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The Prevalence and Risk Factors of White Spot Syndrome Virus in Tiger Shrimp at Traditional Ponds

(PREVALENSI DAN FAKTOR RISIKO WHITE SPOT SYNDROME VIRUS PADA TAMBAK UDANG WINDU TRADISIONAL)

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Abstrak

Telah dilakukan penelitian untuk menentukan status epidemi *White Spot Syndrome Virus* (WSSV) pada budidaya tradisional udang windu di kabupaten Demak, propinsi Jawa Tengah. Metode penelitian yang dipilih adalah *cross-sectional,* dengan jumlah sampel tambak dihitung berdasarkan asumsi prevalensi 70% dan galat (p<10%). Penelitian ini juga menentukan faktor risiko apa saja yang berpengaruh dalam kejadian wabah yang disebabkan WSSV pada budidaya udang windu. Area yang disurvai meliputi Kecamatan Sayung dan Bonang yang merupakan sentra yang aktif melakukan budidaya udang windu. Sebanyak 90 tambak udang windu tradisional berhasil dikumpulkan sebagai sampel dalam penelitian ini. Hasil analisa menunjukkan prevalensi WSSV adalah sebesar 65,6% (*Confident Interval,* CI=55,7%-75,4%). Hasil analisis secara regresi logaritmis didapatkan bahwa faktor yang berpengaruh terhadap terjadinya wabah WSSV adalah pemilahan benih (koefisien =1,517; P<0,049), ukuran tambak (koefisien =0,0002; P < 0,0089) dan pengeringan tambak (koefisien =-3,756; P<0,001). Faktor yang berperan penting dalam produksi udang juga dilakukan analisis dengan regresi linier *multivariate* adalah umur benih tebar (koefisien =19,091; P<0,19), kedalaman air tambak (koefisien =1,343; P<0,0024) dan udang terinfeksi WSSV (koefisien =-44,369 , P<0,001). Hasil penelitian dapat disimpulkan bahwa prevalens di daerah kajian adalah 65,6%, yang termasuk kategori hiperendemik. Faktor yang berpengaruh terhadap infeksi WSSV di tambak adalah pemilihan benih, ukuran tambak dan pengeringan tambak. Faktor risiko yang mempengaruhi produksi udang di tambak adalah stadia udang yang ditebar, kedalaman air di tambak dan dipengaruhi secara negatif oleh infeksi WSSV.

Kata-kata kunci : prevalensi WSSV, faktor risiko WSSV, tambak udang windu tradisional, *Penaeus monodon*

Abstract

A research has been conducted to determine the status of the White Spot Syndrome Virus (WSSV) epidemic on traditional shrimp ponds in Demak regency. A cross-sectional research method was selected, by calculating samples using statistical calculation based on the assumption of 70% prevalence rate and error (p<10%). Additionally, this research also determined the risk factors in the onset of WSSV shrimp pond. Surveyed areas included Sayung and Bonang, subdistrict in Demak district, because the area are actively engaged with shrimp farming. A total of 90 traditional tiger shrimp ponds were sampled in this study. The results showed that the prevalence of WSSV infected farms was 65.6% (Confident Interval, CI=55.7%-75.4%). Factors that affected high prevalence of WSSV were screening of seed (Coefficient=1.517, P<0.049), pond size (coefficient=0.0002, P<0.0089) and drying ponds (coefficient=3.756, P<0.001). Factors that affected the production of shrimp in ponds were analysed using multivariate regression analysis. Based on the analysis, some factors had an important role in production, i.e. : stadia of shrimp seed (coefficient=19.091, P<0.19), water depth in pond (coefficient=1.343, P<0.0024) and WSSV-infected shrimp (coefficient=-44.369, P<0.001). It was concluded that WSSV prevalence in studied area was 65.6%, and it considered as hyperendemic. Factors affected the WSSV prevalence were selection of seed, pond's size and drying ponds. Risk factors affected shrimp production in the pond were stocking seed stadia, whereas WSSV-infection of shrimp was negatively affected factor.

