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Current status of fish vaccines in Japan

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ABSTRACT

Aquaculture is an important industry in Japan for the sustainable production of fish. It contributes to the diversity of Japanese traditional food culture, which uses fish such as “sushi” and “sashimi”. In the recent aquaculture setting in Japan, infectious diseases have been an unavoidable problem and have caused serious economic losses. Therefore, there is an urgent need to overcome the disease problem to increase the productivity of aquaculture. Although our country has developed various effective vaccines against fish pathogens, which have contributed to disease prevention on fish farms, infectious diseases that cannot be controlled by conventional inactivated vaccines are still a problem. Therefore, other approaches to developing effective vaccines other than inactivated vaccines are required. This review introduces the vaccine used in Japan within the context of the current status of finfish aquacultural production and disease problems. This review also summarizes the current research into vaccine development and discusses the future perspectives of fish vaccines, focusing on the problems associated with vaccine promotion in Japan.

1. Introduction

Various marine and freshwater fish species have been cultured in Japan in recent years, including about 30 marine species [1]. Because various kinds of fish are cultured throughout the coastal and freshwater areas of Japan, diverse infectious diseases have been reported in a variety of species [2–5]. Today, antibiotics are essential for the treatment of bacterial infections in aquaculture. However, the Japanese Government recently formulated a national action plan on antimicrobial resistance (AMR) and has encouraged the development of fish vaccines as an alternative to antibiotic use (https://www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/0000138942.pdf). Under these circumstances, it is not only necessary to develop vaccines, but also to make the rules for approving existing vaccines for multiple species more flexible, efficient, and rapid to effectively prevent the many infectious diseases of fish, including both viral and bacterial infections. In this paper, we summarize the current status of fish vaccines and the issues involved, focusing on the characteristics of recent fish diseases and trends in Japan.

2. General overview of aquaculture in Japan

2.1. Trends in aquacultural production

Because it allows sustainable production, aquaculture has become more important than ever, especially as fisheries production has declined dramatically with the exhaustion of fish resources and/or environmental changes. Aquacultural production accounted for more than 20% of the total national fisheries production and 30% of the total national fisheries value in Japan for the last decade or more (Fig. 1A). Marine finfish aquaculture in Japan accounts for around 90% of its total finfish production because Japan is an island nation, with many coastal areas suitable for marine aquaculture, and has a diverse food culture based on marine fish. The aquaculture industry includes fish species that are predominantly produced in Japan, including those from the genus *Seriola*, such as the yellowtail (*Seriola quinqueradiata*), greater amberjack (*S. dumerili*), and yellowtail kingfish (*S. lalandi*), and other important fish species, such as the red seabream (*Pagrus major*). *Seriola* species are the predominant aquacultured fish, accounting for more than half the total production of finfish by aquaculture. Cultured *Seriola* fish fill both domestic demand and an export demand, and are therefore one of the most economically important fish genera in Japan. The production of other marine finfish, including the coho salmon (*Oncorhynchus kisutch*) and bastard halibut (*Paralichthys olivaceus*),

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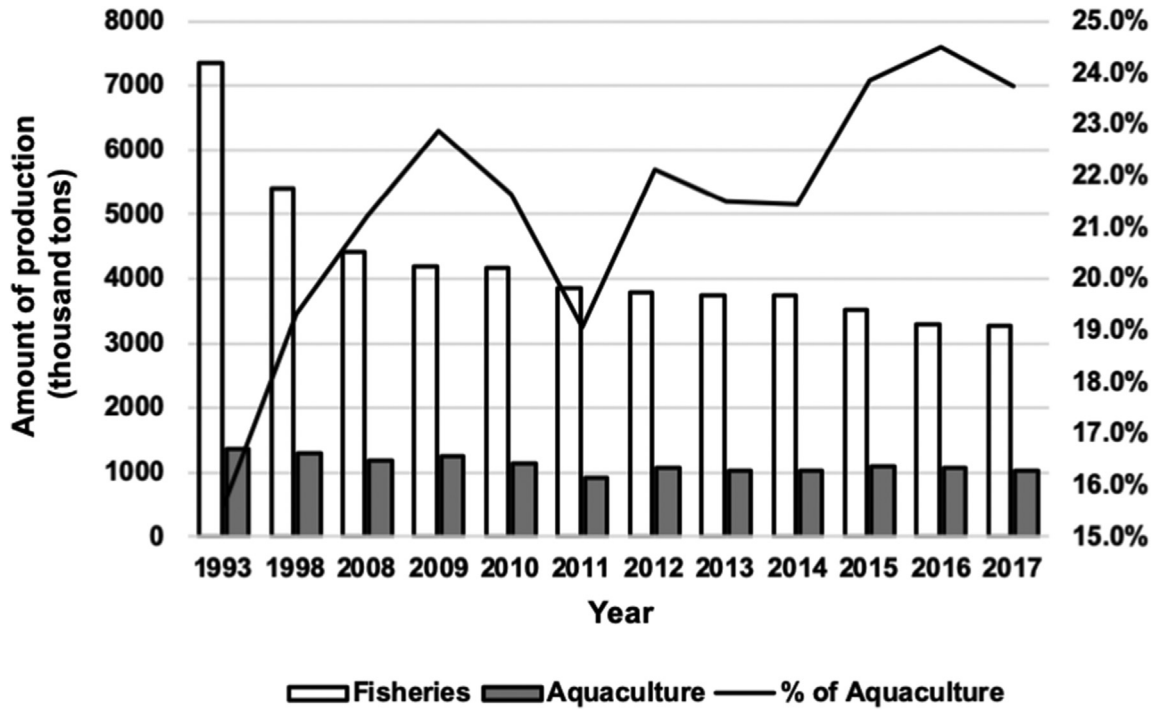

Fig. 1a. Comparison of the production by fisheries and aquaculture in Japan in 1993–2017.
Production by fisheries and aquaculture is shown on the left axis. The ratio of aquacultural production to the total amount of national fisheries production is shown on the right axis. Production of shellfish and seaweed is also included in the statistical data. Data are based on fisheries and aquaculture production statistics from the Ministry of Agriculture, Forestry, and Fisheries of Japan (MAFF).

