish daily and inspection of every shipment by veterinary officials it is likely to be impractical [8]. When Kleingold *et al*. [20] measured antibiotic residues in ornamental fish, only 1/8 species examined was free of antimicrobials. In a separate study, Kleingold *et al*. reported a marked increase in resistance index to enrofloxacin in all fish (food and ornamental species) attributing this phenomenon to the use of this antimicrobial by cold-water ornamental fish hobbyists [5].

The findings of our review and survey indicate that government authorities should seriously consider public health risks associated with zoonotic pathogens and AMR in ornamental fish. In Canada, others [3] have reported salmonellae in aquariums in 1981 recommending surveillance consideration at that time [3]. Risk-based surveillance is defined as 'a surveillance programme in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to ensure appropriate and cost-effective data collection' [102]. This framework provides a sound approach for the development of effective and feasible surveillance that should be considered within the context of each country. It requires prior epidemiological knowledge in order to determine occurrence of disease in differing population strata or the influence of risk factors [102]. Our review provides some relevant and important baseline information for consideration of such potential surveillance or research programmes, globally and within the Canadian context. In 2008, Canada imported ornamental fish from over 110 different countries worth over Can\$9·7 million [2], primarily from the USA, Singapore and Thailand [103]. Given the almost non-existent state of domestic ornamental fish production in Canada, targeting ornamental fish imports with risk-based sampling and testing for *Salmonella* and *Mycobacterium* spp. and AMR in zoonotic or indicator bacteria might be a reasonable focus of such potential surveillance efforts. Within the Canadian context, the baseline prevalence studies of healthy ornamental fish should be undertaken in a systematic manner to determine the bacterial flora, AMR patterns, and resistance genes present in fish and their transport/holding water, as well as countries of importation. The financial sustainability of such initiatives is very challenging, and requires considerable efforts from various government and industry stakeholders.

User-friendly educational material needs to target ornamental fish hobbyists and employees in the ornamental fish industry in order to increase awareness of the zoonotic potential of ornamental fish species. A study in 2003 by Schmoor *et al*. [104] in France surveyed tropical fish salespeople on their knowledge of *M. marinum*. Only 15% of respondents had in-depth knowledge of the pathogen and 75% ignored the problem. Among 22·5% of the respondents who were fish salespersons with some formal training, only one-third had been taught about the pathogen. Most of the workers immersed their hands in the tanks without wearing gloves, and only a few reported destroying all the fish from an infected tank. An analogous questionnaire to fish hobbyists in the UK revealed similar lack of knowledge both in terms of fish and zoonotic diseases [55]. A public education campaign aimed at Canadian hobbyists and aquarium workers should be considered to make them aware of the potential for illness and the steps they can take to prevent disease. A letter by Hay & Seal [105] responding to a study purporting the beneficial physiological and psychological effects of watching ornamental fish, sums up the dangers of aquarium fish, advising 'ornamental fish – look but do not touch!'

NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

ACKNOWLEDGEMENTS

The authors thank Drs Carol McClure, Sofie St-Hilaire, Victoria Alday and Roy Yanong for their assistance with the development and pre-testing of the questionnaire; and Dr America Mederos for translating the questionnaire from English to Spanish and for her generous help with administration of the non-respondent questionnaire in Spanish. We also thank Dr Chantal Vincent of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. This study was funded by the Public Health Agency of Canada-Laboratory for Foodborne Zoonoses in Guelph, Canada.

The authors are saddened to report the sudden passing away of co-author Lucie Dutil, on 15 August 2011.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Livengood EJ, Chapman FA.** The ornamental fish trade: an introduction with perspectives for responsible aquarium fish ownership. University of Florida IFAS Extension Publication FA124, 2007 (http://glc.org/ans/documents/OrnamentalFishTradeIntroduction-PersepctivesforResponsibleOwnership.pdf). Accessed 12 February 2011.
2. **Canadian Food Inspection Agency (CFIA).** Global and Canadian trade in ornamental fishes. Final Report. Prepared by Canadian Aquaculture Systems Inc., Cobourg, ON, Canada, 2007, pp. 3.