Keywords : WSSV infection risk factors , traditional farms , prevalence, *Penaeus monodon*

INTRODUCTION

Tiger shrimp is one of the areas of aquaculture sector that is expected to provide foreign exchange for the country through exports. Tiger shrimp has several advantages, in addition to a local species (endemic) and cultivation also known by farmers in Indonesia. Dahuri (2013) states that 30% of the potential of the existing ponds will be generated production of 400,000 tons of shrimp in semi-intensive if it is well managed .

Constraints faced by the shrimp farming is mainly a matter diseases, especially those caused by White Spot Syndrome Virus (WSSV) causing huge economic losses and become the most devastating pathogen of farm shrimp (Bondad-Reantaso *et al.,* 2005; Walker and Mohan, 2009). To reduce further loss due to WSSV, more research need to be conducted, especially related to epidemiological study. According to Lotz *et al.,* (2001) epidemiology or population medicine is considered as another weapon that can be used in fight against disease. Through epidemiological study, some risk factors affecting WSSV outbreak can be analyse to give understanding on how far any risk factors have influence on the onset. Research related to epidemilogical study have been done by researchers, mostly on intensive vannamei shrimp farming, on Taura Syndrome Virus (Lotz *et al.,* 2001; Pinheiro e*t al.,* 2007). Very view research on epidemiology in black tiger shrimp (*Penaeus* monodon), especially traditional shrimp culture (Corsin *et al.,* 2001; *Corsin et al.,* 2002; Tendencia e*t al.,* 2011). Given the limitations of the aspects of cost, due to a if large samples to be taken epidemiological is considered costly, we therefore select a specific area. Demak regency is one of regencies in Central Java which has considerable potential for shrimp farms with 34.71 km long coastline. Potential land available for aquaculture is an area of 10,000 ha, spread across four districts, Sayung, Karangtengah, Bonang and Wedung. A total of 4,022 people rely income through aquaculture, generally with traditional technology and is the type of commodity shrimp. Ponds are not large enough to provide optimal results, due to a disease caused by WSSV. Objectives of the study were to determine WSSV prevalence, factors that may influence on WSSV infection and risk factors affecting on shrimp production in the shrimp pond.

RESEARCH METHODS

The Amount and Manner of Sampling

The study was conducted in areas of traditional shrimp farming, located in Demak district, Central Java province. Determination of the amount of pond and the number of samples from each pond was determined based on the presence or absence of disease cases. Assessments of a sample of pond number using the formula: $n = 4PQL^{-2}$, where n = number of samples ponds, p = prevalence rate ; q = 1 - p ; L = error. Assumptions for farm prevalence was 70%, the sensitivity 95% and specificity of 95%, and the error was set at 10%, Then the number of samples taken (n) was 84 ponds. Sampling method for shrimp pond selection was random, while for shrimp from each ponds was purposive sampling.

Risk Factors

Risk factors selected in the research included basic information regarding shrimp pond such as pond preparation, water preparation, source of seeds, water quality, and biosecurity aspects. Basic information included the pond location, the general condition of the dike, and water depth in the pond can be maintained. Pond preparation included drying and duration of the pond bottom drying, removal of sludge from the groove inside the pond and or court, presence or absence of leaks in the embankment. Water supply preparation included water filtration system before it went into the pond and the type of water filter was used. Seeds stocked include size (stadia), whether seeding or seed originating and examination procedures with *Polymerase Chain Reaction* (PCR) screening, and length of seed transportation. Water quality, included temperature, dissolved oxygen, pH, and water salinity of rearing media. Biosecurity aspects included the presence or absence of buffer ponds to prevent the possibility of contracting out of the pond next to it or not .

On farms that were no cases of WSSV disease outbreaks, sample collection techniques and storage was done in the field. Organ to be preserved were gills, swimming legs, put in 80% alcohol and then stored in referigerator until further processing. Samples were observed clinically by looking at specific signs, and to confirm further analyzed by PCR based protocol OIE 2008 (OIE, 2008).

