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Note

Prevalence of *Perkinsus* spp. in selected shellfish species collected off China coast

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ABSTRACT

We investigated the prevalence of *Perkinsus* spp. in 13 species of shellfish collected from the coastal areas of China between 2006 and 2012. A total of 11,581 shellfish specimens belonging to 13 different species were collected from seven bay areas in China *viz*., Dalian (Bohai Sea), Qingdao (Yellow Sea), Wenzhou (East China Sea), Xiamen (East China Sea), Zhanjiang (South China Sea), Haikou (South China Sea) and Qinzhou (South China Sea). The shellfish samples collected were tested for the prevalence of *Perkinsus* spp. using a PCR protocol. The detection rates of *Perkinsus* spp. by PCR method ranged from 0.38 to 26.37%, with *Ruditapes philippinarum* (26.37%) and *Paratapes undulatus (=Paphia undulata)* (20.21%) showing the highest detection rate. The detection rate of *Perkinsus* spp. by PCR in oyster samples were highest in Wenzhou (17.25%), Qinzhou (15.98%) and Zhanjiang (11.67%) bays. Samples taken in autumn showed the highest positive detection rate. Mixed infection by *Perkinsus* spp. and other protozoan species *viz*., *Haplosporidium nelsoni* and *Marteiliia* spp. was found to be a common phenomenon (10.75%).

Keywords: China coast, PCR detection, *Perkinsus* spp., Shellfish

As per the report of Ministry of Commerce of the People's Republic of China, a total area of 1,409,000 ha was used for annual shellfish production of 12,666,500 t in the year 2011, which formed more than 60% of the total global output (Xie and Xie, 2018). In recent years, however, the annual production of shellfish has declined due to environmental changes and shellfish diseases. In particular, parasitic diseases caused by *Perkinsus* spp. infection pose a serious threat to the coastal ecosystem and the supply of shellfish worldwide (Park and Choi, 2001; Fernandez-Robledo *et al.,* 2008).

Perkinsus spp. is listed as a notifiable parasite by the Office International des Epizooties (OIE). It is the major protozoan parasite infecting shellfish, causing mortality among cultured shellfish since 1988 (Andrews, 1988; Burreson *et al.,* 1996; Hamaguchi *et al.,* 1998; Park and Choi, 2001; Villalba *et al.,* 2004; Shimokawa *et al*., 2010). However, very little information is available on the prevalence of *Perkinsus* spp. in some species of shellfish in coastal areas of China. The present study investigated prevalence of *Perkinsus* spp. in selected shellfish species occurring along the coast of China.

Samples of shellfishes belonging to 13 different species were collected between 2006 and 2012 from seven bay areas in China *viz*., Dalian (Bohai Sea), Qingdao (Yellow Sea), Wenzhou (East China Sea), Xiamen (East China Sea), Zhanjiang (South China Sea), Haikou (South China Sea) and Qinzhou (South China Sea). A total of 11,581 shellfish samples were collected and the specific details are described in Table 1.

Genomic DNA was extracted from shellfish samples as described by Xie *et al.* (2013). Polymerase chain reaction (PCR) was performed with primers PerkITS-85: 5'-CCG-CTT-TGT-TTGGATCCC-3' and PerkITS-750: 5'-ACA-TCA-GGC-CTT-CTA-ATG-ATG-3'(Audemard *et al.,* 2004), yielding an expected amplicon of 703 base pairs (bp). Out of the 11581 shellfish samples, 1153 (9.96%) were *Perkinsus* spp. positive by PCR. The detection rate in the 13 shellfish species tested ranged from 0.38 to 26.37% (Table 1). *Ruditapes philippinarum* (26.37%), *Paratapes undulatus* (=*Paphia undulata*) (20.21%) and *Crassostrea rivularis* (15.16%) showed the highest detection rate. The presence of *Perkinsus* spp. in the other ten shellfish species was lower than 6.63%.

The presence of *Perkinsus* spp. infection and the diseases it causes have resulted in population decline of clams since 1980s (Hamaguchi *et al*., 1998, Shimokawa *et al.,* 2010). Little is known about the pathological effects, including rates of mortality due to *Perkinsus* spp. infection in *Meretrix* spp., *Conus* (=*Eugeniconus*) *nobilis*, *Perna viridis*, *Tegillarca granosa*, *Sinonovacula constricta*, *Haliotis discus hannai*, *P. undulatus*, *Tapes conspersus* (=*dorsatus*) and *Mactra* (=*Coelomactra*) *antiquata* in the seven bay areas in China, although these effects have

been documented for shellfish species infected with *Perkinsus* spp. in other parts of the world (Hamaguchi *et al.,* 1998; Park and Choi, 2001; Shimokawa *et al.,* 2010). Therefore, further studies are needed in this direction.

Oysters are the main shellfish products in China. In order to find out the difference in the prevalence of *Perkinsus* spp. among different oyster species, at different locations and in different seasons, 5609 samples of different oyster species were collected from different locations within four seasons which comprised 3397 *C. rivularis*, 1351 *Crassostrea. gigas* and 861 *Crassostrea angulata.* The detection rates among these three oyster species differed, ranging from 3.95% in *C. angulata* to 15.16% in *C. rivularis*, which suggests that the oyster species *C. rivularis* may be more susceptible to *Perkinsus* spp. than other oyster species. The detection rates of oysters were also found to be different among the seven tested locations, ranging from 1.59% in Dalian Bay to 17.25% in Wenzhou Bay (Table 2). The areas of high prevalence included Wenzhou Bay (17.25%), Zhanjiang Bay (11.67%) and Qinzhou Bay (15.98%). These results suggest that the waters in Wenzhou, Zhanjiang and Qinzhou bays provided a more conducive environment for *Perkinsus* spp. to infect oysters. The biological and environmental factors that may affect *Perkinsus* spp. infection of oysters in Dalian Bay need to be investigated further.