3. **Trust TJ, Bartlett KH, Lior H.** Importation of *Salmonellae* with aquarium species. *Canadian Journal of Microbiology* 1981; **27**: 500–504.
4. **Chapman FA.** Ornamental fish culture, freshwater. In: Stickney RR, ed. *Encyclopedia of Aquaculture*. New York: Wiley and Sons Inc., 2000, pp. 603–605.
5. **Kleingold DW, *et al.*** Administration of antibiotics and chemotherapeutents and development of resistance in coldwater ornamentals. *Bulletin of the European Association of Fish Pathologists* 1996; **17**: 4–7.
6. **Cole B, *et al.*** Shipping practices in the ornamental fish industry. Center for Tropical and Subtropical Aquaculture Publication no. 131, 1999, pp. 14. (http://documents.ponics.org/sections/aquaculture/ornamental-fish/Shipping-Practices-in-the-Ornamental-Fish-Industry.PDF). Accessed 12 February 2011.
7. **Chong R, Whittington R.** Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Preventive Veterinary Medicine* 2007; **81**: 92–116.
8. **Jing LJ, Chuan LL.** Quality assurance programmes for Singapore (http://www.ornamental-fish-int.org/data-area/conservation/untitled2/quality-assurance-programmes-for-singapore). Accessed 6 September 2010.
9. **Biosecurity New Zealand.** Importing ornamental fish and marine invertebrates from all countries. (http://www.biosecurity.govt.nz/imports/animals/standards/fisornic.all.htmc). Accessed 20 September 2010.
10. **Council Directive** 2006/88/EC (http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L: 2006:328:0014:0056:EN:PDF). Accessed 20 September 2010.
11. **Fisheries and Oceans Canada.** Importation of ornamental fish (http://www.dfo-mpo.gc.ca/science/enviro/aah-saa/ornamental-aquarium-eng.htm). Accessed 20 September 2010.
12. **United States Department of Agriculture.** Importation of pets and other animals into the United States (http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports_pets.shtml). Accessed 20 September 2010.
13. **Lowry T, Smith SA.** Aquatic zoonoses associated with food, bait, ornamental and tropical fish. *Journal of the American Veterinary Medical Association* 2007; **231**: 876–880.
14. **Lehane L, Rawlin GT.** Topically acquired bacterial zoonoses from fish: a review. *Medical Journal of Australia* 2000; **173**: 256–259.
15. **Novotny L, *et al.*** Fish: a potential source of bacterial pathogens for human beings. *Veterinarni Medicina* 2004; **49**: 343–358.
16. **Alderman DJ, Hastings TS.** Antibiotic use in aquaculture: development of antibiotic resistance – potential for consumer health risks. *International Journal of Food Science and Technology* 1998; **33**: 139–155.
17. **Cabello FC.** Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology* 2006; **8**: 1137–1144.
18. **Sargeant JM, *et al.*** The process of systematic review and its application in agri-food public health. *Preventive Veterinary Medicine* 2006; **75**: 141–151.
19. **Windsor R, *et al.*** *Evaluation of Health Promotion, Health Education and Disease Prevention Programs*, 3rd edn. New York: McGraw Hill, 2004, pp. 44–45.
20. **Kleingold DW, *et al.*** Bacterial isolations, resistance development and risk analysis in the ornamental fish trade. *Bulletin de l'Institut Océanographique* 2001; **20**: 193–200.
21. **Boubaker K, *et al.*** Atypical mycobacterial tenosynovitis [in French]. *Revue Médicale Suisse* 2004; **506** (http://revue.medhyg.ch/) Accessed 6 September 2010.
22. **Beliè M, Miljkoviè J, Murko PB.** Sporotrichoid presentation of *Mycobacterium marinum* infection of the upper extremity: a case report. *Acta Dermatovenereologica Alpina, Pannonica et Adriatica* 2006; **15**: 135–139.
23. **Trampuz A, *et al.*** Persistent ulcers on the hand of an aquarium owner. *Scandinavian Journal of Infectious Diseases* 2002; **34**: 630–632.
24. **Speight EL, Williams HC.** Fish tank granuloma in a 14-month-old girl. *Pediatric Dermatology* 1997; **14**: 209–212.