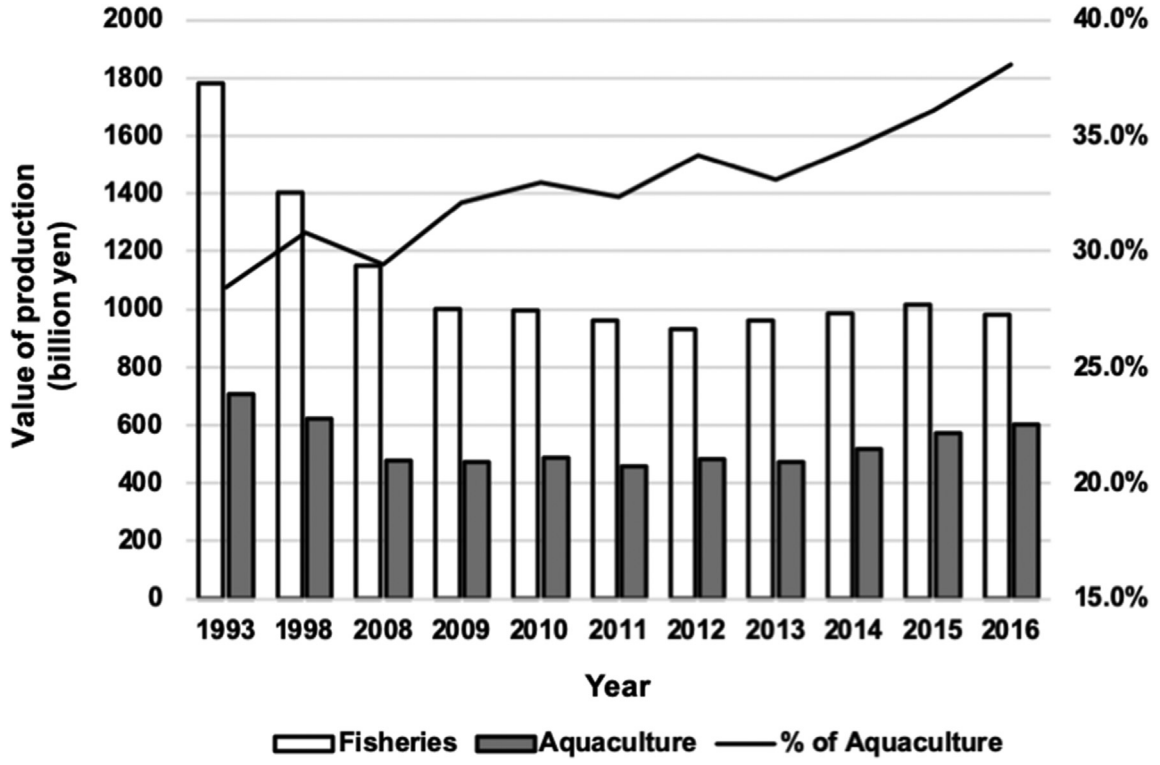

Fig. 1b. Trends in the value of fisheries and aquaculture in Japan in 1993–2017.
Values of fisheries and aquaculture are shown on the left axis. The ratio of the aquacultural value to the total value of national production is shown on the right axis. Values of shellfish and seaweed are also included in the statistical data. Data are based on fisheries and aquaculture production statistics from the MAFF.

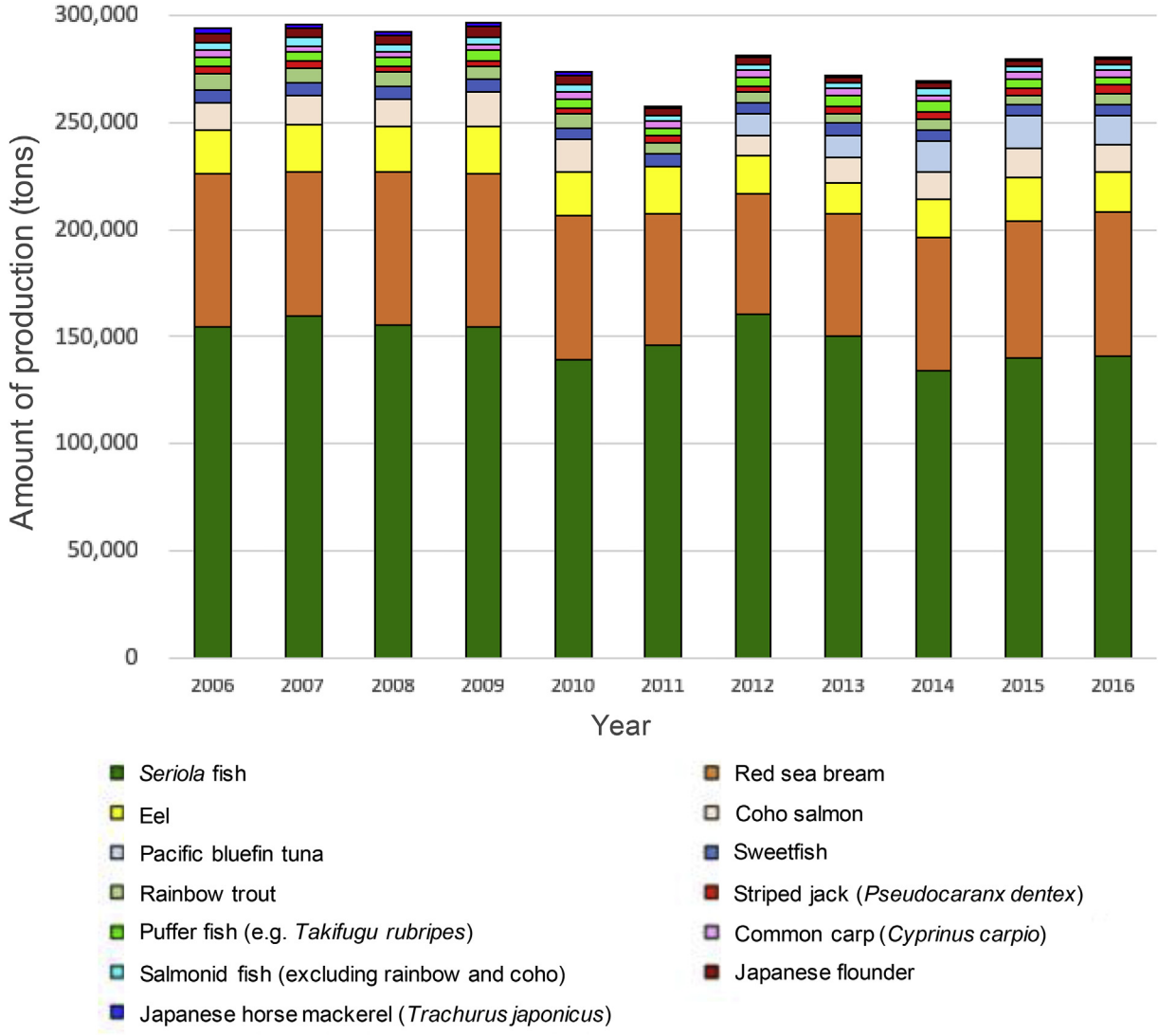

Fig. 2a. Aquacultural production of finfish in Japan in 2006–2016.
Data are based on fisheries and aquaculture production statistics from the MAFF.

corresponds to around 10% of the total cultured finfish production in Japan. The dominant freshwater species cultured is the eel (*Anguilla* spp.), followed by the rainbow trout (*O. mykiss*) and ayu (sweetfish, *Plecoglossus altivelis*), which combined account for around 10% of the total cultured finfish production. In addition to these species, various marine and freshwater finfish species are produced on a smaller scale (see Fig. 1b). Data on the aquacultural production of different finfish are summarized in Fig. 2.

2.2. Economic losses caused by infectious diseases

Before 1996, no vaccines for marine fish had been approved in Japan, and the administration of antibiotics was the major way to prevent outbreaks of fish diseases, although antibiotics are only effective for bacterial infections and potentially stimulate the development of AMR. Because the strategies for disease prevention were limited, the economic losses caused by infectious diseases is aquaculture exceeded 20 billion yen before the 21st century (equivalent to US$0.18 billion in 1996) (Fig. 3). In 1997, a vaccine against *Lactococcus garvieae* was approved for use in *Seriola* fish and reduced the losses caused by this disease every year (Fig. 3). Other vaccines have also been developed, and economic losses from fish infectious diseases were reduced by 70% in the decades since 1995 (Fig. 3). In recent years, the Japanese Government has formulated a national action plan on AMR, so the importance of vaccines against infectious fish diseases is still growing.