Polymerase Chain Reaction

Polymerase Chain Reaction procedure consist of isolation of DNA, by phenol extraction. A total of 0.2 mL hemolymphe, or as much muscle tissue 0.2 gram, one or two pieces swimming legs or gills, or five PLs put in 1.5 mL microtubes then crushed with a mortar. Digestion buffer (50 mM Tris-Cl pH 7.4, 10 mM NaCl, 10 mM EDTA, 1% SDS) and 400 µL proteinase K was added to a final concentration of 300 µg/mL. Microtube was then incubated in a shaking incubator or incubator adjusted at 37°C for one hour. After added with equal volume of phenol, then centrifuged at a speed of 11,600 g for five minutes. The aqueous phase was separated and transferred to a new microtube, then added with equal volume of phenol CIAA and centrifuged at a speed of 11,600 g for five minutes. The aqueous phase was taken carefully and transferred to a new tube, then added 1/10 volume of 2 M Na-acetate pH 4.8 and 2x the volume of cold absolute ethanol and incubated in the freezer, twisted around the tube, and centrifuged at a speed of 11,600 g for five minutes to pellet precipitate, the solution was poured subsequently disposed of by way of air-dried. The final stage was to dissolve the pellet in TE buffer (100 mM Tris pH 8.0; 0.5 mM EDTA pH 8.0). Concentration of DNA was measured by optical density (OD, optical density) at a wave length of 260 nm and 280 nm. DNA concentration is calculated as follows : Levels = value OD x dilution factor x 50 ug / mL. Furthermore, empirically, DNA levels were also observed by means of electrophoresis on 0.8 % agarose gel.

Gene amplification, prepared mixture (50 µL) consists of 5 µL of 10x PCR buffer (500 mM KCl, 100 mM Tris-Cl pH 8.4 , 1 mg/mL gelatin), or PCR buffer that was already available at the time of purchase Enzyme. Add 0.5 µL 10x dNTPs (consisting of dATP, dTTP, dCTP and dGTP each at a concentration of 2 mM), 2.5 µL $MgCl_2$ at a concentration of 20 mM, each 1 µL solution of oligonucleotide sense and antisense primers at a concentration of 20 ìM, enzyme Taq DNA polymerase 0.5 units. Amplification were performed on four stages, namely hot start, cycle, extra extention, and cooling. Cycle I (1x cycle) consist of hot start = 95°C for 5 min, Cycle II (30 cycles) consist of denaturation = 94°C for 30 seconds, annealing = 52.5°C (1st step PCR); 54°C (2nd step PCR) followed by extension = 72°C for one minute. Cycle III (1x cycle) as extra extension = 72°C for five minutes. Observation of amplification products on agarose electrophoresis performed with a concentration of 2% ethidium bromide containing 0.5 mg/mL, with a current strength of 100 volts in TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA). As result, WSSV considered positive if 1447 bp product as 1st step and 947 bp 2^{nd} step product observed on agarose gel.

Statistical Analysis

The collected data was then analyzed statistically using Statistix Version 4.0 Copyright 1996 Analytical Software.

RESULTS AND DISCUSSION

Total samples of 90 samples were taken randomly on traditional tiger shrimp ponds in Demak. Some of the limitations in sample collection pond because of it was not all farms in operational condition, it was mainly caused by WSSV disease encountered continuous research area. Some farms even lies idle that reached more than 60%, due to continuous losses that pond owners prefer to work as a fisherman than as farmer. Some fish filled ponds just like milk fish which can be expected to be harvested, although the gains were relatively small.

Conditions in some areas of aquaculture pond looks shallow, overgrown grass because it was not longer cultivated shrimp. This condition must be extremely detrimental, given the shrimp has been known and practiced in Demak regency for long periods of time. Even the location of the pond was a transition from tidal rice fields into shrimp ponds.

White Spot Syndrome Virus Prevalence in the Study Area

Ponds were sampled was about 90, of the plan as much as 84 shrimp farms based on the formula $n = 4 PQL^{-2}$.

