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## REVIEW

# *Anguilla* sp. Diseases Diagnoses and Treatments: the Ideal Methods at the Cross Roads of Conservation and Aquaculture Purposes

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## **Abstract**

*Anguilla anguilla*, *A. japonica* and *A. rostrata* are the most fished and consumed eel species. However, these species are Critically Endangered, Endangered and Endangered respectively. A combination of factors is thought to be responsible for their decline including fisheries, climate change, habitat destruction, barriers to migration, pollution and pathogens. Among them, viruses, bacteria and parasites are causing weakening of wild eels and serious economic losses for fishermen and eel farmers. Early detection of pathogens is essential to provide appropriate responses both for conservation reasons and to limit economic losses. Classic diagnosis approaches are time consuming and invasive and usual treatments e.g. antipathogenic substances are becoming obsolete because of pathogen resistance and environmental impact problems. The need for early and non-invasive diagnostic methods as well as effective and environmentally friendly treatments has increased. Vaccine development and diet supplementation have known a growing interest since their use could allow prevention of diseases. In this review, we summarize the main pathogens -viruses, bacteria and parasites- of the three northern temperate eel species, the methods used to detect these pathogens, and the different treatments used. We discussed and highlighted the need for non-invasive, rapid and efficient detection methods, as well as effective and environmentally friendly treatments for both conservation and aquaculture purposes.

**Keywords: Eels, Illness, Pathogens, Detection, Control**

### **1. Introduction**

*Anguilla* sp. are widely distributed throughout the world (Aoyama 2009). Their life cycle starts in the ocean where they hatch as leptocephali (leaf-like small larvae of some millimeters) and migrate for several months toward their growing habitat (i.e. inland and transitional waters). After metamorphosing into glass eels (small translucent cylindrical stage of less than 10 cm), they enter inland and transitional waters, become pigmented and develop as yellow eels (growth stage). After several years of growth, they metamorphose into silver eels (future spawners) and return to the ocean to migrate to their spawning ground, reproduce and die (Van Ginneken and Maes 2005, Cresci 2020).

Eels are of ecological importance (as food source and top predator in their freshwater and brackish habitats) and of economic importance. Huge quantities of eels, mainly *Anguilla japonica*, *Anguilla anguilla* and *Anguilla rostrata* used to be caught for direct consumption or aquaculture and this number increased up to the 70s (Dekker 2008; FAO 2021). While the demand for these fish is increasing, there has been a global collapse of eel populations since the end of the 70's. *A. rostrata* and *A. japonica* are now considered threatened while *A. anguilla* is critically endangered, its population being thought to have been reduced by a factor 10 (Feunteun 2002, Dekker 2008; Jacoby *et al.* 2015; IUCN 2022). Since humans consume glass, yellow and silver stage eels, overfishing could be one factor in the population collapse of the three species; climate change, barriers to migration, pollution and pathogens (mainly viral, bacterial or parasitic infections) are also considered to be factors contributing to the decline of eel populations (Elie and Rigaud 1987; Bruslé 1994; Kirk 2003; Knights 2003; Palstra *et al.* 2006; Haenen *et al.* 2009; Esteve and Alcaide 2009; Arai 2014; Drouineau *et al.* 2018).

Efforts have been made for the recovery of eel stocks and management plans have been developed. They include fishing restriction, habitat restoration, facilitation of migration (e.g. eel ladders) and restocking programs (Moriarty and Dekker 1997; Moriarty 1990; Feunteun 2002; Lamson *et al.* 2009; Bierman *et al.* 2012; Righton and Walker 2013; Kaifu *et al.* 2018; Kaifu and Yokouchi 2019). However, there is still a lack of knowledge about pathogens (e.g. prevalence of infections) as well as reliable, non-invasive detection tools and appropriate control methods.

The most cited pathogens are: viruses EVE (Egusa 1970; Haenen *et al.* 2009), EVEX (Sano 1976; Haenen *et al.* 2012; Bellec *et al.* 2014), HVA/AngHV-1 (Békési *et al.* 1986; Sano *et al.* 1990; van Beurden *et al.* 2010; Kempter *et al.* 2014), bacteria *Edwardsiella anguillarum* (Hah *et al.* 1984; Joh *et al.* 2013), *Vibrio vulnificus* (Amaro *et al.* 1995; Amaro and Biosca 1996; Høi *et al.* 1998; Dalsgaard *et al.* 1999; Esteve *et al.* 2007; Esteve and Alcaide 2009; Haenen *et al.* 2014), *Pseudomonas anguilliseptica* (Wakabayashi and Egusa 1972; Nakai and Muroga 1982; Ellis *et al.* 1983; Andree *et al.* 2013; Joh *et al.* 2013), *Vibrio anguillarum* (Hah *et al.* 1984; Mellergaard 1987; Frans *et al.* 2011), and parasites *Pseudodactylogyrus anguillae* and *P. bini* (Buchmann *et al.* 1987) and *Anguillicola crassus* (Køie 1991; Kirk 2003). The latter is thought to have a significant impact on swim bladder function, especially in European and American eels (Kirk 2003; Sokolowski and Dove 2006; Barry *et al.* 2014). More generally, infections by pathogens can cause tissue damages, hemorrhages, anemia and thus general weakening and death. As a diadromous fish, migration is a critical period in the life cycle of eel and the renewal of population. Thus, infections and the resulting weakening can exacerbate the collapse of eel populations. In addition, the demand for these fish is high which implies an increased production of eels in aquaculture (FAO 2021). In some countries such as in the Republic of Korea, eel farming is the most important freshwater aquaculture industry (Joh *et al.* 2011; Yi *et al.* 2013). Eels are fished in the juvenile stages and reared in intensive aquaculture systems. The constant renewal of juvenile eel stocks, combined with stressful farming conditions, can lead to the introduction of pathogens, development of diseases (Bruslé 1990; Haenen *et al.* 2012) and important economic loss for eel farmers (Assefa and Abunna 2018). Thus pathogens are problematic both for life cycle, renewal of the natural population and in anguiculture.

Knowledge on pathogens and outbreaks in wild and farmed eels is necessary in order to react in the right way. For example, using fish devoid of the most harmful pathogens is essential for the success of restocking programs and must only be possible via pathogen detection and the knowledge of the prevalence of infection. Early pathogen detection and effective treatments of diseases are also essential for the sustainability of eel farming. The most common methods used to detect and identify pathogens include pathogen cultures from diseased organs, serology and histology (Altinok and Kurt 2003; Noga 2010) but these can be time consuming and performed on already dead fish or after their sacrifice. The arrival of molecular techniques such as Polymerase Chain Reaction (PCR) amplifications, qPCR and radio techniques methods has led to the development of non-invasive methods that can detect pathogens prior to an outbreak (Altinok and Kurt 2003). However, effectiveness is sometimes below the one of more traditional methods and their cost is often relatively high

(Jousseau *et al.* 2021). Taken together these elements constitute a real obstacle to the use of these methods on a larger scale.

Regarding treatments, modulation of the environment and intensive use of chemicals have been employed for a long time. However, modulation of the environment, that requires a compromise between optimal conditions for growth and those that prevent the development of diseases, could result in economic loss and is not applicable in the wild. Antipathogenic substances, even though efficient for the short term, are increasingly banned because of their potential environmental impact and their ability to generate the development of resistant pathogens (Alcaide *et al.* 2004; Romero *et al.* 2012; Lin *et al.* 2016). There is therefore a critical need for efficient and environmentally friendly treatments for fish diseases (Lieke *et al.* 2020). Vaccinations and diet supplementations with plants or probiotics have received a growing interest since their use could allow the prevention of fish diseases through the stimulation of fish immunity (Gudding and van Muiswinkel 2013; Reverter *et al.* 2014).

In this review, we have focused on the three northern temperate and most fished eel species: the European eel *A. anguilla*, the American eel *A. rostrata* and the Japanese eel *A. japonica*. Focusing only on viruses, bacteria and protozoan (although a non-monophyletic group) and metazoan parasites, we have presented and summarized those that are commonly described as pathogenic for eels. We have listed the diagnosis tools used and the different treatments intended directly applied to eels. Finally, we discussed the methods, their applicability at a higher scale and we highlighted the need for more environmentally friendly and non-invasive diagnoses and treatments.

## **2. Eel pathogens, damages and symptoms**

### **2.1 Viruses**

Various viruses were isolated from eels and are summarized in **Table 1**. They cause major viral diseases (van Beurden *et al.* 2012) triggered by the Anguillid herpesvirus 1 (HVA/AngHV-1) (Békési *et al.* 1986; Sano *et al.* 1990; van Beurden *et al.* 2010; Kempter *et al.* 2014), the Eel Virus European (EVE) (Egusa *et al.* 1970; Haenen *et al.* 2009) but also the two closely related rhabdovirus Eel Virus America (EVA) (Sano 1976) and Eel Virus European X (EVEX) (Sano *et al.* 1977; Haenen *et al.* 2012; Bellec *et al.* 2014). Other viruses causing diseases in eels were also identified such as the Japanese eel endothelial cells-infecting virus (JEECV) that causes viral endothelial cell necrosis (VECNE) in Japanese eels and large economic losses for aquaculture (Ono *et al.* 2003; Mizutani *et al.* 2011; Okazaki *et al.* 2015; Okazaki *et al.* 2016a,b; Kim *et al.* 2018). Finally, infectious pancreatic necrosis (IPN) viruses (Jørgensen *et al.* 1994), IHNV viruses (McAllister *et al.* 1977; Bergmann *et al.* 2003 in van Beurden *et al.* 2012) were also isolated from eels. Although haemorrhagic septicaemia viruses (HSV) have also been isolated from *A. anguilla*, but no data confirmed the pathogenicity of this virus for eels (Castric *et al.* 1992; Jørgensen *et al.* 1994). In a similar way, orthomyxoviruses EV-1 and EV-2 were isolated from tumours but no clear relationship between these viruses and tumours could be demonstrated (Wolf *et al.* 1973; Neukirch 1985; Nagabayashi and Wolf 1979).

Among various direct or indirect symptoms, infected eels present haemorrhagic gills or fins and organ disorders (Shchelkunov *et al.* 1989; van Ginneken and Maes 2005; van Beurden *et al.* 2012). For example, AngHV1 has been reported to cause serious destructions of the gill filaments (Rijsewijk *et al.* 2005). The presence of viruses may also impact swimming behaviour and thus have significant effect, at least for the European eel as reviewed in Haenen *et al.* (2009), on eel spawning migration and thus on their overall recruitment.

Eel viruses have been detected both in wild and farmed eels (Haenen *et al.* 2010; van Beurden *et al.* 2012; Danne *et al.* 2022). Viruses are particularly problematic because of their ability to remain dormant, to spread through seemingly healthy populations and become virulent under certain conditions (Haenen *et al.* 2009).

Virulence is usually associated with stress such as temperature increase (Haenen *et al.* 2009) or husbandry practices i.e. high stocking densities or poor water quality (Hangalapura *et al.* 2007, Muñoz *et al.* 2019). In anguiculture, reported mortalities due to viruses were, for European eels, up to 50% for EVE, around 20% for EVEX and 10% for HVA (Haenen *et al.* 2009). HVA infections resulted in mortality of 7% for Japanese eel (Sano *et al.* 1990) while experimental infection with JEECV caused 60% mortality (Ono *et al.* 2003). Viral infection often co-occurs or occurs with bacterial or parasitic infections (Haenen *et al.* 2009; van Beurden *et al.* 2012; Muñoz *et al.*, 2019).