2.3. Infectious diseases that cannot be controlled by vaccines

Intracellular bacterial infections, such as nocardiosis, and edwardsiellosis, are major problems in various fish species (Table 1) because no effective vaccines are available. The pathogens are naturally eliminated by cell-mediated immunity, which can be enhanced by live-attenuated vaccines but is difficult to induce with inactivated vaccines [7,8]. Unfortunately, live-attenuated vaccines are not permitted for use in aquaculture in Japan because the attenuated pathogens can potentially revert to a pathogenic form, risking their environmental spread. Therefore, the lack of effective methods for the prevention of these infectious diseases is a major issue that must be resolved.

Infection with difficult-to-culture or unculturable pathogens is also difficult to control with vaccines because inactivated vaccines cannot be prepared against them. For instance, a pathogen causing bacterial hemolytic jaundice (BHJ) in the yellowtail, *Ichthyobacterium seriolicida* [9], belongs to the novel bacterial family Ichthyobacteriaceae and is difficult to culture [10,11], so a stable supply of vaccine antigen cannot be ensured. Some viral pathogens of fish cannot be cultured because there are no suitable cell lines. Atypical cellular gill disease, which

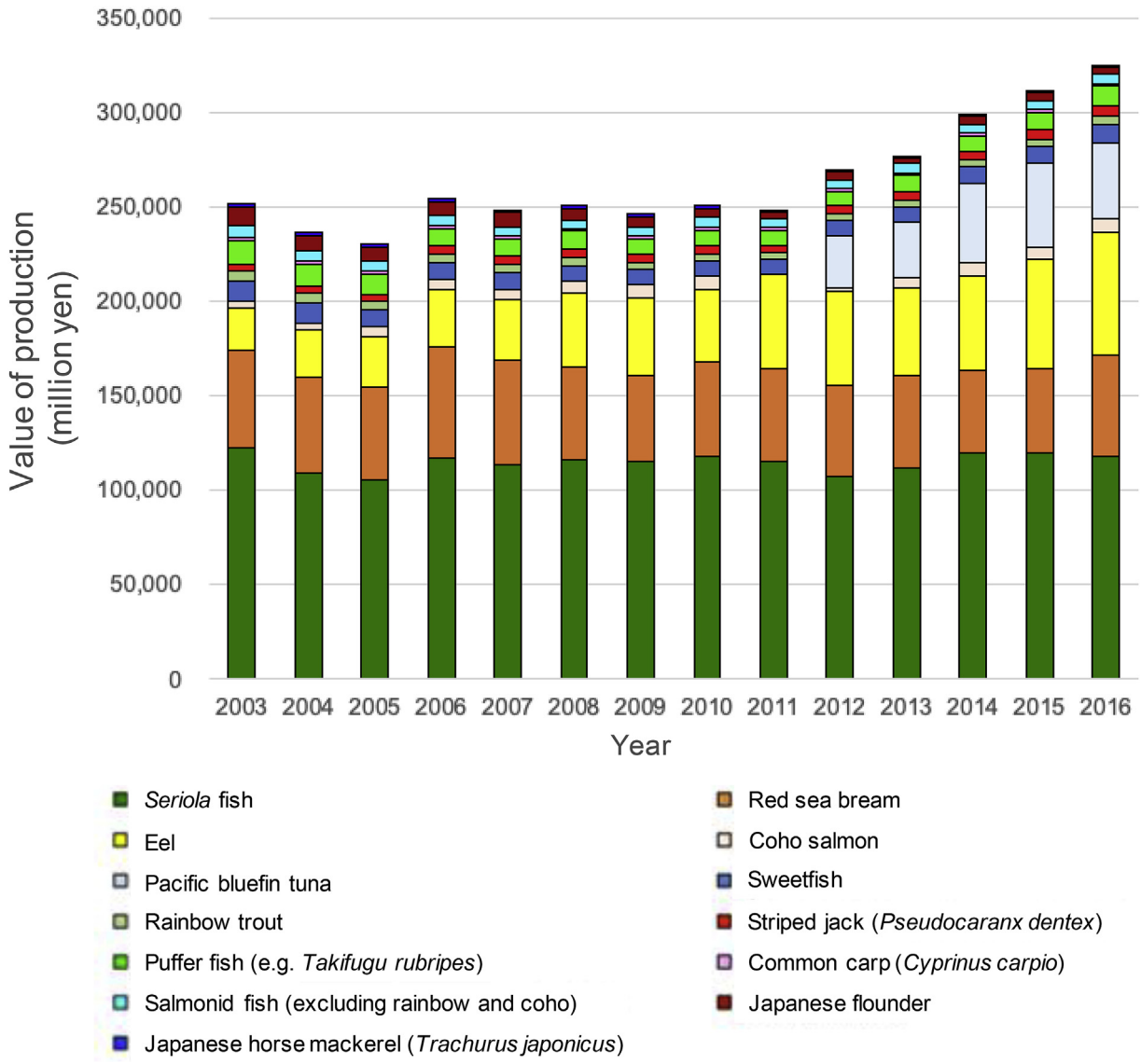

Fig. 2b. Value of finfish production by aquaculture in Japan in 2003–2016.
Data are based on fisheries and aquaculture production statistics from the MAFF.

occurs in sweetfish, is a viral infection thought to be caused by an unculturable poxvirus (*Plecoglossus altivelis* poxvirus-like virus, PaPV) [12], and has spread widely in Japan. The disease is the second or third deadliest disease in sweetfish since records of the disease began in 2012 [12]. Viral erythrocytic inclusion body syndrome (EIBS), which occurs in salmonid fish, has also threatened coho salmon farms and has caused the second-highest economic loss in salmonids (Table 1). The causative agent of the disease is an unculturable virus designated Piscine orthoreovirus 2 (PRV-2) [13].

Japan has imported large amounts of marine fish fingerlings from Asian countries and the eggs of salmonids from the United States for sustainable aquacultural production. Several fish pathogens have been introduced into Japan during the importation of these fingerlings and eggs. Infectious hematopoietic necrosis virus (IHNV) in salmonid fish is considered to have been introduced into Japan from Alaska, USA, in 1970 with the eggs of imported sockeye salmon [14], and has become the worst contributor to mortality on rainbow trout (*O. mykiss*) farms (data not shown). No effective vaccine for the practical prevention of IHN has been developed in Japan, although a DNA vaccine (Apex-IHN®: Elanco Canada Ltd, Charlottetown, Canada) has been approved for use in the aquaculture of Atlantic salmon in Canada [15]. *Flavobacterium psychrophilum*, the etiological agent of bacterial cold-water disease (BCWD), has caused the most serious mass mortality in the aquaculture of sweetfish in recent years (Table 1). BCWD is also considered to have been introduced from abroad, with the imported eggs of salmonid fish [16], and has affected farmed sweetfish since the 1990s. The use of several antibiotics is permitted for the treatment of BCWD in sweetfish, but no effective vaccine has yet been developed for this disease.