Prevalence rates based on the results of both clinical diagnosis characterized by occurence of white spot on shrimp carapace (Figure 1) and shrimple field test kit and PCR analysis. Prevalence of each studied area were Sidorejo I has 40% , Sidorejo II 77.5%, Serangan 100%, Tambakpolo I 55.6% and Tambakpolo II was 75%. In cumulative prevalence of WSSV in shrimp pond area of Demak was 65.6% with a lower limit of 55.7% and 75.4% upper limit on the level of 95% (Table 1).

Figure 1. Tiger shrimp (*Penaeus monodon* Fab.) heavily infected with White Spot Syndrome Virus. Gross sign are the occurence of white spot on carapace (arrow)

Table 1 . White Spot Syndrome Virus infected farm level prevalence

Area study	Total Ponds	Positif* ponds	Prevalence (%)
Sidorejo I	25	10	40
Sidorejo II	40	31	77,5
Serangan	4	4	100
Tambakpolo I	9	5	55,6
Tambakpolo II	12	9	75,0
Total	90	59	Average: 65,6% 95% Confident Interval: 55,7% - 75,4%

*) Diagnosis based on clinical signs such as white patches on the carapace and the use of PCR , 2nd step

Calculation of prevalence rates obtained from the results of this study did not differ much with the original assumption, which was 70%. The samples taken were as many as 90 samples of the pond, so it gained the standard error of 0.096. High prevalence not only in shrimp farms in Asia, even in Louisiana, United States, the WSSV prevalence of farm crawfish, *Procambarus (P.) clarkii* and *P. zonangulus* was also high, 90% (Baumgartner *et al*., 2009).

The high prevalence of WSSV in the pond due to white spot disease was a viral disease that has been found for a long time, and until now was still the most common diseases and lead to large losses on shrimp farms. White spot syndrome disease not only attack the tiger shrimp but also vannamei shrimp as well as the latest shrimp is a shrimp introduced in Indonesia.

On traditional farms, the incidence of WSSV outbreaks continue to be cyclical (Hoa e*t al.,* 2005). High mortalities of shrimp related to WSSV infection has also reported by Corsin *et al,* (2001). During outbreak, mass deaths occured, but there were some shrimps surviving and persistently infected by WSSV with low virus levels. Besides shrimp surviving, other crustaceans such as mysids also acted as a career, crabs (Wongteerasupaya e*t al.,* 1998) then infected a new shrimp stocked .

The high prevalence of WSSV in this area of research was partly due to the practice in the traditional shrimp farmers that performed continuously cultivate shrimp and almost without pause. Shrimp farms were harvesting early due to the outbreak of WSSV then stocked again after shrimp taken from the pond, this resulted in repeated transmission. White spot syndrome virus transmission repeatedly in traditional farms also reported by Hoa *et al.*, (2011) occurred in Vietnam, and has the potential to transmit the virus to other farms that implement more advanced technologies. Traditional farms experiencing outbreaks and mortality of shrimp harvesting and water was generally discharged into public waters, further water containing virion particles seeping through the dike embankment.

Risk Factors for WSSV Outbreak

Totally, 21 factors related to the occurrence of outbreaks of WSSV examined in this study, which were grouped into general condition of ponds, pond preparation, seed selection and stocking, maintenance conditions during maintenance, water quality management and biosecurity conditions. Some parameters were binary, with answers Yes and No to the presence or absence parameters WSSV infection, outbreak, water filtration, availability reservoir. The results of numerical parameters such as the observation of water quality conditions such as water temperature, dissolved oxygen, water pH was observed in the morning and afternoon, salinity performed once a day. Production-related factors, including survival, production etc.

The analysis of several factors that allegedly triggered an outbreak of WSSV were analyzed using Statistics. Parameters analyzed between the size of farms, water filtration, removal of sludge in *caren* (groove inside the bank of pond to fascilitate water collected so pond bottom lawn set dry), stadia stocked seed, drying ponds, water replacement, the use of buffer pond, makroalgae growth and PCR screening of seed.