## **2.2 Bacteria**

A significant number of bacteria has been identified as causative agents of eel diseases. Among them the most reported are: *Edwardsiella tarda*, *Aeromonas hydrophila*, *Vibrio vulnificus* and *V. anguillarum* (Tison *et al.* 1982; Hah *et al.* 1984; Biosca *et al.* 1991; Amaro and Biosca 1996; Joh *et al.* 2013; Haenen *et al.* 2014). About ten other bacteria potentially pathogens and isolated from diseased eels were reported: they include *Aeromonas jandaei*, *A. aquariorum*, *A. bestiarum*, *A. caviae*, *A. salmonicida*, *A. sobria*, *A. veronii*, *Citrobacter freundii*, *Delftia acidovorans*, *E.tarda/piscida/anguillarum*, *Flavobacterium columnare*, *F. psychrophilum*, *Lactococcus garvieae*, *Mycobacterium marinum*, *Pleisomonas shigelloides*, *Pseudomonas anguilliseptica*, *P. fluorescens*, *V. harveyi*, and *Yersina ruckeri* (**Table 2**). For most of them, their pathogenicity was confirmed by infecting healthy eels and by recording the resulting mortalities and symptoms (**Table 2**).

Some bacteria have been isolated from apparently healthy farmed eels, such as *E. tarda*, *A. hydrophila* and *V. anguillarum* (Hah *et al.* 1984; Amaro *et al.* 1995; Joh *et al.* 2013). These bacteria might turn into pathogenic under stress conditions such as presence of pollutants in the environment (Rødsæther *et al.* 1977; Esteve *et al.* 2012), or during warmer periods (Davis and Hayasaka 1983; Haenen 2019). For example, *A. hydrophila* appears to be pathogenic when the water temperature is between 17 and 22°C (Esteve *et al.* 1993). Similarly, at low temperature <20°C *V. vulnificus* did not induce mortality with the 50% lethal dose (LD<sub>50</sub>) below 10<sup>8</sup> CFU/mL, but the infection trial at 27-29°C led to high mortality rate with LD<sub>50</sub> of 8.4x10<sup>4</sup> CFU/mL (Amaro *et al.* 1995). Bacterial infections can cause external damages to the skin, fins, mouth, eyes, tail, gills and affect internal organs: heart, liver, spleen, kidney, muscles associated with global hemorrhages and septicaemia (**Table 2**). Disease outbreaks caused by bacteria can result in important economic losses for eel farmers (Joh *et al.* 2013). For example, several outbreaks have been reported in Spanish eel farms resulting in the loss of 80% of elvers. The prevalence of bacterial diseases in wild eels is also of concern as it can reach significant levels. For example, 34.4% of wild eels from the Albufera lake had bacterial diseases (Esteve and Alcaide 2009).

Among all the bacteria cited, some of them may act as primary pathogen while some others may be opportunistic acting as secondary pathogen and leading to eel co-infections (Joh *et al.* 2010; Joh *et al.* 2013). For example, a co-infection with *P. anguilliseptica* and *D. acidovorans* occurred in *A. anguilla* glass eels (Andree *et al.* 2013).

## **2.3 Protozoan and Metazoan Parasites**

Various parasites can affect eels; these are protozoan or metazoan parasites. Parasites can be ecto- or endo-parasite, they can be found on the gills, the skin and fins, in the blood and on/in almost all organs (e.g. kidney, liver, intestine, stomach...) (Jakob *et al.* 2016; Nagasawa and Katahira 2017). 161 species of parasites have been isolated from specimens of *A. anguilla* in European and north African countries and are reviewed in Jakob *et al.* (2016). This checklist summarizes data on parasites acquired up to 2007. In the meantime, other parasite species have been isolated from European eels, including *Henneguya psorospermica*, *Cystidicola farionis*, unidentified species of *Dactylogyrus* (Dzido *et al.*, 2020) but also *Bucephalus anguillae* (Giari *et al.*, 2020). 50 species from Ciliophora, Microspora, Myxozoa, Trematoda, Monogenea, Cestoda, Nematoda, Acanthocephala, Hirudinida, Bivalvia, and Copepoda taxa have also been reported as parasites for *A. japonica* and *A. anguilla* in

Japan and are reviewed in Nagasawa and Katahira (2017). Finally, about 63 parasites (Monogenea, Trematoda, Cestoda, Nematoda and Acanthocephala) have been reported in *A. rostrata* (Hanek and Threlfall 1970; Kennedy 2007; Hoffman 2019). Among all reported species some are eel-specific parasites such as *Bothriocephalus claviceps*, *Proteocephalus microcephalus*, *A. crassus*, *P. anguillae*, or *P. bini* (Jakob *et al.* 2016). Although the diversity and number of parasites found in eels is huge, only a small proportion of species have been identified as true pathogens. The other species cause mainly little damage, either locally by attaching to tissues or more globally by diverting nutrients from the host to themselves, as intestinal acanthocephalans can do (Kennedy 2007; Gérard *et al.* 2013). However, those little damages can become dangerous under stressful conditions especially to the gills (Kjøie 1988; Kennedy 2007). Of the parasites found in eels, the gill parasites *Pseudodactylogyrus* spp. (Buchmann *et al.* 1987) and the swim bladder parasite *A. crassus* (Kirk 2003) often represent the highest prevalence (Sures *et al.* 1999). They are also the main confirmed harmful pathogens, although with different intensities depending on the eel species (Sokolowski and Dove 2006) but also on factors such as temperature, salinity, eels foraging and ontogeny, or intermediate host density (Jakob *et al.* 2009, Li *et al.* 2015, Barry *et al.* 2017, Giari *et al.* 2021).

*Pseudodactylogyrus bini* and *P. anguillae* are two species of *Pseudodactylogyrus* occurring in eels (Buchmann *et al.* 1987). They were first described on *A. japonica* (Kikuchi 1929), and have been reported in *A. anguilla* in different European countries (Golovin 1977; Molnár 1983; Lambet *et al.* 1984; Mellergaard and Dalsgaard 1986 in Buchmann *et al.* 1987) and a few years later in the American eel (Cone *et al.* 1993; Hayward *et al.* 2001). The parasites attach themselves to the gills with hooks called “hamuli” that can cause damage to the gill tissue. When high in number and/or under stressful conditions (lack of oxygen), damage to the gills can impair the eel's gas exchange and have a sublethal effect leading to mortality (Buchmann *et al.* 1987; Kjøie 1991).

*Anguillicola crassus* is a nematode, originally found on Japanese eel, which was introduced into Europe in the 1980s through the eel trade (Koops and Hartman 1989). This parasite was also reported in the 90s in American eels from North American Atlantic coast rivers and estuaries (Fries *et al.* 1996; Barse and Secor 1999). Differences in eel species susceptibility to *A. crassus* were reported. The European and American eel are apparently more susceptible to the parasite than the original host species, the Japanese eel (Egusa 1979). Indeed, it has been shown that the survival rate of the parasite larvae was lower in the Japanese eel compared to the European eel (60% of Japanese eels had live worms compared to 100% for European eels) (Knopf and Mahnke 2004; Knopf 2006). Regarding the effects of the presence of parasites on the swim bladder, no clear damage was found in Japanese eel (Nagasawa *et al.* 1994) whereas it causes direct damage such as inflammations, reduction of elasticity and thickening of the swim bladder wall in American and European eels (Molnár *et al.* 1993; Kirk 2003; Sokolowski and Dove 2006; Barry *et al.* 2014; Pester 2015; Dezfuli *et al.* 2021). These damages could lead to altered swimbladder functions and swimming ability (Sprenkel and Lüchtenberg 1991; Palstra *et al.* 2007; Sjöberg *et al.* 2009; Newbold *et al.* 2015; Pester 2015). Sprenkel and Lüchtenberg (1991), showed a 19% reduction in the swimming speed of infected European eels and Newbold *et al.* (2015) observed a delay in downstream passage of eels with high abundance of *A. crassus*. In Hungary, an important mortality episode was attributed to *A. crassus*, eels being found to be heavily parasitized with 30-50 parasites in the swim bladders (Molnár *et al.* 1991). The presence of the parasite may also make eels more susceptible to secondary bacterial infection (van Banning and Haenen 1990).

#### **2.4 Are external symptoms sufficient to identify a causative agent ?**

As explained above, pathogens can cause several types of external symptoms (**Figure 1**). For example, clinical symptoms have been recorded in 122 *A. anguilla* from the Albufera Lake such as haemorrhagic fins (55%), ulcers on the opercula and anal regions (29%), reddened mouth (25%), necrosis of the tail (23%), petechiae on the belly (11%), over secretion of skin mucus (4%), and discoloured skin spots (3%). Microbial

isolation was performed and multiple bacteria were identified including *V. vulnificus*, *E. tarda*, *A. hydrophila*, *A. bestiarum* or *A. jandae*. When eels were intraperitoneally infected with the bacteria and the external symptoms were recorded, bacterial infections seemingly caused the same type of clinical symptoms: haemorrhagic fins, ulcers on opercula and anal regions, reddened mouth, oversecretion of skin mucus, discoloured skin spots (Esteve and Alcaide 2009). Thus, the observation of external symptoms may be the first step in identifying the presence of a pathogen or the development of a disease but is not specific enough to identify the causative agent. Moreover, infections are not always accompanied by external symptoms (Hudson *et al.* 1981; Stewart 1983) particularly in the early stages of infections, when pathogens are present at low levels. Finally, external symptoms such as haemorrhagic fins could be the result of a fight or an aggression between eels or with another species. Therefore, research and identification of a possible pathogen is essential in order to implement appropriate treatments.

### **3. Causative agent identification**

Detecting and identifying the pathogens responsible for a disease is an essential step in providing appropriate solutions to control outbreaks or limit the spread of pathogens. In parallel, knowledge on prevalence of pathogens in wild eel populations is essential to adapt management plans. Several methods exist to detect pathogens including pathogen cultures followed by morphological and biochemical identifications, serology, molecular techniques such as PCR amplification (Adams and Thompson 2011) but also radio or biomarkers detections. A summary of the methods used and applied to eels is presented in **table 3**.

#### **3.1 Classic isolation**

The classical identification approach consists of combining isolation, culture (specially for viruses and bacteria) and/or identification of pathogens by microscopic/histological, chemical or molecular methods. For example, the *Herpesvirus anguillae* (HVA) was isolated through organ grinding, centrifugation, and culturing on eel kidney (EK-1) cells. Electron microscopy was used to confirm the type of virus (rhabdovirus in this case) and specific identification was achieved by seroneutralization (van Nieuwstadt *et al.* 2001). Similarly, after inoculation of organ samples from eels (single organ or pool of various crushed organs) on specific cell lines: RTG2 - Rainbow Trout Gonad cells, FHM – Fat Head Minnow cells, and EK-1, observation of cytopathic effect and microscopy, EVEX, HVA and EVE were detected, isolated and identified (van Ginneken *et al.* 2004).