25. **Bleiker TO, Bourke JE, Burns DA.** Fish tank granuloma in a 4-year-old boy. *British Journal of Dermatology* 1996; **135**: 863–864.
26. **Meffert JJ.** A finger infection in a pet store employee. *American Family Physician* 1999; **60**: 245–246.
27. **Ekerot L, Jacobsson L, Forsgren A.** *Mycobacterium marinum* wrist arthritis: local and systemic dissemination caused by concomitant immunosuppressive therapy. *Scandinavian Journal of Infectious Diseases* 1998; **30**; 84–87.
28. **Barton A, *et al.*** *Mycobacterium marinum* infection causing septic arthritis and osteomyelitis. *British Journal of Rheumatology* 1997; **36**: 1207–1209.
29. **Borradori L, *et al.*** *Mycobacterium marinum* granuloma in a fish fancier. Apropos of a case with sporotrichoid lesions. *Schweizerische Medizinische Wochenschrift* 1991; **121**: 1340–1344.
30. **Wolfe SF, Gurevitch AW.** Fish-tank granuloma. *New England Journal of Medicine* 1997; **336**: 1065.

31. **Barr KL, Lowe L, Su LD.** *Mycobacterium marinum* infection simulating interstitial granuloma annulare. *American Journal of Dermatopathology* 2003; **25**: 148–151.
32. **Enzensberger R, *et al.*** Disseminated cutaneous *Mycobacterium marinum* infection in a patient with non-Hodgkin's lymphoma. *Infection* 2002; **30**: 393–395.
33. **Perrot J, Lévigne V, Denis M.** Fatal disseminated *Mycobacterium marinum* infection. *International Journal of Dermatology* 1992; **31**: 286–287.
34. **Vazquez JA, Sobel JD.** A case of disseminated *Mycobacterium marinum* infection in an immunocompetent patient. *European Journal of Clinical Microbiology and Infectious Diseases* 1992; **11**: 908–911.
35. **Parent LJ, *et al.*** Disseminated *Mycobacterium marinum* infection and bacteremia in a child with severe combined immunodeficiency. *Clinical Infectious Diseases* 1995; **21**: 1325–1327.
36. **Holmes GF, *et al.*** Recurrent, disseminated *Mycobacterium marinum* infection caused by the same genotypically defined strain in an immunocompromised patient. *Journal of Clinical Microbiology* 1999; **37**: 3059–3061.
37. **Torres F, Hodges, Zamora MR.** *Mycobacterium marinum* infection in a lung transplant recipient. *Journal of Heart and Lung Transplantation* 2001; **20**: 486–489.
38. **Iijima S, Saito J, Otsuka F.** *Mycobacterium marinum* skin infection successfully treated with levofloxacin. *Archives of Dermatology* 1997; **133**: 947–949.
39. **Brady RC, *et al.*** Facial sporotrichoid infection with *Mycobacterium marinum*. *Journal of Pediatrics* 1997: **130**: 324–326.
40. **Alinovi A, Vecchini F, Bassissi P.** Sporotrichoid mycobacterial infection. A case report. *Acta Dermatovenereologica Alpina, Pannonica et Adriatica* 1993; **73**: 146–147.
41. **Bowen-Jones EJ, Mlisana KP, Peer AK.** *Mycobacterium marinum* hand infection. *British Journal of Plastic Surgery* 2001; **54**: 181.
42. **Ter Borg EJ, Tersmette M.** Unusual and memorable a tender swelling of the dorsum of a hand in a middle-aged woman. *Annals of the Rheumatic Diseases* 2001; **60**: 559.
43. **Cassetty CT, Sanchez M.** *Mycobacterium marinum* infection. *Dermatology Online Journal* 2004; **10**: 21.
44. **Guarda R, *et al.*** Cutaneous infection by *Mycobacterium marinum*: case report. *Revista Medica de Chile* 1992; **120**: 1027–1032.
45. **Palomo C, *et al.*** Lymphocutaneous syndrome due to *Mycobacterium marinum* in a young aquarium fond. *Enfermedades Infecciosas y Microbiología Clínica* 2001; **19**: 506–507.