The results of the multivariate analysis found the variables that influence the occurrence and outbreak of WSSV infection were seed by PCR screening, pond size, and drying ponds prior to seeding. Screening by PCR was one factor that gave the role as a cause of WSSV infection in ponds. This result is contrary to previous study (Mohan *et al.,* 2008) because PCR could screened post larvae (PL), so that only non-infected PL was selected. May some factors affecting of this result, such as stadia seed that treaten for screening is PL-10, where are some ponds stocked with larger PL, which was PL40. Young stadia of PL has more stressfull and can easily getting infected with WSSV transmitted from neighbouring ponds or from infected water. Pond size also affects the occurrence of WSSV

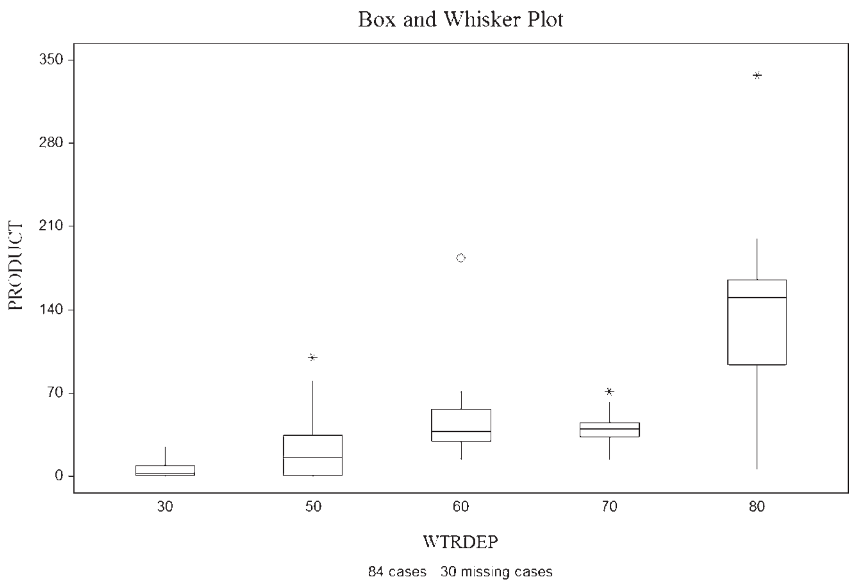

Figure 2 . The relationship between the depth of the water with the production (n = 90). Remarks : WTRDEP = Water Depth in the shrimp pond.

infection, the wider ponds provide great impact for ease of WSSV infection. Farm size that is too wide will lead difficulty in farm management, especially at the time of preparation. Farms that were too wide lead to the possibility of draining water difficulty, causing preparation was not optimal. Improper water drying, it lead as media some kind of wild shrimp containing WSSV.

Pond drying also appear in the multivariate analysis, with a minus notation which means that drying ponds negatively impact WSSV infection. Results obtained from this study contrasts with the results obtained by other researchers. Tendencia *et al*., (2011) mentioned the removal of mud and pilling them on the inside of the dike after culture period prior to WSSV infection risk factors.

Production of Shrimp and WSSV Infection

Multivariate analysis performed on several variables that affected the production of shrimp. Variables that were included in the analysis such as the WSSV infected shrimp, pond preparation, water quality, survival rate and maintenance. Outcomes after screening the final equation with variable significant at level $p < 0.1$.

Result of the multivariate analysis showed that the variables that affect the production of shrimp in ponds were stadia seeds, water depth and the status of WSSV infected shrimp. Stadia of seeds were stocked in the pond and it had a positive role, with a coefficient of 19.09. Notation of positive coefficient on the variable means greater seed stadia stocked will have a positive impact on the survival of shrimp and ultimately provide high production.

Water depth was also a positive impact on production, with a coefficient of 1.343. Positive coefficient means that the deeper the pond water will have a positive impact on increasing shrimp production. The pond water will provide a greater volume of water thus increasing the oxygen in the water reserves. The results of the analysis showed that pond depth of 80 cm produced higher production than farms with lower depths. Pond depth of 30 cm gave lowest production compare to pond depth of 50 cm, 70 cm and 80 cm (Figure 2).

Stadia seeds and water depth had a positive influence on the production of shrimp, meaning the seeds were stocked large stadia will get the higher production. Traditional pond generally done very simple. Pond preparation such as drying was done in a limited amount of time, even some farmers do not draining during pond preparation.