In a similar way, pathogenic bacteria can be isolated from lesions or crushed organs using antibiotic-enriched media or specific agar plates (e.g. TCBS plates for *Vibrio* sp., Sheep Blood Agar, Cytophaga Agar or Shieh Medium for *Flavobacterium* spp.) (Alcaide *et al.* 2004). Then, the morphology, phenotype and biochemistry of the isolates can be compared with those of reference bacteria (Haenen and Davidse 2001; Caruso *et al.* 2014). Usually, to be identify as the causative agent for an occurring outbreak, the bacterium must fit the Koch's postulates: the microorganism is found in diseased organisms, is grown in pure culture, induces the disease when inoculated into healthy organisms and must be reisolated from these newly diseased organisms. For example, in an outbreak in an eel farm in Japan, the observed clinical symptoms, apparently those of the red spot disease, were attributed to *P. anguilliseptica*. The causative bacterium was isolated from the blood, liver or kidneys of diseased eels on blood-enriched media, the morphology described and physiological and biochemical tests were performed for its identification. The morphological characteristics led to the classification of the bacterium in the genus *Pseudomonas*, and due to some specific differences with other *Pseudomonas* sp., the bacterium was apparently attributed to *P. anguilliseptica*. Finally, the isolate obtained was used to experimentally infect healthy eels. The infected eel developed the same symptoms as those of the farmed eels. However, the bacterium was not reisolated from the newly infected organisms (Wakabashi and Egusa 1972). Thus, even if application of the Koch's postulates is quite robust in identifying pathogens, recent studies have highlighted the need to adapt Koch's postulates to include the whole bacterial communities and their interactions as they can influence diseases (Byrd and Segre 2016). In addition, some bacterial species

responsible for new diseases might not be cultured under laboratory conditions (Austin 2017). In addition to, or instead of, morphological and biochemical tests, and, to increase the robustness of bacterial identification at a species level, 16S rDNA sequencing can be performed.

The isolation and identification of parasites is a very long, fastidious and delicate process: the whole body of the fish being observed under stereomicroscope, opened following standard protocols, then parasitic species isolated for direct morpho-anatomical observations under a microscope or after histological staining (Sures *et al.* 1999). Thus, the identification of parasites is based on the observer's knowledge, ability to distinguish morphologically similar parasites, skills in taxonomy and available literature. Nevertheless, in the case of targeted studies on known eel parasites such as *Pseudodactylogyrus spp.* and *A. crassus*, their presence can be detected more easily by targeting the examination to specific organs: the gills for the former and the swim bladder for the latter. In the case of *Pseudodactylogyrus sp.* the classical approach consists of microscopic observations of the gill filaments and the parasites themselves (morpho-anatomical recognition on the sclerotized parts involved in their fixation) (Monni *et al.* 2002). Gill biopsies can also be performed but the prevalence of parasites using this technique was lower (20%) than the prevalence found by complete microscopic observation of the entire gill arches (70%) (Larrat *et al.* 2012). For *A. crassus*, its morphological identification is quite easy, the only tricky point being the identification of L3 and L4 larval stages, very difficult to distinguish, and which is often done only according to their size (Blanc *et al.* 1992; Sures *et al.* 1999).

Thus, classical methods are easy to set up and relatively cheap but the identification of pathogens is long and may require very special qualifications as for parasite identifications. These methods have the disadvantage of, most of the time, having to sacrifice organisms. In a context of conservation of eels and optimization of eel farming as well as routine detection of pathogens, these processes appear less and less feasible. Thus, there is a need for rapid and non-invasive identification methods to quickly apply treatment after detection of pathogens and to select the appropriate treatment depending on the pathogen.

### **3.2 Antibodies detection**

Pathogens can also be detected by the presence of specific antibodies or antigens, mainly using enzyme-linked immunosorbent assay (ELISA) tests. The advantages are that these tests are relatively non-invasive since they can be performed on blood samples from eel's caudal vein and do not need a pathogen isolation to be performed. This type of detection method has been employed for detection of various types of eel pathogens. ELISA tests have been used to detect the presence of HVA antibodies in eel sera (van Nieuwstadt *et al.* 2001). They have also been used to detect Anti-*A. hydrophila* antibodies in *A. anguilla* after experimental infection. Blood samples were collected at 1, 4, 7, 14 and 28 days post-infection and the optical density of serum was read to infer the presence and amount of antibodies. Serum OD values were significantly higher since the first day post-infection, which confirmed the possibility of detecting *A. hydrophila* infection by ELISA tests (Guo *et al.* 2013). Finally, ELISA tests have been performed to detect the presence of *A. crassus* in eels (Höglund and Pilstrom 1994; Inui *et al.* 1999; Knopf *et al.* 2000). However, these tests based on *A. crassus* wall antigens performed on blood samples from European eels showed a positive predictive value but low specificity and predictive negative value (Knopf *et al.* 2000). Various types of ELISA tests exist, such as the competitive ELISA developed to detect antibodies to *A. crassus* in *A. japonica*. The positive detection rate and the false positive rate were compared with those of a conventional indirect ELISA method. They were 95% and 5% for the competitive ELISA and 80% and 20% for the indirect ELISA (Inui *et al.* 1999).

Thus, the above studies showed that ELISA tests are relatively successful and could be applied on some types of pathogens that can be viruses, bacteria or parasites. The identification and detection of a pathogen by antibodies and antigens detection have the advantages of being relatively non-invasive, easy to set up and results can be obtained rapidly. However, when using these methods, specific features must be taken into account such as specificity or the risk of false positives, especially in the case of parasites sero-diagnosis where antibodies



may cross-react (Knopf *et al.* 2000). In addition, a positive test based on antibodies detection against a specific pathogen may result from a previous infection and may not reveal a current infection. For example, HVA antibodies have been detected before an experiment, and did not increase after a recrudescence of the virus (van Nieuwstadt *et al.* 2001). Knopf *et al.* (2000) also pointed out that ELISA tests may not be applicable for *A. crassus* diagnostic purposes since in wild populations with high parasite prevalence, the currently non-infected (negative) eels could not be detected, probably because of previous contact with these parasites and of antibodies persistence in these individuals. For aquaculture purposes, the test remains very specific but needs an *a priori* idea of the type of pathogens responsible for the disease. In conclusion, ELISA tests are more applicable for management and eel immune responses studies (if the aim is to detect and follow the presence of a single pathogen), but may only be applicable in certain conditions, such as experimental studies or field survey where low prevalence of the pathogen allow the detection of the negative eels.

### **3.3 PCR amplification**

#### **3.3.1 Invasive methods**

Most of the methods mentioned above have been described as laborious and time consuming. Also, in addition to the detection issues previously listed, infections may not be detected if the amount of pathogen present in the sample is below the test's sensitivity threshold (Adams and Thompson 2011). The development of molecular techniques using Polymerase Chain Reaction (PCR) amplification of DNA can provide other possibilities of pathogen identification (Ador *et al.* 2021). PCR methods allow rapid identification of genetic material from pathogens with results available within a day or less (Coleman *et al.* 1996). Routinely deployed, PCR could allow early detection of pathogens, and appropriate actions could be quickly set up before outbreaks. Detection of various types of eel pathogens such as viruses, bacteria or parasites can be performed using PCR amplification. For example, a sensitive PCR was developed to detect HVA in eel tissue (Rijsewijk *et al.* 2005). In the same perspective, a two-step real time reverse transcriptase PCR assay has also been developed to detect EVEX virus (van Beurden *et al.* 2011). Similarly, PCR amplification has been developed to detect *V. vulnificus* in *A. anguilla* pre-infected with the bacterium (Coleman *et al.* 1996). Although PCR currently allows detection of a single pathogen per reaction, the development of Multiplex-PCR assays with the development of multiplex kits including specific primers directed against more than one pathogen could allow detection of multiple pathogens in a single reaction (Adams and Thompson 2011). To date, multiple pathogens have not yet been detected using these techniques on eels, but they have already been used to identify different types of parasites in a single reaction on goldfish (Jaruboonyakorn *et al.* 2022), different species of bacteria (*Vibrio parahaemolyticus*, *Salmonella spp.* and *Escherichia coli*) on fish products (Triwibowo *et al.* 2020).

It should be noted, however, that genetic material of pathogens may persist over time, preventing any evidence of ongoing infection or infectious agents (Hiney 2001). Classical isolations could improve PCR detections but have the disadvantage of adding steps to the detection processes. PCR applicability in the wild or in ponds is still low since it requires specific and costly machines. In addition, a first DNA extraction step is required which is time consuming, requires equipment and can be costly if kits are used. However, the democratization of these techniques could make them more accessible with regard to their cost. Finally, PCR amplification is mainly performed on pooled organs (**Table 2**) which makes this method invasive.

#### **3.3.2 Non-invasive methods**

Non-invasive methods for the detection of DNA from *A. crassus* in eel faeces have also been developed (De Noia *et al.* 2022; Jousseume *et al.* 2021). De Noia *et al.* (2022) used a pair of parasite-specific DNA primers (designed from the most conserved regions within the ten cytochrome oxidase subunit 1 (COI) available gene sequences) for PCR amplification while Jousseume *et al.* (2021) designed three new pairs of primers to amplify three *A. crassus* specific microsatellite markers to optimize specificity. In both cases the robustness of the

method was determined by comparing results with microscopic examinations of the swim bladder to check for the presence of the parasite. Time for DNA extraction and PCR amplification was relatively quick. The method could also be performed in the wild since it was highlighted that the whole machinery necessary for performing the test was small and could be used with a battery (De Noia *et al.* 2022).

### **3.4 Other non-invasive methods**

Various other less invasive approaches have been developed. A radio diagnostic based method has been used to detect inflammation on the swim bladder caused by *A. crassus* (Beregi *et al.* 1998). However, this method needs costly equipment and the success of the method is dependent on the quality of the image. Ultrasound has also been used as a detection tool for *A. crassus* but, although able to detect moderately infected animals, the technique was not sensitive enough for the diagnosis of low-infected eels. Finally, this technique could not be applied on severely infected animals (Frisch *et al.* 2016). Although non-invasive, these methods do not make it possible to give more precise information on the parasite such as its developmental stage or the presence of eggs. The color of the anal region of eels was also used as a diagnostic tool for *A. crassus* infection, with greater redness of the anal region of eels being significantly correlated with *A. crassus* infection. Thus, the use of anal redness as a rapid indicator of the infection by the parasite has been proposed with caution as anal redness can have multiple other causes and validation of the indicator is therefore required (Crean *et al.* 2003).

Finally, the detection of diseases-specific biomarkers using metabolomics could be a non-invasive alternative method since metabolomics analyses can be performed on blood, mucus or faeces samples with minimal impact on the animal. In addition, it is an inexpensive, quick and easy method to identify the presence of pathogens (Low *et al.* 2017). Metabolomics relies on the study of small molecules in an organism. Thus, any change in metabolite levels can be statistically detected between groups (Johnson *et al.* 2016). To our knowledge, this method has not yet been evaluated on eels, but metabolomics using GC-MS has already been used in survival vs death crucian carps (*Carassius auratus*) challenged with  $5 \times 10^6$  CFU/mL *E. tarda*. 67 metabolites including amino acids, carbon sources, lipids and nucleotides were found to be differentially detected in the different fish groups (Guo *et al.* 2014). Similarly, <sup>1</sup>H-NMR metabolomics was used to study the responses of metabolite expression in Atlantic salmon *Salmo salar* exposed to *A. salmonicida*. Lipids and choline-residues were metabolites that most contributed to the observed differences in the metabolite profiles of survivors, control and dead fish (Solanky *et al.* 2005). These molecules could be used as biomarkers for disease detections, however the specificity of the method is poor and does not allow a precise identification of pathogens.

The different methods listed above present a combination of advantages and disadvantages, none of them being applicable to all situations. The use of one method over another is often dependent on factors such as ease of implementation, speed of action, cost, invasiveness but also the purpose of the identification. For example, budgets allocated to projects to identify sites with the lowest prevalence of parasites for conservation purposes are different from those allocated for the identification of pathogens in aquaculture outbreaks. The following table summarizes the main factors in choosing a method over another (Table 4).