3. Overview of fish vaccines in Japan

An overview of the authorization system for medicinal products for veterinary use in Japan, including fish vaccines, has been summarized well by Holm et al. [17]. Veterinary medicines are regulated by the Japanese Pharmaceutical and Medical Devices (PMD) Act. The PMD Act provides a legal framework for the regulation of medicinal products on the Japanese market. The law came into force on November 2, 2014, replacing the Pharmaceutical Affairs Law. Those who intend to distribute veterinary medicinal products (VMPs) must obtain marketing permission for each product from the Minister of Agriculture, Forestry, and Fisheries. A general scheme for the regulatory approval of VMPs intended for use in aquaculture is shown in Fig. 4. The National

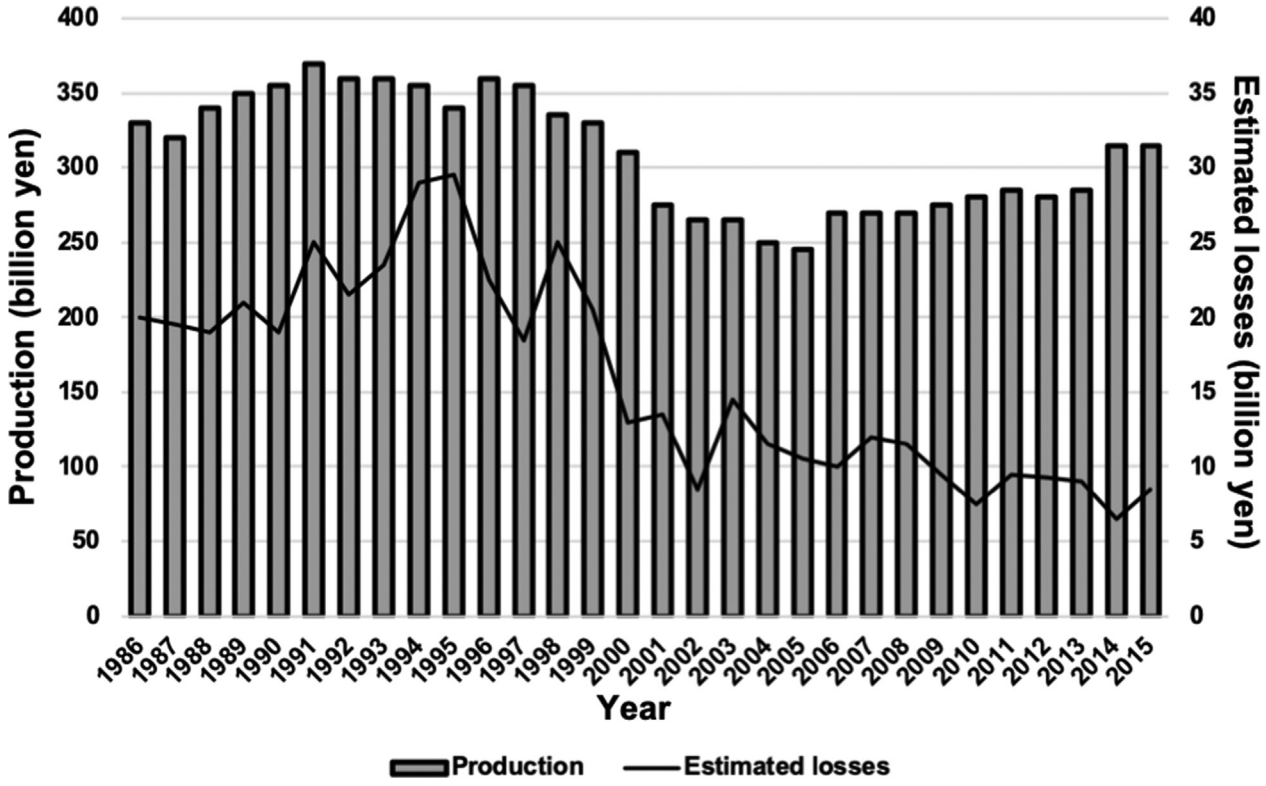

Fig. 3a. Economic losses caused by infectious disease in fish aquaculture in Japan.
The data are based on values estimated from a questionnaire-based survey of fish farmers.

Veterinary Assay Laboratory reviews the availability, safety, and reliability of the subjects of the application, in cooperation with the Food Safety and Consumer Affairs Bureau. The application for a new VMP is investigated by a committee of the Pharmaceutical Affairs and Food Sanitation Council. Applications for the approval of fish-related VMPs are then reviewed by the Advisory Committee on Fishery Medicinal Products, the Council for Veterinary Products, and the Pharmaceutical Affairs Council. The possibility of residues of VMPs intended for use in food-producing animals is investigated by the Food Sanitation Council. VMPs for food-producing animals must be assessed for their safety to human health by the Food Safety Commission and, when required, maximum residue limits are set for the relevant tissues by the Minister of Health, Labor, and Welfare. The processes and data collection required to develop a new fish vaccine must meet the requirements of Good Laboratory Practice, Good Manufacturing Practice, and Good Clinical Practice. A full dossier of data, including the origin and history of development; physical, chemical, and biological properties; manufacturing process; indications, effects, and potency; administration and dosage; stability; target animal safety; pharmacological action; and the results of clinical trials, must be submitted by the applicant before a fish vaccine can be approved [17].

The Japanese legislation on vaccines for fish, unlike vaccines for other food-producing animals, does not require a veterinarian's prescription for the use of a vaccine. However, the protective efficacy of a vaccine cannot be conferred unless the vaccine is used appropriately, so the users of fish vaccines must receive guidance from prefectural departments on the use of any vaccine. The departments that provide this guidance are selected according to provisions of each prefecture (e.g., prefectural livestock hygiene service center, prefectural fisheries research institute). Those who intend to use a fish vaccine must obtain certification from their prefectural departments to purchase the desired vaccine from a distributor. The prefectural department that issued the certificate must also keep track of the situation at the time that the relevant vaccine is used and the results of its use.

A list of VMPs approved for use in aquaculture, including fish vaccines and antibiotics, can be found at the website of the Fish and Fishery Products Safety Office, Animal Products Safety Division, Ministry of Agriculture, Forestry, and Fisheries of Japan (http://www.maff.go.jp/j/syouan/suisan/suisan_yobo/) (in Japanese). Nine pharmaceutical companies manufacture fish vaccines for the Japanese market, and 29 vaccine formulations were approved by 2018. Vaccines against two viral species and eight bacterial species have been approved, and are used in more than 13 fish species (Table 2). All the fish vaccines approved in Japan are inactivated vaccines. No other types of vaccines, such as live-attenuated vaccines, recombinant subunit vaccines, or DNA vaccines have been licensed for aquaculture in Japan.

4. Status of new vaccine research and development

In this section, we describe the status of vaccine development against the diseases that occur in Japan but are rare or do not occur in other countries: nocardiosis of *Seriola* fish, BHJ of yellowtail, BCWD of sweetfish, and EIBS of coho salmon.