46. **Sánchez R, Ortiz C, Muòoz-Sanz A.** Cutaneous sporotrichoid infection by *Mycobacterium marinum*. *Enfermedades Infecciosas y Microbiología Clínica* 1999; **17**: 219–222.
47. **Contios S, *et al.*** *Mycobacterium marinum* flexor tenosynovitis [in French]. *Presse Médicale* 2005; **34**: 587–588.
48. **Saint-Blancard P, *et al.*** Cutaneous nodular infection with haemopathy [in French]. *Annales de Pathologie* 2000; **20**: 346–348.
49. **Seiberras SD, *et al.*** *Mycobacterium marinum* nodular lymphangitis [in French]. *Presse Médicale* 2000; **29**: 2094–2095.
50. **Sene D, *et al.*** Cutaneous nodules in an aquarium lover [in French]. *Revue de Medecine Interne* 2003; **24**: 328–329.
51. **Vandenbos F, Rahelintrina V, Dellamonica P.** Nodular dermo-hypodermitis on right upper limb [in French]. *Presse Médicale* 2005; **34**: 617.
52. **Ryan JM, Bryant GDR.** Fish tank granuloma – a frequently misdiagnosed infection of the upper limb. *Journal of Accident and Emergency Medicine* 1997; **14**: 398–399.
53. **Laing RBS, *et al.*** Antimicrobial treatment of fish tank granuloma. *Journal of Hand Surgery* 1997; **22B**: 135–137.
54. **Lewis FTM, Marsh BJ, von Reyn CF.** Fish tank exposure and cutaneous infection due to *Mycobacterium marinum*: Tuberculin skin testing, treatment and prevention. *Clinical Infectious Diseases* 2003; **37**: 390–397.
55. **Gray SF, *et al.*** Fish tank granuloma. *British Medical Journal* 1990; **300**: 1069–1070.
56. **Aubry AO, *et al.*** Sixty-three cases of *Mycobacterium marinum* infection. *Archives of Internal Medicine* 2002; **162**: 1746–1752.
57. **Kullavanijaya P, Sirimachan S, Bhuddhavudhikrai P.** *Mycobacterium marinum* infections acquired from occupations and hobbies. *International Journal of Dermatology* 1993; **32**: 504–507.
58. **Devchaud P, Gullick P.** *Mycobacterium marinum* skin infections: two case reports. *Journal of Family Practice* 1993; **36**: 336–338.
59. **Gkrania-Klotsas E, Lever AML.** A husband and wife with left hand rashes. *Lancet Infectious Diseases* 2005; **5**: 802.
60. **Blackwell VC, Hamilton AJ, Hay R.** Analysis by enzyme-linked immunosorbent assay and immunoblot of the antibody responses of patients with *Mycobacterium marinum*. *FEMS Immunology and Medical Microbiology* 2000; **27**; 185–190.
61. **Ang P, Rattana-Apiromyakij M, Goh C.** Retrospective study of *Mycobacterium marinum* skin infections. *International Journal of Dermatology* 2000; **39**: 343–347.
62. **Wu T, *et al.*** *Mycobacterium marinum* infection in Taiwan. *Journal of Microbiology, Immunology and Infection* 2002; **35**: 42–46.
63. **Bhatty MA, Turner DPJ, Chamberlain ST.** *Mycobacterium marinum* hand infections: case reports and review of literature. *British Journal of Plastic Surgery* 2000; **53**: 161–165.
64. **Casal M, del Mar Casal M.** Multicenter study of incidence of *Mycobacterium marinum* in humans in Spain. *International Journal of Tuberculosis and Lung Disease* 2001; **5**: 197–199.
65. **Brutus JP, *et al.*** Atypical mycobacterial infections of the hand: report of eight cases and literature review. *Chirurgie de la Main* 2001; **20**: 280–286.

66. **Dorronsoro I, *et al.*** Cutaneous infections by *Mycobacterium marinum*. Description of 3 cases and a review of the literature [in Spanish]. *Enfermedades Infecciosas y Microbiológicas* 1997; **15**: 82–84.
67. **Groupe AZAY-mycobacteries**, ***et al.*** Investigation on *Mycobacterium marinum* infection in France from 1996 to 1998 [in French]. *Bulletin Épidémiologique Hebdomadaire* 2003; **44**: 215–216.