## **4. Potential treatment**

### **4.1 Modulation of abiotic parameters in anguilliculture**

Due to the interaction between pathogen virulence and abiotic factors (temperature, salinity...), the spread of the disease in aquaculture can often be controlled by a simple modification of these factors. For example, an increase or a decrease in temperature or salinity might turn off the pathogenicity of some pathogens. This practice has been successfully applied to control eels outbreaks (Møllergaard 1987).

The virulence of various eel viruses was described as temperature dependent (van Beurden *et al.* 2012). Consequently, the modulation of the rearing temperature might avoid intense mortalities. For example, the

optimal temperature for virus development were established for EVE (15°C and 23°C), for EVEX (10°C and 15°C), and for HVA (20°C and 26°C) (Shchelkunov *et al.* 1989; Davidse *et al.* 1999 and Smail and Munro 2001; in Haenen *et al.* 2009). Viruses can survive outside of these temperature ranges at a dormant state, but illness does not develop in the host (Haenen *et al.* 2009). To illustrate the impact of water temperature, mortality of fry rainbow trout infected with EVA virus was found to decrease with the decrease of the rearing temperature (26 % at 20°C, 0 % at 15°C and 2 % at 10°C) (Nishimura *et al.* 1981). The same observations were made for fry rainbow trout infected with EVEX (Nishimura *et al.* 1981). Conversely when Japanese eels were infected with a rhabdovirus (EVA/EVEX-like AM92 strain), Kobayashi *et al.* (1999) reported a maximum rate of moribund individuals and/or exhibiting cutaneous lesions for a rearing temperature of 15°C this rate decreased to zero at 25°C. From these previous studies, it appears that the temperature-dependent susceptibility of fish to a given virus may differ according to the fish species. For example, at low temperature, eels could be more susceptible to EVEX virus than trouts. In case of bacterial infection, mortality due to a disease outbreak apparently caused by *P. anguilliseptica* was controlled by increasing water temperature in a Danish eel farm (Muroga and Yano 1973; Mellergaard 1987; Ellis *et al.* 1983). Similarly, in a Scottish eel farm, an epizootic of *P. anguilliseptica* was eradicated, when raising the water temperature to 26-27°C (Stewart *et al.* 1983).

Regarding the impact of salinity on the pathogenicity of bacteria and therefore their control, it has been shown that vibriosis caused by *V. anguillarum* does not occur in freshwater (Mellergaard 1987). On the contrary, the pathogenicity of *A. hydrophila* could be inactivated by an increase of salinity (Mellergaard 1987). In general, low salinity has been associated with lower prevalence of *A. crassus* in eels (Li *et al.* 2015, Giari *et al.* 2021). This was verified in particular through a compilation of *A. crassus* prevalence data from 28 sampling sites in Europe (Giari *et al.* 2021). Lower salinity was also reported to impact hatching, survival and infectivity of *A. crassus* larvae (Kennedy and Fitch 1990; Kirk *et al.* 2000). For example, larval infectivity lasted 8 days in 100% sea water versus 80 days in freshwater. Similarly, salinity had a negative impact on hatching and survival of the larvae (Kirk *et al.* 2000). Finally, no *Pseudodactylogyrus* sp. has been reported in *A. anguilla* sampled in a marine environment with salinity between 32 and 35 (Jakob *et al.* 2009). As for *A. crassus*, a modulation of salinity could allow the control of *Pseudodactylogyrus* sp. infections.

These studies highlight the importance of global knowledge on pathogens and conditions of their development as well as the complexity in choosing the rearing conditions to optimize the growth of the fish while avoiding pathogens development. Modulating rearing conditions is a quick, easy and inexpensive way to control pathogens virulence and may be sufficient, in the short term, to stop mortalities. However, for long term efficiency, conditions may need to be maintained which may lead to additional costs in aquaculture and may provide ideal conditions for other pathogens. Finally, this practice is absolutely not applicable in the wild for conservation purposes.

#### 4.2 Antipathogenic substances administration

Another possibility to treat or prevent diseases is by chemical administration. These may include antivirals, antibiotics and anthelmintic molecules. They can be administered through food or bath treatments and usually lead to good results. Some of them also have a broad range of activity and may act on different pathogens. However, a growing number of studies have highlighted the emergence of antimicrobial resistance (Defoirdt *et al.* 2011; Romero *et al.* 2012; Santos and Ramos 2018). The susceptibility of *A. salmonicida* and *A. hydrophila* strains isolated from American eels to 18 different antibiotics has been assessed. While most antibiotics were effective against the isolates, the susceptibility of *A. hydrophila* to nitrofurazone isolated monthly varied greatly over time which could be a barrier to the use of this antibiotic in aquaculture (Davis and Hayasaka 1983). In two eel Spanish farms, the first with high and the second displaying lower densities of fish, the potential resistance of some fish pathogenic bacteria to oxolinic acid (OXA), oxytetracycline (OTC), sulfamethoxazole-trimethoprim (SXT) and nitrofurantoin (NIT) was assessed. A significant number of the isolated bacteria were

resistant to the antibiotics tested especially against SXT in the farm with high density of fish (Alcaide *et al.* 2004). This latest study highlights the limits of the use of antibiotics in eel farming in a context of high demand for eels and therefore an increase in eel production.

The efficiency of nematicidal drugs on eel platyhelminths is rather limited as they present several disadvantages. For example, drugs (such as metrifonate, fenbendazole, mebendazole, and ivermectin), used both in food and water baths to control *A. crassus*, may have solubility problems which constitute a limiting factor for treatment efficiency. No clear results (elimination of the parasites) were found although damaged worms were detected (Taraschewski *et al.* 1988). Potassium permanganate, sodium chloride, ammonia, formaldehyde and trichlorfon have been used against *Pseudodactylogyrus* sp. as reviewed in Buchmann (1987). In most cases, the intensity of the infection was reduced, but some molecules had an impact on eels: ammonia induced mortality at a certain concentration and sodium chloride induced mucus sloughing. In European eels, for example, a treatment with 0.5 and 1 mg/mL mebendazole used against *Pseudodactylogyrus* sp. infection eliminated all adults and postlarvae after 4 days but also induced an oversecretion of mucus as a side effect (Møllergaard 1990). Finally, in some cases, specimens of *Pseudodactylogyrus* sp. from European eels have shown resistance to mebendazole and flubendazole treatments while the use of praziquantel, another anthelmintic drug, has significantly reduced the prevalence and abundance of this parasite in eels (Buchmann *et al.* 2011).

These studies highlight the fact that chemical, although easy to use, efficient and with a broad range of action can induce pathogen resistances to drugs not only for bacteria but also for parasites as well as negative impacts on the fish (Buchmann *et al.* 1987). In addition to drug resistances and negative impacts on fish, the use of chemicals can lead to the release of products whose potential persistence might induce negative impact in the environment (Weston 1996; Gothwal and Shashidhar 2014; Preena *et al.* 2020). Moreover, some studies have shown that, regarding parasite infections, although effective in the first few days, stopping treatment led to a resumption of the parasitosis (increase in parasites number) (Møllergaard 1990; Geets *et al.* 1992). In summary, there is a strong need for new treatments: that are effective in the long term (or very effective in the short term and capable of reducing the presence of pathogens to zero), do not induce pathogen resistance phenomena, do not alter negatively the physiology of fish and have no impact on the environment.

#### 4.3 Vaccination

Vaccination can prevent the development of diseases. Vaccines against several species of bacteria and parasites, including *V. vulnificus*, *E. tarda*, *A. hydrophila*, *A. sobria* and *A. crassus* (Table 5) have been developed. Most of them consist of formalin-killed cells or outer membrane protein (Omp) of the pathogens (Table 5). For example, a vaccine was developed against *V. vulnificus* in Spain (Collado *et al.* 2000). After testing different types of vaccine, a final formulation consisting of a toxoid-enriched bacterin from *V. vulnificus*, inactivated with formalin and heated, was selected for its efficiency. This vaccine, called Vulnivaccine, was used in a Spanish eel farm (Fouz *et al.* 2001). Vaccination consisted in the immersion of glass eels in water containing the vaccine at three different times after the arrival of the glass eels. The immune response and protection induced by vaccination was studied by the presence of antibodies and by challenging glass eels with the pathogen. The relative percentage of survival ranged from 62% to 86% demonstrating the protective effect of the vaccination. Vulnivaccine was then administrated by oral and anal intubation, intraperitoneal injection and prolonged immersion (Esteve 2004a). Oral and anal intubation showed the best efficacy in protecting eels against vibriosis, with a cumulative mortality under 10%. Since efficacy is limited to 6 months, the possibility of reimmunization has also been studied and an oral vaccine that could be administered to any stage of eel's life has been developed (Esteve 2004b). Other outer membrane proteins (Omp)-based vaccines have been developed, the most used being OmpU, OmpA and OmpII from *V. vulnificus*, *E. anguillarum* and *A. hydrophila* respectively (Table 5). After vaccination and challenge with a given pathogen, eels vaccinated with Omp

generally had significantly higher relative survival than those vaccinated with control solution (Le *et al.* 2018; Guo *et al.* 2019; LiHua *et al.* 2019; He *et al.* 2020; Guo *et al.* 2020).

Vaccination has also been used to prevent infection and development of *A. crassus* in *A. japonica* and *A. anguilla*. *A. crassus* infective larvae (L3) were attenuated by irradiation with 135Cs and then orally administered to several groups of eels. After challenging immunized and control eels with L3 larvae, results showed that the number of *A. crassus* adults was significantly reduced in immunized *A. japonica* compared to the control. However, the treatment did not appear to be effective for *A. anguilla*. (Knopf *et al.* 2008). While most vaccines are developed to protect against a single pathogen, promising bivalent (Guan *et al.* 2011; Guo *et al.* 2019) and trivalent (Zhao *et al.* 2020) vaccines are also being developed and could protect against different pathogens.

Vaccination appears to be a good alternative to the use of chemicals, and it may prevent diseases before their apparition. However, in most studies vaccines are intraperitoneally-injected, which is difficult to implement on a larger scale and even more in natural conditions. Vaccination by immersion has shown good results and would be suitable for eel farms. The development and democratization of vaccines on a larger scale could make them even more accessible. Although vaccination has already been tested against many bacterial infections and one eel parasite, to our knowledge no vaccine has yet been developed against eel viruses. Thus, complementary studies are necessary for this purpose but also to study the long term efficiency of the protection against pathogens. The inclusion of vaccinated eels in restocking programs could be considered in the future.

#### 4.4 Diet supplementation:

Alongside vaccination and in response to the issues surrounding the use of antibiotics, plant, probiotic and other natural product derivative supplementations to enhance fish immunity and their disease resistance have shown promising results in the past leading today to their growing interest. Hundreds of studies have reported that their use in diet positively impacts fish physiology, immunity and disease resistance (Thanigaivel *et al.* 2016; Vallejos and Vidal 2016; Reverter *et al.* 2014, Reverter *et al.* 2021). By colonizing the gut, probiotics may increase host resistance to pathogens (Gatesoupe 1999). On the other hand, plants and algae, are known for their high nutritional value (proteins, B12 vitamins...) and for exhibiting several activities (antioxidant, antibacterial, antiviral...) which can protect but also optimize fish growth (Thanigaivel *et al.* 2016; Wan *et al.* 2019). Several attempts to add probiotics and/or plants to eels diet followed by pathogen challenges have also been made. These include probiotic bacteria (Chang and Liu 2002; Lee *et al.* 2013; Lee *et al.* 2017), association of a bacterium and mannooligosaccharides (Lee *et al.* 2018), alternative protein sources (García-Gallego 1998), and various other natural product derivatives (Bae *et al.* 2008; Choi *et al.* 2008; Bae *et al.* 2012; Lee *et al.* 2018; Huang *et al.* 2020) (**Table 6**).