4.1. Nocardiosis

Nocardiosis, caused by *Nocardia seriolae*, was first reported as *N. kampachi* in yellowtail and greater amberjack in the middle region of Japan [19], spreading rapidly to the western parts of Japan and causing mass mortality in *Seriola* fish [20]. Although the economic damage caused by nocardiosis was minor at the beginning of the epidemic, the disease has become a major cause of mortality [21,22]. *Lactococcus garvieae* is the most serious problem for the culture of *Seriola* fish, but since it has been controlled with a vaccine, nocardiosis has become the most economically damaging disease in the aquaculture industry in Japan. Nocardiosis is characterized by ulcers on the body surface and tubercles on the internal organs, such as the gill, kidney, and spleen [19]. The development of a vaccine against this disease is highly

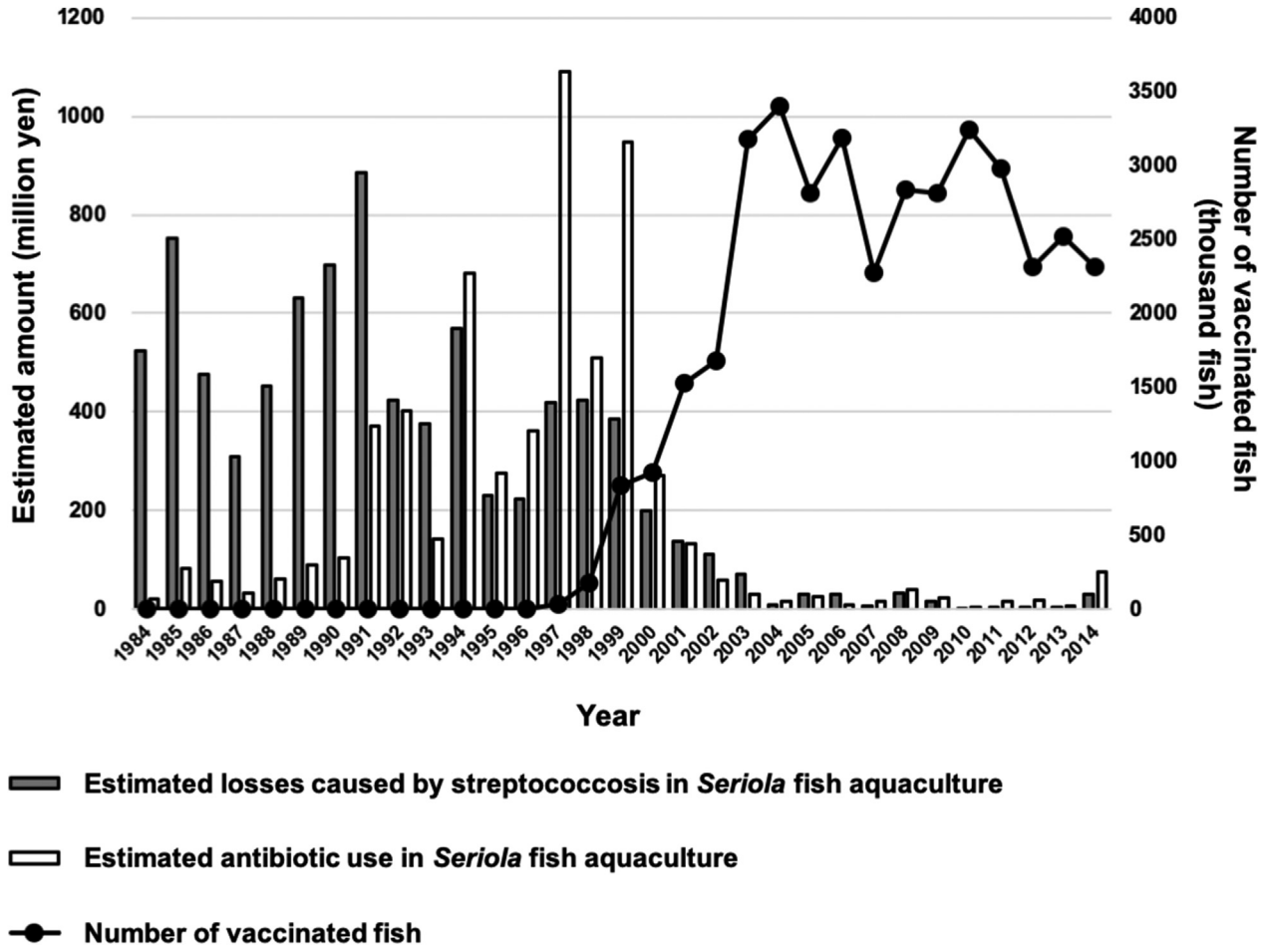

Fig. 3b. Veterinary medicinal product use for streptococcosis in farmed *Seriola* fish in a single prefecture.
Estimated economic losses and antibiotic use are shown on the left axis. The number of fish injected with a vaccine against streptococcosis is shown on the right axis. Data are based on the statistics of the Fisheries Research Division, Oita Prefectural Agriculture, Forestry and Fisheries Research Center [6].

anticipated, given its economic impact and the ineffectiveness of chemotherapy for bacterial nodules. Although immunization with formalin-killed cells (FKC) of *N. seriolae* or FKC with Freund's incomplete adjuvant induced a humoral immune response, no protective effects were observed [22]. The highly protective effects of live vaccines based on the related species *N. soli*, *N. fluminea*, and *N. uniformis* [23] or live-attenuated *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) [24], DNA vaccines encoding the wild-type or codon-optimized antigen-85-like gene of *N. seriolae* [25], and an FKC vaccine supplemented with recombinant interleukin-12, have been reported [26].

4.2. Bacterial hemolytic jaundice

Bacterial hemolytic jaundice (BHJ) in farmed yellowtail resulting in losses of 5%–20% of the total *S. quinqueradiata* population have been reported [11]. The affected fish are characterized by yellow coloration of the skin and muscle tissue caused by the hemolytic activity of the bacterium [11,27]. The disease first appeared in the 1980s and is now widely spread across western Japan [28]. Because the disease affects large fish before shipment, it causes further serious economic losses after the losses caused by nocardiosis in yellowtail culture. The pathogenicity of *I. seriolicida* for the greater amberjack and yellowtail kingfish is low. Routine bacteriological techniques using agar plates are not appropriate for this bacterium because the addition of agar, even low-concentration agar, inhibits bacterial growth [10]. Although the bacterium grows in L-15 medium, Eagle's minimal essential medium, 0.85% NaCl solution, and 30% seawater, supplemented with 10% fetal bovine serum [10], its growth rate is slow and the density of bacterial cells in the medium is low. Subculture also reduces the hemolytic activity of the bacterium. Improvements in the culture technique are required to develop a stable vaccine. In our study, infection with cultured bacteria via the immersion route or by cohabitation with diseased fish was unsuccessful. To evaluate vaccine efficacy, a suitable infection method close to the natural infection route is desirable, so it is necessary to improve the artificial infection method. However, some promising findings for the development of vaccines have been reported. Survivors of natural infections with *I. seriolicida* achieve significant protection, and display antibody-mediated killing of the causative agent [29]. Although the bacterial serotype has not been clarified, in molecular epidemiological analysis of *I. seriolicida* populations, they were shown to be clonal populations with limited polymorphisms [28]. Therefore, it may be possible to develop an effective vaccine if low-cost culture techniques are established, with stable bacterial quality. Research on the development of a recombinant vaccine is underway using the genome information of *I. seriolicida* [30].

4.3. Bacterial cold-water disease (BCWD)

Flavobacterium psychrophilum is the causative agent of BCWD [31] and rainbow trout fry syndrome [32]. In Japan, BCWD was also reported in cultured coho salmon in 1990 [33] and in wild sweetfish in 1993 [34], and has caused serious damage to sweetfish populations in many rivers. The characteristics of BCWD in sweetfish differ from those in salmonid fish, including the water temperature at which it develops

Table 1
Ten fish species with the largest economic losses caused by infectious diseases in 2015.