68. **Gaulin C, Vincent C, Ismail J.** Sporadic infections of *Salmonella* paratyphi B, var. Java associated with fish tanks. *Canadian Journal of Public Health* 2005; **96**: 471–474.
69. **Gaulin C, Vincent C, Alain L, Ismail J.** Outbreak of *Salmonella paratyphi* B linked to aquariums in the province of Quebec, 2000. *Canada Communicable Disease Report* 2002; **28**: 89–96.
70. **Levings RS, *et al.*** Aquariums as reservoirs for multidrug-resistant *Salmonella* Paratyphi B. *Emerging Infectious Diseases* 2006; **12**: 507–510.
71. **Musto J, *et al.*** Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003–04. *Communicable Diseases Intelligence* 2006; **30**: 222–227.
72. **Riley A, Hanson M, Ramsey C.** Tropical fish as a source of *Salmonella* Java infection. *Communicable Disease and Environmental Health in Scotland Weekly Report* 1992; **26**: 4–5.
73. **Boxall N, *et al.*** *Salmonella paratyphi* B var. Java in tropical fish and turtle aquaria. *New Zealand Public Health Surveillance Report* 2005; **3**: 4–5.
74. **Verner-Jeffreys DW *et al.*** High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. *PLOS ONE* 2009; **4**: 1–9.
75. **Ansary A, *et al.*** Plasmids and antibiotic resistance in *Aeromonas hydrophila* isolated in Malaysia from healthy and diseased fish. *Journal of Fish Diseases* 1992; **15**: 191–196.
76. **Redondo PN, Jarero JR, Figueroa JLA.** Antibiotic resistance and presence of plasmids in *Aeromonas hydrophila*. *Vibrio fluvialis* and *Vibrio furnissii* isolated from *Carassius auratus auratus*. *Veterinaria México* 2004; **35**: 21–30.
77. **Redondo PN, *et al.*** Presence of plasmids in pseudomonas isolated from ornamental fish. *Veterinaria México* 2003; **34**: 289–295.
78. **Čižek A, *et al.*** Antimicrobial resistance and its genetic determinants in aeromonads isolated in ornamental (koi) carp (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio*). *Veterinary Microbiology* 2010; **142**: 435–439.
79. **Dixon BA, Issvoran G.** Antibacterial drug resistance in *Aeromonas* spp. isolated from domestic goldfish and koi from California. *Journal of the World Aquaculture Society* 1993; **24**: 102–104.
80. **Huys G, *et al.*** Biodiversity of chloramphenicol-resistant mesophilic heterotrophs from Southeast Asian aquaculture environments. *Research in Microbiology* 2007; **158**: 228–235.
81. **Del Rio-Rodriguez RE, Turnbull JF.** Aerobic microflora of imported tropical ornamental fish from Singapore and South America. Part 2: Antimicrobial resistant profiles of motile Aeromonads. *Fish Veterinary Journal* 2002; **6**: 1–21.
82. **Dixon BA, Yamashita J, Evelyn F.** Antibiotic resistance of *Aeromonas* spp. isolated from tropical fish imported from Singapore. *Journal of Aquatic Animal Health* 1990; **2**: 295–297.
83. **Jongjareanjai M, Assawawongkasem N, Chansue N.** *In vitro* antibiotic susceptibility of *Aeromonas hydrophila* isolated from disease ornamental fish. *Thai Journal of Veterinary Medicine* 2009; **39**: 225–229.
84. **Dixon BA, Issvoran GS.** The activity of ceftiofur sodium for *Aeromonas* spp. isolated from ornamental fish. *Journal of Wildlife Diseases* 1992; **28**: 453–456.
85. **Blanch AR, *et al.*** Comparison of *Vibrio* spp. populations found in seawater, in exhibition aquaria, in fish intestine and in fish feed. *Journal of Applied Microbiology* 2009; **106**: 57–65.
86. **Cremonesini D, Thomson A.** Lung colonization with *Aeromonas hydrophila* in cystic fibrosis believed to have come from a tropical fish tank. *Journal of the Royal Society of Medicine* 2008; **101**: S44–S45.