Most of the experiments were conducted with *A. japonica*, few ones with *A. anguilla* and to our knowledge none with *A. rostrata*. They showed an increase in growth performance, e.g. Japanese eels whose diet was supplemented with  $10^7$  or  $10^8$  CFU/mL *B. subtilis* had significantly greater weight gain compared to the control group (106 and 107% vs 101% respectively) (Lee *et al.* 2017). An increase in disease resistance has also been highlighted in some studies. For example, the survival rate of *A. japonica*, challenged with *E. tarda* and fed with a supplemented diet comprising quartz porphyry and stimulants BAISM, ranged from 40 to 60% 9 days post-infection compared to 0% in control fish (Bae *et al.* 2008). **Table 6** summarizes the protocols and results of the studies in which eel diets were supplemented and challenged with pathogens to study their disease resistance.

Thus, food supplemented with probiotics or other compounds or stimulants have shown good results in strengthening eels' immune system and their disease resistance against several known eel pathogens: *V. anguillarum*, *E. tarda*, *A. hydrophila* and *P. fluorescens*. Long term studies are needed to find out whether resistance to disease decreases over time and therefore whether the supplemented diet should be applied

continuously. In terms of applicability in the wild, if the increase in disease resistance is permanent, the use of supplemented feed could be a good alternative to boost the immunity and resistance of eels that are part of restocking programs. To be suitable, the diet should be constituted of available and stable resources such as cultivable or invasive plants or algae. Furthermore, there is a necessity of data regarding disease resistance against viral and parasitic infections because to date, the first feed supplementation trials applied to eels have only focused on resistance to bacterial diseases.

Finally, as for pathogen identification, the selection of a treatment over another depends on factors such as its cost, ease of implementation, speed of action, short and long term efficiencies, but also its potential impact on the environment. The following table/figure summarizes the main factors discussed above in choosing a treatment over another (**Table 7**).

## **5. Conclusion**

While the consumption and demand for eel continue to increase, *A. japonica*, *A. anguilla* and *A. rostrata* still experience very low stocks. Some organisms, including viruses, bacteria and parasites, can be pathogenic to eels which can lead to economic losses for eel farmers and could even be a significant factor in overall populations weakening of eels.

The early detection of pathogens and their identification is therefore an essential step in the fight against diseases. Effective methods of early detection of disease could also lead to effective and relevant management plans based on knowledge of diseases (appearance, prevalence, environmental factors...) and their potential preventions. Particular attention should be paid to the management of stocks, both in the context of aquaculture and the restocking of natural populations, in order to avoid the exchange of pathogens and their introduction into new environments as in the case of *A. crassus*.

Most detection methods are invasive and results are usually obtained in more than one day. Regarding parasite detections, non-invasive methods such as radio/ultrasonic detection or detection from eel faeces have been developed, but they may not be as effective as conventional methods. Finally, the development of portable devices and multiplex kits including specific primers directed against more than one pathogen could allow detection of multiple pathogens in a single reaction and, applied on non-invasive matrices appears as the most promising pathogen detection method applicable in both wild environment and anguilliculture.

It should also be noted that many potential pathogens have been isolated from apparently healthy eels showing no signs of disease (EVE, EVA, EVEX, *E. tarda*, *A. hydrophila*, *V. anguillarum*...) emphasizing that an eel can carry pathogens without ever developing disease. Also, as methods become more sensitive, they could detect past infections, intact/fragmented or non-viable pathogen fragments. Thus the reaction to a positive result should always be accompanied by a consideration of the risk involved (risk of symptom development, risk of spread, risk of economic loss) and thus an appropriate response.

Following pathogen identification, a rapid, effective and adapted action is often necessary. In anguilliculture, modulation of abiotic parameters and antivirals, antibiotics and/or antiparasitic drugs have long time been used and are still used. However, their use is and will be reduced due to supplementary cost, drug resistance for pathogens and molecules persistence in the environment. Thus, other types of treatments that can allow the prevention of diseases are being developed. Vaccination for example shows promise especially since development of bi and tri-valent vaccines could provide one-step protection against multiple pathogens species at once. However, to date, few vaccines have been developed against eel parasites or viruses. Efforts should therefore be focused on this as well as on strengthening the data on long-term protection and the need for reimmunization or not. Finally, the incorporation of probiotics and plants in eel food is now known to enhance their immune system and make them more resistant to multiple pathogens such as *E. tarda*, *V. anguillarum* or *A. hydrophila*. However, more data on the resistance of these eels challenged with other types of bacteria, viruses and parasites,

on whether or not continued supplementation is necessary to maintain protection as well as long-term follow-ups on the protection provided by supplementation are needed to conclude on the potential of supplemented diets as a replacement for chemical use. These methods however, seem difficult to apply in the natural environment unless diet supplemented or vaccinated eels are included in restocking programmes.

To conclude, in this review, we have summarized i) the main pathogens, viruses, bacteria and parasites, of the three northern temperate eel species *A. anguilla*, *A. japonica* and *A. rostrata*, ii) the methods used to detect diseases and pathogens and, iii) the different treatments used. Finally, we have highlighted the need for non-invasive, rapid and efficient detection methods as well as effective and environmentally friendly treatments, as an essential prerequisite to be taken into account in management plans, particularly when considering endangered species, or during any action intended to preserve biodiversity.

### **Conflict of interest :**

The authors declare no conflict of interest.

### **Authors contributions :**

Conceptualization, C.P.; writing—original draft preparation, C.P.; Writing—review and editing, C.P., P.S., E.F. and N.T. All authors have read and agreed to the published version of the manuscript.

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## **Table and Figure Legends:**

**Table 1:** Potential and confirmed pathogenic viruses for eels. External symptoms and Internal abbreviations: Abd: Abdomen, Cong: congestion, Dark: Darkened, Dep: Depigmentation, F: Fins, G: Gills, Hem: Hemorrhage, In: intestine, Ki: Kidney, Les: Lesion, Li: liver, Mu: muscles, Nec: Necrosis, Pet: Petechiation, Red: Reddening, Rot: Rot, S: Skin, Sp: Spleen, Swo: Swollen, Tum : Tumor, Ulc: Ulcerations. <sup>1</sup>Lists of symptoms and damages are not intended to be exhaustive, but include commonly encountered symptoms and damages.

**Table 2:** Potential and confirmed pathogenic bacteria for eels. External symptoms and Internal Damages abbreviations: A: Anal region, Abd: Abdomen, Cong: congestion, Dark: Darkened, Dep: Depigmentation, E: Eye, Exo: Exophtalmos, F: Fins, G: Gills, He: Heart, Hem: Hemorrhage, In: intestine, Inf: inflammation, Ki: Kidney, Les: Lesion, Li: liver, M: Mouth, Mu: muscles, Nec: Necrosis, O: Opercula, Perf: Performance, Pet: Petechiation, Red: Reddening, Rot: Rot, S: Skin, Sto: Stomach, Sp: Spleen, Swo: Swollen, T: Tail, Ulc: Ulcerations, Vaso: Vasodilatation. <sup>1</sup>*Edwardsiella tarda* species were recently re-affiliated to *Edwardsiella piscida* and *Edwardsiella anguillarum* species (Bujan, 2018). <sup>2</sup>*Aeromonas sobria* was isolated from *Anguilla anguilla* but no health status was communicated. <sup>3</sup>*Delftia acidovorans* was isolated in a co-infection event with *Pseudomonas anguilliseptica* (Andree, 2013). ND: Not determined. <sup>4</sup>Lists of symptoms and damages are not intended to be exhaustive, but include commonly encountered symptoms and damages.

**Table 3:** Methods used to identify the main reported eel pathogens.

**Table 4:** Comparison of the different methods used to identify pathogens responsible for a disease. <sup>1</sup> Whether methods can be applied on Bacteria, Viruses or Parasites. <sup>2</sup> Scale based on whether a non-specialist could easily use the method. <sup>3</sup> Scale based on the estimated cost of consumables and heavy equipment <sup>4</sup>High for results obtained in few hours, Medium for more than 12 hours. <sup>5</sup> High for identification to species level, low for detection of disease state only. <sup>6,7</sup>Differences lie in the spatial scale in which to implement the method which is higher in the natural environment. <sup>8</sup> High: the use of the method results in the sacrifice of the animal, Low: low animal impact.

**Table 5:** Summary of the different vaccines developed and used to protect eels against bacteria and parasites. List of abbreviations: Omp: Outer membrane protein; FKC: Formalin-killed cells. IP: Intraperitoneal injection; I: Immersion; OA: Oral Administration; AA: Anal administration. CM: Cumulative mortality (%); RPS: Relative percentage survival (%); SR: Survival rate (%); RIPR : Relative Immune Protection Rate. Vv: *Vibrio vulnificus*; Va: *Vibrio anguillarum*; Ea: *Edwardsiella tarda/anguillarum*; Ah: *Aeromonas hydrophila*, As: *Aeromonas sobria*.

**Table 6:** Summary of methods and results obtained for diet supplementation to enhance eel disease resistance. Abbreviations: IP: Intraperitoneal injection, OA: Oral administration ND: Not determined.

**Table 7:** Comparison of the main potential treatments to control or improve disease resistance in fish. <sup>1</sup> Scale based on whether a non-specialist could easily use the method. Scale based on the estimated cost of consumables and heavy equipment. <sup>3</sup> High for complete disappearance of disease symptoms of mortality few days after administration, Medium in case of treatment more preventive than curative. <sup>4</sup> Low when use leads to unsustainable additional cost or development of other pathogens or resistant organisms, "?" for data that are still preliminary or not available. <sup>5</sup> Medium: can be applied under specific conditions (release of vaccinated or diet supplemented eels in the natural environment). <sup>6</sup> Limited: due to cost and ease of implementation.



**Figure 1:** Illustration of common symptoms of diseases: haemorrhagic fins (a,i), necrosis of the tail (b), discolored skin spots (c,e), mucus oversecretion (f,g), haemorrhagic and reddened head and mouth (h), petechiae on the body, redness and swelling of the peritoneal cavity part (d). All photos belong to the author.

**Table 1:** Potential and confirmed pathogenic viruses for eels. External symptoms and Internal abbreviations: Abd: Abdomen, Cong: congestion, Dark: Darkened, Dep: Depigmentation, F: Fins, G: Gills, Hem: Hemorrhage, In: intestine, Ki: Kidney, Les: Lesion, Li: liver, Mu: muscles, Nec: Necrosis, Pet: Petechiation, Red: Reddening, Rot: Rot, S: Skin, Sp: Spleen, Swo: Swollen, Tum : Tumor, Ulc: Ulcerations. <sup>1</sup>Lists of symptoms and damages are not intended to be exhaustive, but include commonly encountered symptoms and damages.