Highest detection rate (15.39%) of *Perkinsus* spp. in oysters was observed in the bay areas of China in Autumn season and lowest detection rate (3.70%) was noticed in summer (Table 3). This is consistent with findings showing that infection rates in Korean waters were lowest during summer (Park *et al.,* 2006). The association between season and oyster mortality, however, has not been determined during the present study.

The amplified PCR products (*Perkinsus* spp. positive) were ligated into pMD18-T cloning vector (Takara, Dalian, China) and sequenced. The *Perkinsus* spp. sequences of the individual positive samples from *C. rivularis*, *C gigas* and *Meretrix meretrix* had high sequence homology (98.1 to 99.9%) with the sequences of *Perkinsus atlanticus*

Table 1. Prevalence of *Perkinsus* spp. in 13 shellfish species in China

Shellfish species	No. of shellfish tested	No. of positive samples	Detection rate (%)	No. of mixed positive	Mixed positive detection rate*(%)
Oyster, *C. rivularis*	3,397	515	15.16	11	2.14
Oyster, *C. gigas*	1,351	70	5.18	28	40
Oyster, *C. angulata*	861	34	3.95	7	20.59
Total (Oysters)	5,609	619	11.04	46	7.43
M. meretrix	1,026	39	3.80	0	0
C. nobilis	1,012	37	3.66	7	18.92
R. philippinarum	1,005	265	26.37	39	14.72
P. viridis	513	19	3.70	0	0
T. granosa	506	12	2.37	3	25
S. constricta	603	40	6.63	11	27.5
H. discus hannai	521	2	0.38	0	0
P. undulatus	569	115	20.21	18	15.65
T. conspersus (= T. dorsatus)	107	3	2.80	0	0
M. (= C.) aniquata	110	2	1.81	0	0
Total	11,581	1153	9.96	124	10.75

* Number of mixed positive / number of positive samples.

Table 2. Prevalence of *Perkinsus* spp. in oysters from different locations

Location (species)	No. of oysters tested	No. of positive samples	Detection rate (%)
Dalian (*C. gigas*)	694	11	1.59
Qingdao (*C. gigas*)	657	59	8.98
Wenzhou (122 *C. Angulata* + 475 *C. rivularis*)	597	103	17.25
Xiamen (*C. angulata*)	739	31	4.19
Zhanjiang (*C. rivularis*)	540	63	11.67
Haikou (*C. rivularis*)	454	44	9.69
Qinzhou (*C. rivularis*)	1928	308	15.98
Total	5609	619	11.04

Table 3. Prevalence of *Perkinsus* spp. in oysters sampled in different seasons

Season	No. of samples tested	No. of positive samples	Prevalence (%)
Spring (February - April)	1,432	175	12.22
Summer (May - July)	1,326	49	3.70
Autumn (August – October)	1,384	213	15.39
Winter (November to January)	1,467	182	12.41
Total	5609	619	11.04

Table 4. Mixed positive infections of *Perkinsus* spp. with other protozoan parasites

Shellfish species	Number of cases			
	Perkinsus spp.+ *H. nelsoni*	*Perkinsus* spp.+ *Marteiliia* spp.	*Perkinsus* spp.+ *H. nelsoni* + *Marteiliia* spp.	*Perkinsus* spp.+ *B. ostreae*
Oysters	37	9	0	0
P. undulatus	18	0	0	0
R. philippinarum	17	14	8	0
C. nobilis	7	0	0	0
S. constricta	6	5	0	0
T. granosa	1	2	0	0
Total	86	30	8	0
Mixed positive detection rate (%)	7.46	2.60	0.69	0

strains in GenBank. However the *Perkinsus* spp. sequences of isolates from *C. angulata*, *S. constricta, P. undulatus* and *R. philippinarum* showed a high degree of homology (98.5 to 100%) with the sequences of *Perkinsus beihaiensis*. Thus, these isolates were classified as *P. atlanticus* and *P. beihaiensis* respectively, similar to previously observed phylogenetic groupings (Moss *et al.,* 2008).

To investigate if *Perkinsus* spp. positive shellfish were also mixed infected with *Marteiliia* spp., *Haplosporidium nelsoni* or *Bonamia ostreae*, 1153 *Perkinsus* spp. positive shellfish samples were also tested for the presence of these three parasites by PCR as previously described (Carnegie *et al.,* 2000; Day *et al.,* 2000; Leroux *et al.,* 2001). Out of 1153 *Perkinsus* spp. positive samples, 124 (10.75%) were mixed positive for *Perkinsus* spp. along with *H. nelsoni* or *Marteilia refringens* (Table 1). Eightysix samples were mixed positive for *Perkinsus* spp. + *H. nelsoni* (86/1153, 7.46%), 30 samples were mixed positive for *Perkinsus* spp. + *M. refringens* (30/1153, 2.60%) and 8 samples were mixed positive for *Perkinsus* spp. + *H. nelsoni* +*Marteilia* spp. (8/1153, 0.69%) (Table 4). None of the samples were mixed positive for *Perkinsus* spp +*B. ostreae*. The effects of mixed infections on shellfish mortality or survival rate needs further investigations.

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