	Fish	Causative agent and rate of disease							
		1st	Total % loss	2nd	Total % loss	3rd	Total % loss	Others (total % loss)
1	*Seriola* fish	Nocardiosis	45%	*Streptococcus dysgalactiae* infection	18%	*Lactococcus garvieae* infection	8%	29%
2	Eel	Edwardsiellosis	65%	Viral endothelial-cell necrosis	10%	*Vibrio anguillarum* infection	6%	19%
3	Japanese flounder	Edwardsiellosis	47%	Streptococcosis	37%	*Aquareovirus* infection	4%	12%
4	Puffer fish	Gill fluke disease	43%	*Myxosporean emaciation* disease	24%	NA	–	33%
5	Other marine fish	*Streptococcus dysgalactiae* infection	50%	Gliding bacterial disease	9%	NA	–	41%
6	Red sea bream	Edwardsiellosis	23%	Scuticociliatosis	20%	Red sea bream iridoviral disease	14%	43%
7	Sweetfish	Bacterial cold-water disease	51%	[b]Boke disease	29%	NA	–	20%
8	Kuruma shrimp	Vibriosis	76%	Black gill disease (fusariosis)	10%	NA	–	14%
9	Pacific bluefin tuna	Blood fluke disease	32%	Vibriosis	24%	Nocardiosis	24%	20%
10	[a]Other salmonids	Furunculosis	46%	Erythrocytic inclusion body syndrome (PRV-2 infection)	20%	Saprolegniasis	7%	27%

Data are based on values estimated from a questionnaire-based survey of fish farmers.
[a] Salmonid species farmed in freshwater, except for rainbow trout.
[b] Diagnosis may have included the following diseases: bacterial gill disease caused by *Flavobacterium branchiophilum*, atypical cellular gill disease (ACGD), unknown diseases with symptoms similar to those of ACGD.
NA = not applicable.

and the clinical signs [34–36]. Genetic [37–40], serotypic [41,42], and pathogenic [39] differences between the *F. psychrophilum* strains isolated from sweetfish and other fish species have also been reported. Sweetfish is a popular game fish and a large number of juvenile fish, reared in hatcheries or collected from Lake Biwa located in central Japan, are released into local rivers to improve sweetfish stocks. BCWD probably spread throughout Japan with the release of juveniles carrying the pathogen and/or the introduction of infected decoy fish, live sweetfish that are used in an angling method called “tomozuri” [38,43].

The development of an effective vaccine is anticipated because passive immunization with antiserum from a survivor of BCWD was protective against the disease [44]. An FKC vaccine with an oil-based adjuvant has also shown a protective effect [45]. However, because the oily-adjuvanted vaccine remains in the body of the sweetfish for more than 2 months, it has not been used in the industry. This is because sweetfish is an annual fish and its internal organs are eaten, so a long-term residual adjuvant cannot be used. The efficacy of a water-soluble adjuvant with a short residence time has been confirmed [46,47], but unfortunately, the toxicity of the adjuvant thwarted the commercialization of the vaccine. Although oral vaccines using FKC from logarithmic bacterial cultures [48] and enteric microcapsulated oral vaccines have been studied, no stable effect has been achieved. Protective efficacies of peritoneal injection with recombinant antigenic proteins against *F. psychrophilum* had been investigated in sweetfish [49]. Further study is necessary to improve protective efficacy, but these proteins are good candidates for the development of vaccines against BCWD.

4.4. Erythrocyte inclusion body syndrome

Piscine orthoreovirus 2 is the causative agent of EIBS in coho salmon [13]. The disease occurs at water temperatures below 10 °C and causes severe anemia and mass mortality. PRV-2 is closely related to PRV-1 [50] and PRV-3 [51], which are pathogens causing heart and skeletal muscle inflammation (HSMI) in Atlantic salmon (*Salmo salar*) and an HSMI-like disease in rainbow trout, respectively. It is possible to control the disease with passive immunization with the serum of infected tolerant individuals [52]. All attempts to culture this virus have failed, but a molecular biological approach may facilitate the development of a vaccine, such as a recombinant vaccine or DNA vaccine. In a recent trial of a DNA vaccine for PRV-1, a moderate protective effect against HSMI was reported when the DNA vaccine encoding the non-structural protein μNS was combined with one encoding the cell attachment protein σ1 [53]. Given the similarity of PRV-1 and PRV-2, this strategy may also be effective for EIBS.

4.5. Parasitosis

The skin-parasitic capsalid monogeneans *Neobenedenia girellae* and *Benedenia seriolae* cause problems on cultured marine fishes in Japan. The former parasitizes various fishes and shows low host specificity [54], and the latter demonstrates high host specificity for members of the genus *Seriola* [55]. Although bath treatments with hydrogen peroxide solution or freshwater and oral administration of praziquantel are effective for control of the parasite, these operations require intensive *labor or costs.* Research on DNA vaccines targeting proteases selected by transcriptome analysis of *B. seriolae* is underway, and good results have been obtained (H. Kondo, unpublished data; personal communication).

The ciliate *Miamiensis avidus* causes scuticociliatosis in many marine fish species and frequently causes the mortality in Japanese flounder *Paralichthys olivaceus*. Because there is no effective chemical treatment method for scuticociliatosis, the vaccine is desired. Japanese flounder immunized with formalin-killed *M. avidus* showed low mortalities in experimental infections with the ciliate [56]. But effective protection was only provided when the same isolate was used in the vaccination and the challenge in a vaccination study with *Philasterides dicentrarchi*