Type	Viruses	<i>Anguilla</i> sp. affected	Disease name	External symptoms <sup>1</sup>	Internal Damages <sup>1</sup>	Source
Alloherpesvirus	Anguillid herpesvirus 1 (HVA/AngHV-1)	All three species	-	S/Hem,Nec ; F/Hem; G/Hem, Nec; Abd/Red	Li/Hem; Sp/Swo,Hem,Nec; Ki/Swo;	Békési 1986; Sano 1990; Haenen 2009; van Beurden 2010; Kempter 2014
Aquabirnavirus	Eel Virus European (EVE)	<i>A. anguilla</i> , <i>A. japonica</i>	Branchionephritis	S/Hem; Head/Cong; G/Swo/Cong	Glomerulonephritis	Egusa 1970; Haenen 2009
	Infectious pancreatic necrosis (IPN) viruses	<i>A. anguilla</i>	Infectious pancreatic necrosis	Overall Darkening; Abd/Swo,Hem;	Li/Dep; Sp/Dep;	Hnath 1983; Jørgensen 1993; Varvarigos 2011
Rhabdovirus	Rhabdovirus Eel Virus America (EVA)	<i>A. rostrata</i>	Rhabdoviral dermatitis	Lethargy; Anorexia; S/Les,Hem ; Head/Red; F/Cong; Abd/Cong	Ki/Hem,Nec; Mu/Hem,Nec; Li/Hem,Nec; Pancreas/Hem,Nec	Sano 1976; Sano 1977; Castric 1980; Castric 1984; Jørgensen 1993; Kobayashi 1996; Haenen 2009; Galiniér 2012; Haenen 2012; Bellec 2014
	Eel Virus European X (EVEX)	<i>A. anguilla</i> , <i>A. japonica</i>				
	Haemorrhagic septicaemia (VHS) viruses	<i>A. anguilla</i>	Haemorrhagic septicaemia	-	-	Castric 1992; Jørgensen 1993
Polyomavirus-like	Japanese eel endothelial cells-infecting virus (JEECV)	<i>A. japonica</i>	Viral endothelial cell necrosis (VECNE)	G/Cong,Dilatation, Abnormalities; F/Red; Abd/Swo	Li/Cong; In/Cong	Ono 2003; Okazaki 2015; Okazaki 2016a,b; Kim 2018
Orthomyxovirus	EV-1	<i>A. anguilla</i> , <i>A. rostrata</i>	Stomatopapillomatos	-	-	Wolf 1973
	EV-2	<i>A. anguilla</i>		-	-	Nagabayashi 1979; Neukirch 1985

**Table 2:** Potential and confirmed pathogenic bacteria for eels. External symptoms and Internal Damages abbreviations: A: Anal region, Abd: Abdomen, Cong: congestion, Dark: Darkened, Dep: Depigmentation, E: Eye, Exo: Exophtalmos, F: Fins, G: Gills, He: Heart, Hem: Hemorrhage, In: intestine, Inf: inflammation, Ki: Kidney, Les: Lesion, Li: liver, M: Mouth, Mu: muscles, Nec: Necrosis, O: Opercula, Perf: Performance, Pet: Petechiation, Red: Reddening, Rot: Rot, S: Skin, Sto: Stomach, Sp: Spleen, Swo: Swollen, T: Tail, Ulc: Ulcerations, Vaso: Vasodilatation. <sup>1</sup>*Edwardsiella tarda* species were recently re-affiliated to *Edwardsiella piscida* and *Edwardsiella anguillarum* species (Bujan, 2018). <sup>2</sup>*Aeromonas sobria* was isolated from *Anguilla anguilla* but no health status was communicated. <sup>3</sup>*Delftia acidovorans* was isolated in a co-infection event with *Pseudomonas anguilliseptica* (Andree, 2013). ND: Not determined. <sup>4</sup>Lists of symptoms and damages are not intended to be exhaustive, but include commonly encountered symptoms and damages.

Bacteria Species	<i>Anguilla</i> sp. affected	Disease	Isolated from diseased eels	Experiment to support pathogenicity against eels	LD50 (intraperitoneal injection)	External symptoms <sup>4</sup>	Internal Damages <sup>4</sup>	Source
<i>Aeromonas aquariorum</i>	<i>A. japonica</i>		Yes	ND	-	ND	ND	Yi 2013
<i>Aeromonas bestiarum</i>	<i>A. anguilla</i>	-	Yes	Yes	$3.3 \times 10^6$ – $2.3 \times 10^7$ cfu.fish <sup>-1</sup>	F/Red, T/Nec		Esteve 2009
<i>Aeromonas caviae</i>	<i>A. japonica</i>	-	Yes	ND	-	ND	ND	Yi 2013
<i>Aeromonas hydrophila</i>	All three species	Red fin disease	Yes	Yes	$10^5$ to $10^{7.5}$ cfu.fish <sup>-1</sup> , $10^{6.2}$ to $10^{7.4}$ cfu.fish <sup>-1</sup> , $3.3 \times 10^{6-2.3} \times 10^7$ cfu.fish	S/Pet, S/Ulc	Global Hem, Septicemia	Rickards 1978; Davis 1983; Hah 1984; Esteve 1993; Esteve 1995; Esteve 2009; Zhang 2010; Hossain 2011b; Joh 2013
<i>Aeromonas jandaei</i>	<i>A. anguilla</i> , <i>A. japonica</i>	-	Yes	Yes	$10^{5.4}$ to $10^{7.5}$ cfu.fish <sup>-1</sup> , $3.3 \times 10^6$ to $2.3 \times 10^7$ cfu.fish <sup>-1</sup> , $10^{6.6}$ cfu.fish <sup>-1</sup>	T/Ulc	ND	Esteve 1993; Esteve 1995; Esteve 2009; Hossain 2011b
<i>Aeromonas salmonicida</i>	<i>A. rostrata</i>	Furunculosis	Yes	Yes	-	skin lesion with softened and haemorrhagic dermis centres	ND	Hayasaka 1981; Davis 1983
<i>Aeromonas sobria</i>	<i>A. anguilla</i>	-	? <sup>2</sup>	Yes	$2 \times 10^7$ cfu.fish <sup>-1</sup> , $>10^{7.9}$ cfu.fish <sup>-1</sup>	ND	ND	Esteve 1993; Guan 2010; Hossain 2011b
<i>Aeromonas veronii</i>	<i>A. japonica</i>		Yes	Yes	$2.15 \times 10^7$ cfu.mL <sup>-1</sup>	ND	ND	Songlin 2012; Joh, 2013
<i>Citrobacter freundii</i>	<i>A. japonica</i>	Tail rot disease	Yes	Yes	$5.62 \times 10^5$ cfu.mL <sup>-1</sup>	T/Rot, F/Rot	ND	Joh 2013; Cao 2016
<i>Delftia acidovorans</i> <sup>3</sup>	<i>A. anguilla</i>	-	Yes	ND	-			Andree 2013

<i>Edwardsiella tarda/piscida/anguillarum</i> <sup>1</sup>	All three species	Edwardsiellosis	Yes	Yes	4.55×10 <sup>4</sup> cfu.g <sup>-1</sup> , between 10 <sup>4.85</sup> to 10 <sup>6.83</sup> cfu.fish <sup>-1</sup> , 1.5×10 <sup>4</sup> to 7.4×10 <sup>5</sup> cfu.fish <sup>-1</sup>	S/Dep,Sw,Les; G/Dep,Ulc; E/Sw; mucus oversecretion,posterior part of the body paralysis	Li/Hem, Sp/Ulc, St/Ulc, Septicemia	Wakabayashi 1973; Miyazaki 1976a ,b ,c; Hah 1984; Nakai 1985; Chang 2002; Alcaide 2006; Mohanty 2007; Esteve 2009; Chen 2011; Joh 2011; Joh 2013; Guo 2013
<i>Flavobacterium columnare</i>	<i>A. anguilla</i>	Columnaris	Yes	Yes	ND	G/nec,sw, hyperplastic gill epithelium, partial or total fusion of secondary gill lamellae	He/Perf,	Egusa 1989; Foscarini 1988; Alvarado 1989
<i>Flavobacterium psychrophilum</i>	<i>A. anguilla</i>	Bacterial Cold Water	Yes	ND	ND	F/Hem; Mu/hem; A/Blister;	In/Inf	Lehmann 1991; Soares 2019
<i>Lactococcus garvieae</i>	<i>A. japonica</i>	Lactococcosis	Yes	ND	-	E/Exo, O/Pet, F/Cong	In,Li,Sp,Ki/Cong,Hem	Kusuda 1991; Vendrell 2006
<i>Mycobacterium marinum</i>	<i>A. anguilla</i>	Mycobacteriosis						
<i>Pleisomonas shigelloides</i>	<i>A. japonica</i>	-	Yes	Yes	> 10 <sup>8.5</sup> cfu.fish <sup>-1</sup>	ND	ND	Esteve 1993; Joh 2013
<i>Pseudomonas anguilliseptica</i>	<i>A. japonica, A. anguilla</i>	Red spot disease/Sekiten-byo	Yes	Yes	ND	M/Pet,Hem; O/Pet,Hem; F/Red; G/Cong,Vaso	Li/Pet,Cong, Licapsule/Nec, Ki/Cong,Vaso	Wakabayashi 1972; Nakai 1982; Ellis 1983; Wiklund 1990; Michel 1992; Haenen 2001; Andree 2013; Joh 2013
<i>Pseudomonas fluorescens</i>	All three species	-	Yes	Yes	10 <sup>7.3</sup> cfu.fish <sup>-1</sup>	S/Pet, S/Ulc	-	Davis 1983; Esteve 1993; Hossain 2013
<i>Vibrio anguillarum</i>	<i>A. anguilla</i>	Vibriosis	Yes	Yes	10 <sup>7.3</sup> cfu.fish <sup>-1</sup>	S/Ulc, S/Pet	Sp/Sw,Cong ; Global Hem, Septicemia	Hah 1984; Mellergaard 1987; Esteve 1993; Frans 2011
<i>Vibrio harveyi</i>	<i>A. rostrata</i>	Vibriosis	Yes	Yes	1.67x10 <sup>3</sup> cfu.g <sup>-1</sup>	S/Ulc, S/Pet	Li/Nec, Ki/Swo, Hem	Wan 2021
<i>Vibrio vulnificus</i>	All three species	Vibriosis	Yes	Yes	<3.6x10 <sup>3</sup> cfu.fish <sup>-1</sup> , 2.6x10 <sup>1</sup> to 1.4x10 <sup>4</sup> cfu.fish <sup>-1</sup> , <9.4 x 10 <sup>3</sup> to 2.3x10 <sup>5</sup> cfu.fish <sup>-1</sup>	M/Red, F/Red, A/Ulc	Global Hem, Mu/Nec, Septicemia	Amaro 1995; Amaro 1996; Høi 1998; Dalsgaard 1999; Esteve 2007; Esteve 2009
<i>Yersina ruckeri</i>	<i>A. japonica</i>	Enteric redmouth disease	Yes	Yes	ND	S/Dep; Abd/Red; G/Ret	Li/Red, Ki/Dark	Joh 2010; Joh 2013

