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Cases of White Spot Disease (WSD) in European shrimp farms

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ABSTRACT

White Spot Disease (WSD) caused by White Spot Syndrome Virus (WSSV) is listed as 'non-exotic' to the European Union in EC Directive 2006/88. Two other viral diseases (Taura Syndrome and Yellowhead Disease) are listed as exotic. Despite the listing of WSD as a non-exotic disease, definitive case reports have not been officially reported, or published in the peer-reviewed literature. Here we report on a series of outbreaks of WSD in three European Union (EU) Member States (Greece, Italy and Spain) and in one non-EU European country (Turkey) over the period 1995 to 2001. Samples were submitted by industry representatives over this period and were therefore not officially reported to the Competent Authorities of respective European Member States. At least one of the cases appeared to be associated with the feeding of imported shrimp carcasses from Asia to broodstock while other cases were associated with the importation of post-larvae between hatcheries and on-growing facilities from outside of Europe and further movements within Europe. These case reports demonstrate the ability for WSSV to cause disease and mortality in penaeid shrimp farmed at European ambient temperatures. Furthermore, they demonstrate potential for the introduction of WSSV to new geographic areas via the movement of live crustaceans and their products, both from outside of the EU, and between EU Member and non-member countries within the European region.

1. Introduction

Alongside several diseases of finfish and molluscs, the crustacean diseases White Spot Disease (WSD) caused by White Spot Syndrome Virus (WSSV), Yellowhead Disease (YHD) caused by viruses in the Yellowhead virus complex, and Taura Syndrome (TS) caused by Taura Syndrome Virus (TSV), have recently been listed in European Community Directive 2006/88/EC. The listing of these diseases recognises their global importance in causing significant economic losses and the potential for their international transfer via trans-boundary trade in live crustaceans and their products. An important aspect of the Directive for both exporting nations (3rd countries) and importing nations (European Union Member States) requires recognition of the fact that some of the listed diseases are considered 'exotic' to the European Union (EU) whilst others, with limited or widespread distribution within Member States are deemed 'non-exotic' (Stentiford et al., 2010). In the case of the crustacean diseases, YHD and TS are currently considered exotic to the EU due to their apparent absence and a lack of report of the presence in farmed or wild susceptible species in aquatic habitats of the EU. Conversely, WSD is listed as 'non-exotic' to the EU, despite a lack of published or official reports to confirm the current or historic presence of the causative pathogen (WSSV) in EU aquatic habitats. The listing of WSD as a non-exotic disease appears to be based upon its anecdotal occurrence in penaeid shrimp farms in southern Europe during the late 1990's (see Stentiford et al., 2009 for context). However, to date, these cases descriptions have not been reported in the peer-reviewed literature. In this paper we summarise the available diagnostic data relating to samples of cultured European penaeid shrimp submitted to an OIE Reference Laboratory for WSSV during the 1990's. This period preceded adoption of EC Directive 2006/88/EC. Furthermore, apart from crayfish plague, crustacean diseases were not listed in previous European legislation dealing with finfish and molluscan health (EC Directive 91/67). The description of WSD in penaeid shrimp sampled from European culture facilities confirms the past presence of WSD in susceptible species within the EU. More recent submissions of farmed penaeid shrimp sampled from farms within the EU, to the OIE Reference Laboratory for WSSV (after 2001), have demonstrated an absence of WSSV. Whilst not making assumptions on the current status for WSD of specific EU Member States, or other countries within Europe, this paper provides a definitive statement for historic cases of WSD in Europe.

2. Materials and methods

Samples of marine penaeid shrimp (*Penaeus japonicus*, *Penaeus monodon*, *Penaeus semisulcatus*, *Metapenaeus kerathurus*) and *Artemia* sp., obtained from aquaculture facilities within EU Member States and

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from out-with the EU, but within the European continental area, were submitted by private industry representatives to the OIE Reference Laboratory for WSD at the University of Arizona, USA between 1995 and 2007. Samples for histology and *in situ* hybridization (ISH) were submitted fixed either in Davidson's AFA fixative or in neutral buffered formalin (Bell and Lightner, 1988). Samples for PCR were submitted preserved in absolute ethanol (see Table 1 for sample details).

Samples were processed for histology following the methods outlined in Bell and Lightner (1988). Samples were processed for ISH using the protocol as outlined in Section 2.5 of Lightner (1996). Samples were processed for nested PCR using the protocol of Lo et al. (1996). According to the OIE manual of diagnostic tests for aquatic animals, histopathology, *in situ* hybridization and PCR are all considered as presumptive and confirmatory tests for use on all life stages for detection of WSSV infection (OIE, 2009).

3. Results

Table 1 summarises the details of samples submitted by anonymous individuals from EU Member States and non-EU European nations during the period 1995 to 2007. Due to the *ad hoc* nature of submissions and the varied format of sample type, submissions from each country are dealt with separately. Specific locations of affected farms within Member States are not provided due to confidentiality agreements between those submitting samples and the testing laboratory.