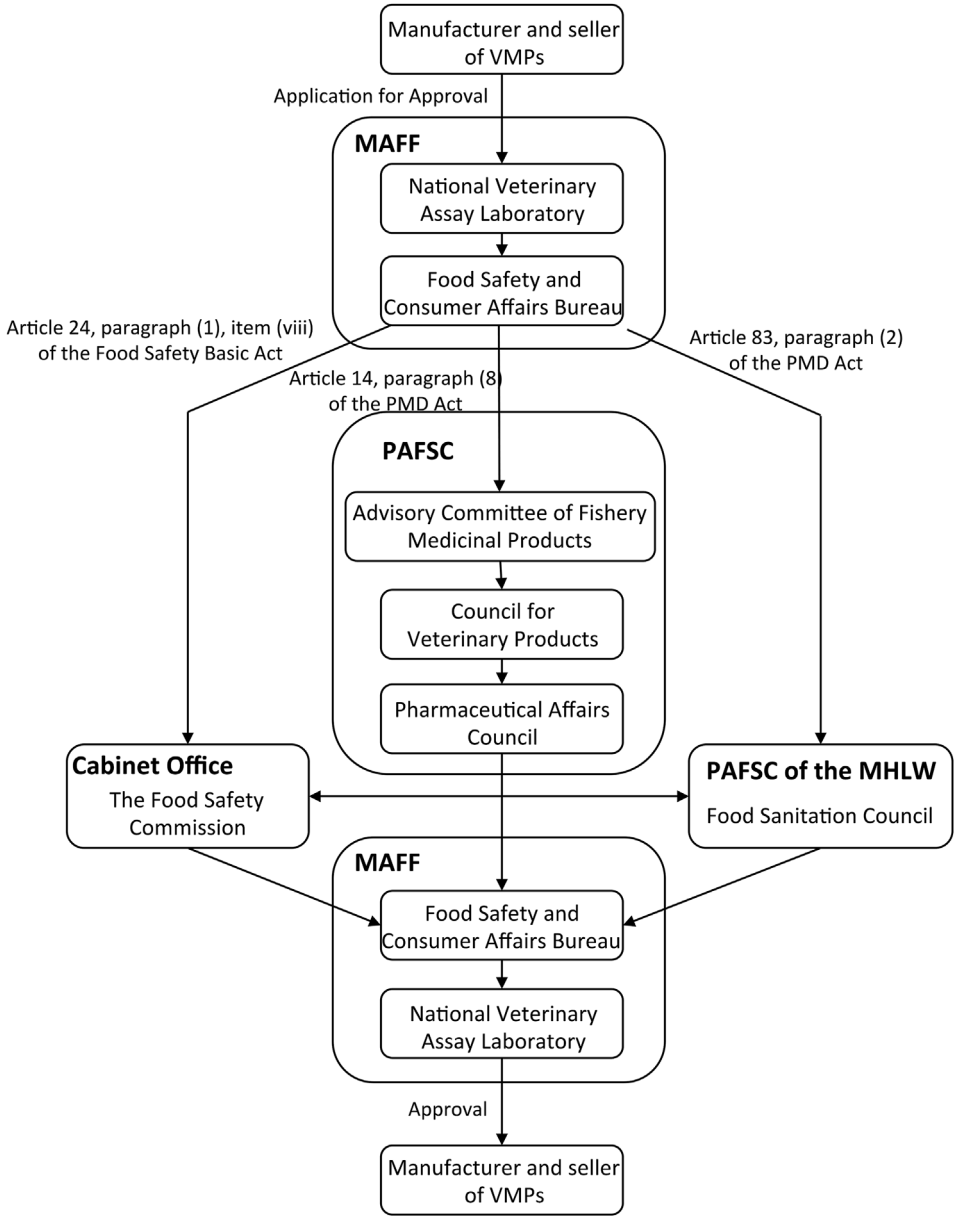

Fig. 4. Scheme for the regulatory approval of veterinary medicinal products (VMPs) and therefore fish vaccines in Japan.
Original figure was published in a Japanese book "Handbook of Fish Vaccines" [18]. MAFF: Ministry of Agriculture, Forestry, and Fisheries; MHLW: Ministry of Health, Labour, and Welfare, PAFSC: Pharmaceutical Affairs and Food Sanitation Council.

(junior synonym of *M. avidus*) against Spanish turbot [57], and at least three types of serotypes of *M avidus* exist in Japan [58]. Highly antigenic polypeptides that correlate with the three serotypes have been reported as surface antigens involved in *M. avidus* immobilization [59]. Therefore, the mixture of multiple serotypes of inactivated M. avidus or that of polypeptides derived from multiple serotypes is considered to be promising antigens for effective vaccines.

5. Concluding remarks and future perspectives

In recent years, global efforts have been made to properly use antibiotics to reduce the risk of drug-resistant bacteria. Within this context, the methods of controlling fish diseases in Japan are shifting from antibiotic treatments to vaccine prophylaxis. In this section, in our concluding remarks, we discuss the issues involved in the promotion of vaccines for use in fish farming in Japan.

5.1. Development of evaluation methods and criteria for vaccine efficacy

A challenge test is essential to the process of vaccine development and is currently considered the most appropriate way to evaluate vaccine efficacy, although large numbers of live fish are sacrificed in this test. From the perspectives of cost, the efficiency of the process, and animal welfare, the development of methods to evaluate vaccine efficacy with little or no sacrifice of fish is preferable. Therefore, it will be necessary to develop a novel method that can measure the immune responses of fish, which correlate with vaccine efficacy.

When the specific antibody titer against the target pathogen correlates with the vaccine efficacy, an assay of the specific antibody titer in the sera of vaccinated fish may be an effective alternative to a challenge test. In Japan, the neutralization test was used to test the efficacy of the licensed vaccines against viral nervous necrosis virus (VNNV). However, the protection afforded by antibody-dependent cellular cytotoxicity (ADCC) activity against infection has not been fully clarified

Table 2
Fish vaccines in Japan.

Pathogen	Recommended species	Administration	Adjuvant	Dose
Monovalent vaccines				
Vibrio anguillarum	Sweetfish (0.6–500 g)	Imm	No adjuvant	Immerse 0.5 kg of fish per 1 L of vaccine for 2 min (1:10 dilution), or immerse 0.2 kg of fish per 1 L of vaccine for 10 min (1:100 dilution)
Viral nervous necrosis virus	Sevenband grouper (8–128 g) Longtooth grouper (7–180 g)	Inj	No adjuvant	0.1 mL/fish
Lactococcus garvieae	*Seriola* fish*[1] (20–300 g)	Inj	No adjuvant	0.1 mL/fish
L. garvieae	*Seriola* fish (100–400 g)	Ora	No adjuvant	0.5 mL/fish/day for 5 days, or boosted 0.125 mL/fish at 3 month later
Streptococcus iniae	Japanese flounder (30–300 g) Thread-sail filefish*[2] (5–100 g)	Inj	No adjuvant	0.1 mL/fish
Edwardsiella tarda	Japanese flounder (20–50 g)	Inj	Fucoidan	0.1 mL/fish
Red sea bream iridovirus (RSIV)	Red sea bream (5–100 g) *Seriola* fish (10–100 g) Groupers*[3] (5–50 g) Striped jack*[4] (10–70 g)	Inj	No adjuvant	0.1 mL/fish
RSIV	Yellowtail (30–90 g)	Inj	Montanide ISA763A	0.1 mL/fish
Bivalent vaccines				
Vibrio sp. (serotype J-O-1), *V. anguillarum* (serotype J-O-3)	Salmonids	Imm	No adjuvant	Immerse 0.5 kg of fish per 1 L of vaccine for 2 min (1:10 dilution)
L. garvieae, *V. anguillarum*	*Seriola* fish (30–2000 g)	Inj	No adjuvant	0.1 mL/fish
RSIV, *L. garvieae*	*Seriola* fish (10–100 g)	Inj	No adjuvant	0.1 mL/fish
L. garvieae, *Photobacterium damselae* subsp. *piscicida*	Yellowtail (30–110 g) Greater amberjack (20–210 g)	Inj	Montanide ISA763A	0.1 mL/fish
S. iniae, RSIV	Red sea bream (5–50 g)	Inj	No adjuvant	0.1 mL/fish
S. iniae, *S. parauberis*	Japanese flounder (30–300 g)	Inj	No adjuvant	0.1 mL/fish
Imm = Immersion vaccine; Inj = Injectable vaccine; Ora = Oral vaccine				
Trivalent vaccines				
RSIV, *V. anguillarum*, *L. garvieae*	*Seriola* fish*[5] (10–860 g)	Inj	No adjuvant	0.1 mL/fish
P. damselae subsp. *piscicida*, *L. garvieae*, *V. anguillarum*	Yellowtail (30–110 g), Greater amberjack (30–200 g)	Inj	Montanide ISA763A	0.1 mL/fish
L. garvieae, *V. anguillarum*, *S. dysgalactiae*	Greater amberjack (20–1600 g)	Inj	No adjuvant	0.1 mL/fish
Tetravalent vaccines				
RSIV, *V. anguillarum*, *L. garvieae*, *P. damselae* subsp. *piscicida*	Yellowtail (20–1000 g)	Inj	Montanide ISA763A	0.1 mL/fish
RSIV, *V. anguillarum*, *L. garvieae*, *P. damselae* subsp. *piscicida*	*Seriola* fish (30–300 g)	Inj	Fucoidan	0.1 mL/fish