**Table 3:** Methods used to identify the main reported eel pathogens.

Pathogen	Detection method	Matrice	Source		
Viruses	Anguillid herpesvirus 1 HVA/Ang HV-1	Cell culture on specific cell-line RTG2, FHM, and EK-1 followed by electron microscopy identification and eventual seroneutralization	pooled organs	van Nieuwstadt 2001; van Ginneken 2004; Rijsewijk 2005	
		ELISA test	blood	van Nieuwstadt 2001	
		PCR	pooled organs, gill fragments	Rijsewijk 2005; Kempter 2014	
		Real-time PCR	organ suspension and infected cell cultures	van Beurden 2016	
		PCR, nested PCR	pooled organs	Nguyen 2017	
	Eel Virus European X EVEX	Cell culture on specific cell-line RTG2, FHM, and EK-1; EPC, BF-2 followed by electron microscopy identification	pooled organs	van Ginneken 2004; Caruso 2014	
		ELISA test	infected cell cultures/whole fish/viscera/fish without heads, tails and musculature	Dixon 1984	
		Real time RT-PCR	infected cell cultures and pooled organs	van Beurden 2011	
	Eel Virus European EVE	Duplex real time RT-PCR	pooled organs	McConville 2018	
		Cell culture on specific cell-line RTG2, FHM and EK-1 followed by electron microscopy identification	pooled organs	van Ginneken 2004	
Bacteria	<i>Vibrio vulnificus</i>	Duplex real time RT-PCR	infected cell cultures/ pooled organ	McConville 2018	
		Cell culture cellobiose-colistin-polymyxin B plates	mucus, gills, and intestinal contents	Høi 1998	
		Antibody detection	blood	Le 2018	
	<i>Edwardsiella tarda/anguillarum</i>	PCR	whole eel, liver and kidney tissues	Colleman 1996	
		Cell culture TSA supplemented with sodium chloride	organs/damaged tissues	Austin 2016	
		Indirect ELISA	blood	Iida 1991	
		PCR	pooled organs	Lee 2013	
	<i>Pseudomonas anguilliseptica</i>	Cell culture TSA, BHIA and TCBS, horse blood or nutrient agar containing 0.5 % (w/v) sodium chloride	blood/liver/spleen/kidney/damaged tissues	Wakabayashi 1972; Haenen 2000; Andree 2013	
	Parasites	<i>Anguillicola crassus</i>	Microscopic examination	dissected swimbladder	Sures 1999
			Competitive ELISA	blood	Inui 1998
Indirect ELISA			blood	Beregi 1998	
Radio detection			swim bladder	Frisch 2016	
Ultrasound			swim bladder	De Noia 2020;	
PCR		faeces	Jousseume 2021		
<i>Pseudodactylogyus spp.</i>		Microscopic examination	gill filaments, entire gill arches	Monni 2002; Larrat 2012	

**Table 4:** Comparison of the different methods used to identify pathogens responsible for a disease. <sup>1</sup> Whether methods can be applied on Bacteria, Viruses or Parasites. <sup>2</sup> Scale based on whether a non-specialist could easily use the method. <sup>3</sup> Scale based on the estimated cost of consumables and heavy equipment <sup>4</sup>High for results obtained in few hours, Medium for more than 12 hours. <sup>5</sup> High for identification to species level, low for detection of disease state only. <sup>6,7</sup>Differences lie in the spatial scale in which to implement the method which is higher in the natural environment. <sup>8</sup> High: the use of the method results in the sacrifice of the animal, Low: low animal impact.

<b>Method</b>	<b>Pathogens<sup>1</sup></b>	<b>Easy to implement<sup>2</sup></b>	<b>Cost<sup>3</sup></b>	<b>Rapidity<sup>4</sup></b>	<b>Specificity of identification<sup>5</sup></b>	<b>Application in natural environment<sup>6</sup></b>	<b>Application in aquaculture<sup>7</sup></b>	<b>Invasiveness<sup>8</sup></b>
<i>Classic isolation (from organs)</i>	Bacteria, Viruses	Relatively High	Relatively low	Medium	Relatively high	Limited	Medium	High
<i>Classic isolation (from wounds)</i>	Bacteria, Viruses	Relatively High	Relatively low	Medium	Relatively high	Limited	Medium	Medium
<i>Classic isolation (parasites)</i>	Parasites	Relatively high	Low	Relatively High	High	Limited	Medium	High
<i>Antibodies (blood from caudal vein)</i>	All	Medium	Relatively High	Medium	Relatively High	Limited	Limited	Relatively low
<i>PCR amplification (from organs)</i>	All	Medium	High	High	High	Limited	Limited	High
<i>PCR amplification (from faeces)</i>	<i>A. crassus</i>	Medium	High	High	High	Medium	Limited	Relatively Low
<i>Radio detection/Ultrasounds through eel</i>	<i>A. crassus</i>	Medium	High	High	Low	Medium	Medium	Low
<i>Detection of diseases-specific biomarkers</i>	All	Medium	Medium	Medium	Low	Medium	Medium	Low

**Table 5:** Summary of the different vaccines developed and used to protect eels against bacteria and parasites. List of abbreviations: Omp: Outer membrane protein; FKC: Formalin-killed cells. IP: Intraperitoneal injection; I: Immersion; OA: Oral Administration; AA: Anal administration. CM: Cumulative mortality (%); RPS: Relative percentage survival (%); SR: Survival rate (%); RIPR : Relative Immune Protection Rate. Vv: *Vibrio vulnificus*; Va: *Vibrio anguillarum*; Ea: *Edwardsiella tarda/anguillarum*; Ah: *Aeromonas hydrophila*, As: *Aeromonas sobria*.

Vaccination against	<i>Anguilla</i> sp.	Type of vaccine	Method of vaccination	Efficiency (Measured Parameter)	Efficiency	Source
<i>Monovalent vaccine</i>						
<i>Ah</i>	<i>A. anguilla</i>	Omp (46kD maltoporin) of <i>Ah</i>	IP	CM	<i>Ah</i> B10: 45% (Control), 20% (FKC), 10% (Omp); <i>Ah</i> B11: 95% (Control), 50% (FKC), 35% (Omp); <i>Ah</i> B15: 35% (Control), 0% (FKC), 0% (Omp); <i>Ah</i> B19: 22% (Control), 0% (FKC), 0% (Omp); <i>Ah</i> B20: 100% (Control), 32% (FKC), 10% (Omp);	Feng 2017
<i>Ah</i>	<i>A. anguilla</i>	OmpF and rOmpK of <i>Ah</i>	IP	RIPR	35.5% (OmpF); 70% (OmpK)	Zhang 2019
<i>Ea</i>	<i>A. japonica</i>	Eel or rabbit hyperimmune anti <i>Ea</i> sera	I	SR	<25% (Control), 30 to 50% (Anti <i>Ea</i> sera)	Kusuda 1991
<i>Ea</i>	<i>A. japonica</i>	FKC or sonicated products (SP) of <i>Ea</i>	OA	RPS	<10% (Control), 75% (FKC), 25% (sonicated products)	Salati 1991
<i>Ea</i>	<i>A. japonica</i>	Egg yolk IgY anti <i>Ea</i>	OA		Absence of <i>Ea</i> in the intestine	Gutierrez 1993
<i>Ea</i>	<i>A. japonica</i>	FKC or lipopolysaccharide (LPS) of <i>Ea</i>	IM	CM	Trial 1: 100% (Control), 87.5% (FKC); Trial 2: 80% (Control), 60% (FKC); Trial 1: 90% (Control), 57% (LPS); Trial 2: 80% (Control), 40% (LPS)	Gutierrez 1994
<i>Ea</i>	<i>A. japonica</i>	Formalin, pressure and electric current inactivated bacterin of <i>Ea</i>	IP	SR	0% (Control), 70% (Formalin), 85% (Pressure), 38% (Electric current)	Hossain 2009
<i>Ea</i>	<i>A. japonica</i>	Formalin, formalin with heat, heat, potassium chloride, tannic acid, citric acid, pressure and electric current killed cells of <i>Ea</i>	IP	CM	>70% (Control), 25% (Formalin), 45% (Formalin with heat), 65% (Heat), ND (potassium chloride), ND (tannic acid), 65% (citric acid), <10% (pressure), 60% (electric current killed cells)	Hossain 2011a

<b><i>Ea</i></b>	<i>A.japonica</i>	Formalin, formalin with heat, citric acid, pressure and electric current inactivated cells of <i>Ea</i>	IP	CM	70% (Control), 10% (Pressure), 30% (Formalin), 50% (Formalin heat), >60% (Electric current and citric acid)	Hossain 2012
<b><i>Ea</i></b>	<i>A. anguilla</i>	<i>Ea</i> ghosts	IP, I, OA	RPS	<i>Ea</i> ghosts: 75% (IP), 52.5% (IM), 37.5% (OA); Formalin killed cells: 55% (IP), 40% (IM), 32.5% (OA)	Li 2014
<b><i>Ea</i></b>	<i>A.japonica</i>	FKC of <i>Ea</i>	I, OA	RPS	67.4% (Control), 64% (Immersion 5mg/mL), 25.2% (Immersion 10 mg/mL), 58.5% (Oral 10mg/g), 65.1% (Immersion (5 mg/mL) + Oral (10 mg/g)), 32.2% (Immersion (10 mg/mL) + Oral (10 mg/g))	Jung 2015
<b><i>Ea</i></b>	<i>A.japonica</i>	OmpA of <i>Ea</i>	IP	CM	90% (Control), <20% (Omp)	LiHua 2019
<b><i>Ea</i></b>	<i>A.japonica</i>	FKC of <i>Ea</i> starch hydrogel-based oral (SHO)	OA	SR	<50% (Control), 75% (SHO1), >80% (SHO4 and 8)	Jun 2020
<b><i>Ea</i></b>	<i>A.japonica</i>	OmpA of <i>Ea</i>	IP	CM	90% (Control), <20% (Omp)	He 2021
<b><i>Vv</i></b>	<i>A. anguilla</i>	Formalin (with or without heating (H) inactivated whole cells bacterin with (WCB) or without toxoids (TWCB); + opaque (o) or translucent cells (t) attenuated live vaccines (LCV) and purified lipopolysaccharides (LPSV) = WCBFo, WCBFt, WCBHo, WCBHt, TWCBHo, TWCBHt	IP and I	RPS	ND (Control), 79.6% (WCBFo), 54.9% (WCBFt), 74.6% (WCBHo), 25.5% (WCBHt), 92.7% (TWCBHo), 49.2% (TWCBHt), ND (LCV), 2.72% (LPSV)	Collado 2000
<b><i>Vv</i></b>	<i>A. anguilla</i>	Vulnivaccine (FKC and extracellular products = toxoid-enriched bacterin)	I	% bacterial survival in surface mucus	300% (Control), 75% (Vaccinated eels)	Fouz 2001
<b><i>Vv</i></b>	<i>A. anguilla</i>	Vulnivaccine (FKC and extracellular products = toxoid-enriched bacterin) Frozen (FV) or lyophilised (LV)	I	RPS	65% (FV), 75% (LV)	Esteve-Gassent 2004b
<b><i>Vv</i></b>	<i>A. anguilla</i>	Omp-ISCOMs of <i>Vv</i>	IP	RIPR	100% (200µg/ml), 87.5% (100µg/ml), 75% (50µg/ml), 50% (25µg/ml)	Xu 2012
<b><i>Vv</i></b>	<i>A.japonica</i>	OmpU of <i>Vv</i> or FKC of <i>Vv</i>	IP	RPS	0% (Control), 80% (Omp) , 60% (FKC)	Le 2018