3.1. Samples submitted from Spain

Six samples of shrimp (*P. japonicus*), one sample of *Artemia* sp., and one sample of pond sediment, collected from Spanish shrimp farms were submitted in 2000, 2001 and 2003 (case numbers 00-251, 00-276, 00-301, 00-333, 01-007, 01-042 and 03-257). One of the samples (00-251) displayed advanced (up to grade 4) WSSV infection by histology (Fig. 1) and ISH (data not shown) and two shrimp samples from 2000 (case numbers 00-301, 00-333) were positive for WSSV using PCR (Fig. 2). The *Artemia* sp. sampled from shrimp ponds in 2001 was negative for WSSV by PCR but WSSV viral DNA was detected in pond sediment collected from the same pond that previously underwent WSSV-associated mortality in 2000. The sample of *P. japonicus* collected during 2003 (case number 03-257) was negative (by histology) for WSSV infection but was positive for 'gut and nerve syndrome' (GNS at grade 3 severity), vibriosis and contained lymphoid organ spheroids (LOS) of unknown cause (Lightner, 1996).

3.2. Samples submitted from Italy

Four samples of *P. japonicus* and *P. semisulcatus* were collected from an Italian shrimp farm stocked with shrimp originating from Turkey in 1997 (case numbers 97-104, 97-110, 97-114 and 97-121). The largest single sample of 100 animals (case number 97-121) revealed a 40% apparent prevalence of WSSV infection based upon histology and ISH (Fig. 3).

3.3. Samples submitted from Greece

One sample of *P. japonicus* (n = 4) collected from a Greek shrimp farm was submitted in 1995 (case number 96-125). Histology revealed grade 3–4 WSSV-associated pathology and grade 2–4 GNS-associated pathology. ISH for WSSV on histological sections of infected shrimp revealed a weak-positive reaction in all 4 cases (data not shown). A second submission of *P. japonicus* collected from Greek shrimp farms in 2005 (case number 05-366) was negative for WSSV (and TSV, YHV and IHHNV) by PCR. A third submission of *M. kerathurus* collected from a Greek shrimp farm in 2007 (case number

Table 1
Summary of cases submitted from Europe to the OIE Reference Laboratory for WSD at the University of Arizona, USA over the period 1995 to 2007.

Case number	Date	Submitting country	Country of origin (if other)	Species	Test(s) ran	Diagnostic findings
96-125	6/21/95	USA	Greece	*P. japonicus*	Histology ISH	Histology: WSSV G3-4; GNS G2-4 ISH: 4/4 weak positive but on re-test 4/4 negative
97-104	6/4/97	Italy	Turkey	*P. japonicus* *P. semiculcatus*	Histology	WSSV G2-4
97-110	6/10/97	Italy	Turkey	*P. japonicus*	ISH	Poorly fixed tissues. ISH — WSSV positive
97-114	6/16/97	Italy	Turkey	*P. japonicus* *P. semiculcatus*	ISH	Poorly fixed tissues. ISH — WSSV positive.
97-121	6/23/97	Italy	Turkey	*P. japonicus*	Histology ISH	Histology: 27/100 WSSV G3-4; 13/100 Susp. WSSV ISH: 4/40 WSSV positive; 2/40 suspect WSSV
97-138	7/15/97	Turkey	n/a	*P. japonicus* *P. monodon* *P. semiculcatus*	Histology	Histology: GNS G4; WSSV not detected.
97-179	9/3/97	Turkey	n/a	*P. japonicus* *P. monodon* *P. semiculcatus*	Histology	Histology: WSSV G3-4; GNS G1-4; MBV G1-4
98-28	3/30/98	Turkey	n/a	*P. japonicus* *P. semiculcatus*	Histology	Not suitable for diagnosis
98-74	4/29/98	Turkey	n/a	*P. japonicus* *P. semiculcatus*	Histology ISH	Histology: WSSV positive ISH: WSSV positive
98-115	6/18/98	Turkey	n/a	*P. japonicus*	Histology	Histology: WSSV G2-3
00-251	7/28/00	Spain	n/a	*P. japonicus*	Histology	Histology: WSSV G1-4
00-276	8/15/00	Spain	n/a	*P. japonicus*	PCR	PCR: WSSV not detected
00-301	9/14/00	Spain	n/a	*P. japonicus*	PCR	PCR: WSSV positive
00-333	10/19/00	Spain	n/a	*P. japonicus*	PCR	PCR: WSSV positive
01-007	1/12/01	Spain	n/a	*P. japonicus*	PCR	PCR: WSSV, IHHNV, YHV not detected
01-042	2/13/01	Spain	n/a	*Artemia* and sediment	PCR	PCR: *Artemia* — WSSV not detected. Sediment — WSSV positive
03-257	8/7/03	Spain	n/a	*P. japonicus*	Histology	Histology: GNS G3; LOS G1; vibriosis, biofouling
05-366	10/4/05	Greece	n/a	*P. japonicus*	PCR	WSSV, TSV,YHV, IHHNV not detected
07-357	11/8/07	Greece	n/a	*P. kerathurus*	Histology ISH PCR	Histology: bacterial infection ISH: bacteria positive WSSV not detected