Imm = Immersion vaccine; Inj = Injectable vaccine; Ora = Oral vaccine.
*[1] *Seriola* fish include yellowtail (*Seriola quinqueradiata*), greater amberjack (*S. dumerili*), and yellowtail kingfish (*S. lalandi*).
*[2] Thread-sail filefish (*Stephanolepis cirrhifer*).
*[3] Malabar grouper (*Epinephelus malabaricus*), orange-spotted grouper (*E. coioides*), longtooth grouper (*E. bruneus*), sevenband grouper (*E. septemfasciatus*).
*[4] Striped jack (*Pseudocaranx dentex*).
*[5] *Seriola* fish include yellowtail (*Seriola quinqueradiata*), greater amberjack (*S. dumerili*), and yellowtail kingfish (*S. lalandi*).

in fish, although alloreactive cytotoxic cells are armed with immunoglobulin M (IgM) to execute ADCC in the channel catfish [60,61]. Therefore, studies of the correlation between specific antibodies against pathogens and the ADCC activity in fish are necessary to evaluate vaccine efficacy more clearly.

The innate immunity and cellular immunity conferred by cytotoxic T lymphocytes (CTL) may also play roles in the protection of vaccinated fish. The conventional analytical methods for biological markers of innate immunity, such as phagocytic activity, the production of reactive oxygen species, complements, and bacteriolytic activities, have already been used in fish [62–64]. However, because the necessary analytical tools have not been developed for most fish species, such as antibodies directed against surface markers of lymphocytes and clonal strains, how to evaluate the cytotoxic activity of CTLs against a specific pathogen has not been clarified, except in the crucian carp (*Carassius auratus langsdorfii*) [65,66] and rainbow trout [67]. It is noteworthy that a functional correlation between perforin and granzyme-B-like activities and the cytotoxic activity of CTLs has been reported in the crucian carp, as in mammals [68,69]. Therefore, an analytical method that targets perforin or granzyme may be useful in evaluating the cytotoxic activity of CTLs in vaccinated fish.

5.2. Introduction of vaccines other than inactivated vaccines

Today, edwardsiellosis is one of the most important diseases in the aquaculture of the eel, Japanese flounder, and red sea bream, in Japan [70]. As mentioned above, infections caused by acid-fast bacteria (*Mycobacterium* spp. and *N. seriolae*) also seriously affect cultured yellowtail and greater amberjack [71–73]. The epidemic of IHNV on rainbow trout farms should not be overlooked, because it is suggested that the nucleotide diversity of IHNV isolates changes rapidly, altering its virulence in rainbow trout farm environments in Japan [74]. The establishment of prophylactic methods for these intracellular pathogens is an urgent issue in Japan. To date, the protective efficacy of live-attenuated vaccines, DNA vaccines, or a combination of killed bacteria and a cytokine adjuvant against these intracellular pathogens has been confirmed for *Edwardsiella tarda* [75], *Mycobacterium* spp. [76,77], *N. seriolae* [24–26,78], and IHNV [79]. Furthermore, among the intracellular pathogens of fish, a live-attenuated vaccine for enteric septicemia of catfish and a DNA vaccine for IHNV infection of Atlantic salmon have been approved in the USA and Canada, respectively. However, all fish vaccines approved for use in Japan are inactivated vaccines, and guidelines for the introduction of other vaccines have not yet been prepared. Therefore, it is necessary not only to confirm the safety and effectiveness of DNA vaccines and live-attenuated vaccines but also to establish an administrative system that can introduce these vaccines into Japanese aquaculture.

5.3. Autogenous vaccines

The use of autogenous vaccines for aquaculture is not permitted in Japan. Autogenous vaccines can be defined as inactivated immunological VMPs that are manufactured from pathogens and antigens derived from an animal or animals at a specific facility, which are used for the treatment of that animal or animals at that facility or in the same locality [17]. An autogenous vaccine for a facility with a specific disease problem can be manufactured much more rapidly than a commercial vaccine can be developed and licensed [80]. Thus, autogenous vaccines prepared from pathogen strains at a particular fish farm or in a specific geographical area may be an alternative strategy when no efficient commercial fish vaccine is available. Generally, when various fish species are cultured, varieties of pathogens and their variants may emerge. The introduction of autogenous vaccines may promote the development of fish vaccines in countries where various fish species are cultured, such as Japan.

5.4. Application of conventional fish vaccines to minor species

Various species of marine and freshwater fish are cultured in Japan. In principle, even though an infectious disease is caused by a specific pathogen, a vaccine requires approval for its application to the disease in individual fish species or genera. In other words, even if a vaccine is already approved for use in a certain fish species, it may not be approved for another fish species. For example, some fish pathogens, including Red sea bream iridovirus (RSIV) [81], *Vibrio* species [82], *L. garvieae*, and *Streptococcus* species [83], seriously affect a wide range of host species in Japan. However, the vaccines against these fish pathogens are restricted for use in the species that are produced at high levels, such as *Seriola* fish, red sea bream, Japanese flounder, and salmonid fish (Table 1). This is because vaccine manufacturers are hesitant to seek approval to apply existing vaccines to fish species that a produced in small amounts (vaccine sales for these fish species are expected to be lower) because applications for such approval requires considerable cost and time. Therefore, it is necessary to establish criteria and analytical methods that will simplify the assessment of existing vaccines for application to various fish species, because this will extend the application of vaccines to low-producing species.

5.5. Vaccine administration in fish that are difficult to handle

Several fish species that are vulnerable to handling stress and/or are difficult to handle are cultured in Japan. For instance, BCWD caused by *F. psychrophilum* develops readily when the skin of sweetfish has abraded by handling [36]. Larger fish, such as the Pacific bluefin tuna (*Thunnus orientalis*), and slippery fish, such as the Japanese eel (*A. japonica*), are representative of these difficult-to-handle species, although both species are important for aquaculture in Japan. For these kinds of species, it is necessary to develop a vaccine administration method other than injection. Because research into mucosal immunity is proceeding actively throughout the world [84,85], it is expected that an efficient antigen-delivering method will be devised in the future.

5.6. Supply of fish for vaccine trials

There are no special agencies that produce and distribute test fish for scientific use in Japan. At this time, vaccine manufacturers and laboratories in Japan must find fish producers, such as private companies or prefectural hatcheries, from which to obtain fish in the necessary numbers and of the appropriate size for vaccine trials. Therefore, establishing a specialist agency for supplying test fish in Japan is warranted, and should promote to development of fish vaccines by ensuring the accuracy and reproducibility of vaccine trials. VESO Vikan in Norway, a facility that supplies specific-pathogen-free fish for scientific use and conducts experimental tests to develop VMPs for use in fish, is an excellent model for this task.

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