<i>A. crassus</i>	<i>A. anguilla</i> , <i>A. japonica</i>	Infective larvae (L3) attenuated with 135Cs irradiation	OA	-	<i>A. japonica</i> : number of parasites significantly decreased. <i>A. anguilla</i> : no decrease	Knopf 2008
<b>Bivalent vaccine</b>						
<i>Ah, As</i>	<i>A. anguilla</i>	OmpG of <i>Ah</i>	IP	SR	<i>As B10</i> : 40% (Control), 85% (Omp); <i>Ah B27</i> : 30% (Control), 60% (Omp); <i>Ah B33</i> : 15% (Control), 75% (Omp)	Guan 2011
<i>Ea, Ah</i>	<i>A. rostrata</i>	Omp with porin II of <i>Ah</i> and OmpS2 of <i>Ea</i> or bivalent FKCs of <i>Ah</i> and <i>Ea</i>	IP	RPS	<i>Ah</i> : 0% (Control), 50% (FKC), 50% (Omp); <i>Ea</i> : 0% (Control), 50% (FKC), 37.5% (rOmp)	Guo 2013
<i>Vv, Ah</i>	<i>A. rostrata</i>	Omp with OmpU of <i>Vv</i> and porinII of <i>Ah</i> or bivalent FKCs of <i>Vv</i> and <i>Ea</i>	IP	CM	<i>Vv</i> : 100% (Control), 60% (FKC), 50% (Omp); <i>Ah</i> : 100% (Control), 37.5% (FKC), 50% (Omp)	Guo 2015
<i>Ea, Vv</i>	<i>A. japonica</i>	Omp with Omp A of <i>Ea</i> and Omp U of <i>Vv</i> or bivalent FKCs of <i>Ea</i> and <i>Vv</i>	IP	SR	<i>Ea</i> : 50% (Control), 100% (Omp); <i>Vv</i> : 40% (Control), 83% (Omp)	Guo 2019
<i>Vv</i>	<i>A. anguilla</i>	Vulnivaccine (FKC and extracellular products = toxoid-enriched bacterin)	IP, I, OA, AA	CM	<i>Vv</i> serovar E: >60% (Control), 35% (I), 10% (IP), 0% (OA), <10% (AA); <i>Vv</i> serovar A: 60% (Control), 37% (I), 0% (IP), <10% (OA), 0% (AA)	Esteve-Gassent 2004a
<i>Ea, Vv, Ah</i>	<i>A. anguilla</i>	Omp with OmpA of <i>Ea</i> and OmpII of <i>Ah</i>	IP	CM	<i>Ah</i> : 60% (Control), 50% (Freund's incomplete adjuvant), 10% (Omp); <i>Ea</i> : 90% (Control), 80% (Freund's incomplete adjuvant), 40% (Omp); <i>Vv</i> : 90% (Control), 80% (Freund's incomplete adjuvant), 50% (Omp);	Guo 2020
<b>Trivalent vaccine</b>						
<i>Ea, Vv, Ah</i>	<i>A. japonica</i>	Omp with OmpU of <i>Vv</i> , OmpA of <i>Ea</i> , OmpII of <i>Ah</i> or trivalent FKCs of <i>Vv</i> , <i>Ea</i> and <i>Ah</i>	IP	CM	<i>Ah</i> : 60% (Control), 50% (Freund's incomplete adjuvant), 20% (Omp); <i>Vv</i> : 90% (Control), 80% (Freund's incomplete adjuvant), 50% (Omp); <i>Ea</i> : 90% (Control), 80% (Freund's incomplete adjuvant), 70% (Omp);	He 2020

<b><i>Ea, Vv, Ah</i></b>	<i>A. japonica</i>	Omp with OmpU of <i>Vv</i> , OmpA of <i>Ea</i> , OmpII of <i>Ah</i> or trivalent FKCs of <i>Vv</i> , <i>Ea</i> and <i>Ah</i>	IP	CM	<i>Vv</i> : B88 100% (Control), 90% (FKC), 80% (Omp); <i>Ea</i> : B79 >90% (Control), 80% (FKC), 50% (Omp); <i>Ah</i> : B11 90% (Control), 80% (FKC), 50% (Omp)	Zhao 2020
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**Table 6:** Summary of methods and results obtained for diet supplementation to enhance eel disease resistance. Abbreviations: IP: Intraperitoneal injection, OA: Oral administration ND: Not determined.

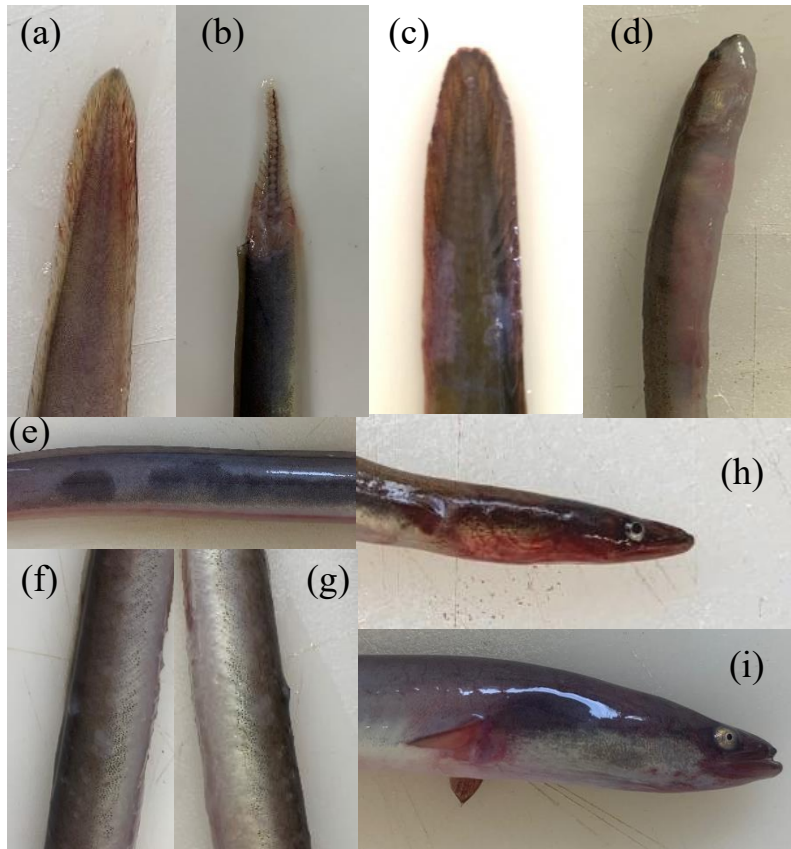
Diet supplemented with	<i>Anguilla</i> sp.	Weight (g)	Administration Dose	Duration (weeks)	Challenge pathogens	Pathogen administration	Dose for bacterial infection (CFU.mL <sup>-1</sup> )	Mortality recorded for (days)	Efficiency against disease (significant survival rates)	Reisolation from infected fish	Source
<b>Probiotics</b>											
<i>Enterococcus faecium</i> SF68 or <i>Bacillus toyoi</i>	<i>A. anguilla</i>	30	Food 1x10 <sup>-2</sup> g/mL sprayed over 1000 g	2	<i>Edwardsiella tarda</i>	OA	1% v/bw 7x10 <sup>8</sup>	14	Yes with <i>E. faecium</i> No with <i>B. toyoi</i>	ND	Chang 2002
<i>Lactobacillus pentosus</i> PL11	<i>A. japonica</i>	25.62 ± 2.54	Food 10 <sup>8</sup> cfu.g <sup>-1</sup>	4	<i>Edwardsiella tarda</i>	IP	0.1 mL of 3.5 × 10 <sup>8</sup>	7	No	Yes	Lee 2013
<i>Bacillus subtilis</i> WB60 (BS) and <i>Lactobacillus plantarum</i> KCTC3928 (LP)	<i>A. japonica</i>	8.29 ± 0.06	Food BS or LP at 10 <sup>6</sup> CFU/g diet at 10 <sup>7</sup> CFU/g diet at 10 <sup>8</sup> CFU/g diet	8	<i>Vibrio anguillarum</i>	IP	0.1 mL of 5 × 10 <sup>7</sup>	10	Yes	Yes	Lee 2017
<i>Bacillus subtilis</i> WB60 (BS) and mannaoligosaccharide (M)	<i>A. japonica</i>	9.00 ± 0.11	Food Combinaison of BS at : 0.0 × 10 <sup>7</sup> CFU/g diet, or 0.5 × 10 <sup>7</sup> CFU/g diet, or 1.0 × 10 <sup>7</sup> CFU/g diet, and M at : 0 or 5 g/kg diet	8	<i>Vibrio anguillarum</i>	IP	0.1 mL of 5 × 10 <sup>7</sup>	10	After 10 days survival range 40- 60% vs <25% control	ND	Lee 2018
<b>Natural product derivatives</b>											
korean mistletoe extract	<i>A. japonica</i>	200	Food 0.1, 0.5, 1%	2	<i>Aeromonas hydrophila</i>	IP	0.1 mL of 3x10 <sup>6</sup>	14	11 days PI: 26,6% (control), 33,3, 66,6 80 (0.1, 0.5, 1%).	ND	Choi 2008
quartz porphyry (QP) and feed stimulants BAISM (BS)	<i>A. japonica</i>	15 ± 0.3	Food 0.7% QP+0,0.3,0.5,0.75,1% BS	8	<i>Edwardsiella tarda</i>	IP	0.1 mL of 1x10 <sup>6</sup>	15	Yes after 4 days PI for all	ND	Bae 2008
propolis	<i>A. japonica</i>	7.7 ± 0.22	Food 0.25, 0.5, 1, 2, 4%	12	<i>Edwardsiella tarda</i>	IP	0.1 mL of 3x10 <sup>6</sup>	10	Significant differences in the first 3 days PI	ND	Bae 2012

yellow loess	<i>A. japonica</i>	11.5 ± 0.4	Food, 5, 10, 20 g/kg	20	<i>Edwardsiella tarda</i>	IP	0.1 mL of 2x10 <sup>7</sup>	14	Relative per cent survival higher in diet supplemented groups (ctrl: 0%, 21 to 38% for diets)	Yes	Lee 2018
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**Table 7:** Comparison of the main potential treatments to control or improve disease resistance in fish. <sup>1</sup> Scale based on whether a non-specialist could easily use the method. Scale based on the estimated cost of consumables and heavy equipment. <sup>3</sup> High for complete disappearance of disease symptoms of mortality few days after administration, Medium in case of treatment more preventive than curative. <sup>4</sup> Low when use leads to unsustainable additional cost or development of other pathogens or resistant organisms, "?" for data that are still preliminary or not available. <sup>5</sup> Medium: can be applied under specific conditions (release of vaccinated or diet supplemented eels in the natural environment). <sup>6</sup> Limited: due to cost and ease of implementation.

Method	Easy to implement <sup>1</sup>	Cost <sup>2</sup>	Short term Efficiency <sup>3</sup>	Long term Efficiency <sup>4</sup>	Application in natural environment <sup>5</sup>	Application in aquaculture <sup>6</sup>	Main risk	Tested against		
								Viruses	Bacteria	Parasites
<b>Modulation of abiotic parameters</b>	High	Low	Relatively High	Low	No	Medium	Trade/off growth/ elimination pathogen	Yes	Yes	Yes
<b>Antipathogenic substances</b>	High	Medium	High	Low	No	Medium	Resistance and environmental impact	Yes	Yes	Yes
<b>Vaccination</b>	Medium	High	Medium	?	Medium	Limited	Weak response Inefficiency on all fishes	No	Yes	Yes
<b>Diet supplementation</b>	Medium	High	Medium	?	Medium	Limited	Inefficiency against all pathogens Weak response	No	Yes	No



**Figure 1:** Illustration of common symptoms of diseases: haemorrhagic fins (a,i), necrosis of the tail (b), discolored skin spots (c,e), mucus oversecretion (f,g), haemorrhagic and reddened head and mouth (h), petechiae on the body, redness and swelling of the peritoneal cavity part (d). All photos belong to the author.