87. **Abalain-Colloc ML, *et al.*** *Mycobacterium szulgai* isolated from a patient, a tropical fish and aquarium water. *European Journal of Clinical Microbiology and Infectious Diseases* 2003; **22**: 768–769.
88. **Senanayake SN, *et al.*** A child with *Salmonella enterica* serotype paratyphi B infection acquired from a fish tank. *Medical Journal of Australia* 2004; **180**: 250.
89. **Booth LV, Lang DA, Athersuch R.** Isolation of *Vibrio cholerae* non-O1 from a Somerset farmworker and his tropical fish tank. *Journal of Infection* 1990; **20**: 55–57.
90. **Mead PS, *et al.*** Food-related illness and death in the United States. *Emerging Infectious Diseases* 1999; **5**: 607–625.
91. **Majowicz SE, *et al.*** Estimating the under-reporting rate for infectious gastrointestinal illness in Ontario. *Canadian Journal of Public Health* 2005; **96**: 178–181.
92. **Evans SJ, *et al.*** Multiple antimicrobial resistant *Salmonella enterica* serovar Paratyphi B variant Java in cattle: a case report. *Veterinary Record* 2005; **156**: 343–346.
93. **Newaj-Fyzul A, Adesiyun AA, Mutani A.** Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from apparently healthy ornamental fish and pond water in Trinidad. *Journal of Food, Agriculture and Environment* 2006; **4**: 27–29.
94. **Taylor PW.** Multiple antimicrobial resistance in chronic bacterial infection of koi carp. *North American. Journal of Aquaculture* 2003; **65**: 120–123.
95. **Mouton A, Basson L, Impson D.** Health status of ornamental freshwater fishes imported to South Africa: a pilot study. *Aquarium Sciences and Conservation* 2001; **3**: 327–333.
96. **Prearo M, *et al.*** Mycobacterioses: emerging pathologies in aquarium fish. *Veterinary Research Communications* 2004; **28**: 315–317.

97. **Pate M, *et al.*** Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. *Diseases of Aquatic Organisms* 2005; **64**: 29–35.
98. **Macri D, *et al.*** Mycobacteriosis in ornamental fish. Case reports in Sicily and medical-legal considerations. *Veterinary Research Communications* 2008; **32** (Suppl. 1): S215–S217.
99. **Evans JJ, *et al.*** Overview of zoonotic infections from fish and shellfish (http://eafp.org/storage/conference-articles/Zoonosis%20workshop% 202009%20EAFP%20Evans%20et%20al%20overview%20zoonotic%20inf%20fish%20shellfish.pdf). Accessed 6 September 2010.
100. **MacDougall C, *et al.*** Antimicrobial drug prescribing for pneumonia in ambulatory care. *Emerging Infectious Diseases* 2005; **11**: 380–4.
101. **Health Canada.** Categorization of antimicrobial drugs based on importance in human health, 2009 (http://www.hc-sc.gc.ca/dhp-mps/consultation/vet/consultations/amr_ram_hum-med-rev-eng.php). Accessed 25 October 2010.
102. **Stark KDC, *et al.*** Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. *BMC Health Services Research* 2006; **6**: 20.
103. **Industry Canada.** Trade data online 2010 (http://www.ic.gc.ca/sc_mrkti/tdst/tdo/tdo.php?hfFileNm=&naArea=9999&lang=30&searchType=KS&toFromCountry=CDN&currency=CDN&hSelectedCodes=%7C30110&period=5&timePeriod=5%7CComplete+Years&periodString=&productBreakDown=Complete+Years&reportType=TI&productType=HS6&areaCodeStrg=9999%7CDET&runReport_x=36&javaChart_x=&runGraph_x=&outputType=RPT&chartType=columnApp&grouped=GROUPED#tag). Accessed 15 September 2010.
104. **Schmoor P, *et al.*** Knowledge and behaviour of exotic fish sellers about aquarian diseases [in French]. *Annales de Dermatologie et de Vénéréologie* 2003; **130**: 425–427.
105. **Hay J, Seal DV.** Ornamental fish: look but do not touch! *Journal of the Royal Society of Medicine* 1996; **89**: 359.