Analysis by Box and Whisker plot in Figure 3 shows the deeper the water the higher the survival rate of shrimp. Pond with deep water can reduce the risk of fluctuations in water quality as well as to provide a deposit more oxygen than ponds with low water depth.

Water depth in the pond neve positive effect on survival and production, as it provides an oxygen deposit, as well has a better buffer than shallow ponds. Pond with too shallow water

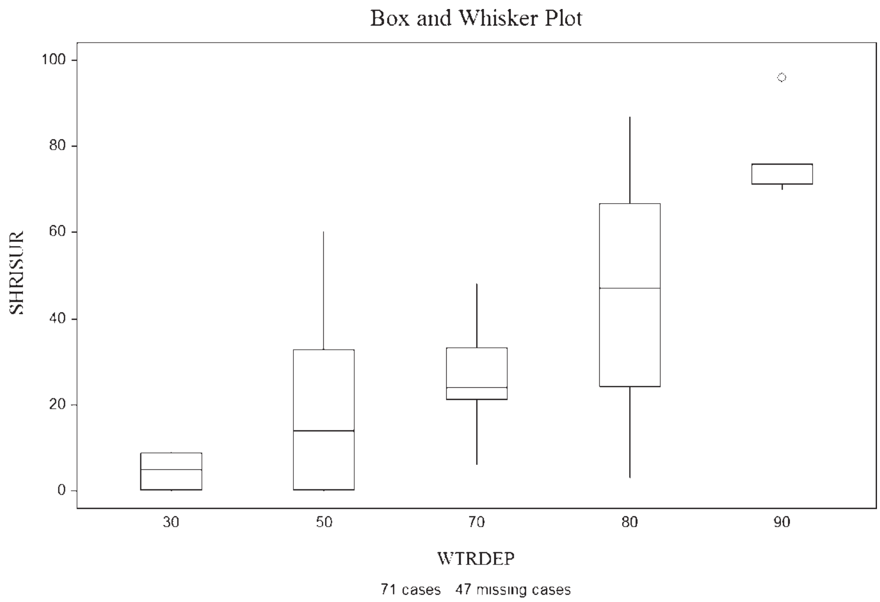

Figure 3. The relationship between the depth of the water with a survival rate of shrimp (n = 90). Remarks : WTRDEP = water depth in the pond

depth will cause greater fluctuations in water and oxygen storage as well as a lower deposit. Disease in shrimp pond, mainly cause by WSSV was triggered by sudden change of water quality such as pH, temperature and low dissolved oxygen (Kautsky e*t al.,* 2000). Water depth of 80 cm therefore seemed as the well depth compared to the lower water depths. Depth of pond water which gives the worst production was a depth of 30 cm .

White spot syndrome virus infected shrimp as variable also emerge on multivariate analysis with coefficient of -44.369. Shrimp with WSSV infection will decrease production significantly. Some researchers claim WSSV as pathogens that occupy the highest priority failure causes the production and economic losses in shrimp culture ponds (Bondad-Reantaso *et al.,* 2005; Hoa *et al.,* 2005; Cuellar-Anjel *et al*., 2010). The mortality in shrimp farming caused by WSSV still quite high because until now there were no medicine either physical or chemical treatment to control the disease (Cuellar-Anjel *et al*., 2010).

The pond size does not indicate a strong role in supporting the successful crop. Results of analysis using Box and Whisker did not show a strong correlation between the pond's size of the production ponds. The pond size of 4,000 m^2 was seen giving high production compared to the smaller size of 2,000-3,000 m^2 and larger ponds (7,000 m^2), but the size of 1,600 m^2 also showed high results. Indonesian traditional farm size varied from 3,000 m^2 to several hectares even reached more than 10 ha.

Pond size as factor is unlikely a direct effect on WSSV status and productivity of shrimp ponds. This finding is contrary to Tendencia *et al.,* (2011) that found wider pond correlated to difficulties to manage. Pond's size 4,000 m^2 based on this research seemed as ideal size for traditional shrimp pond, however not all were true because there were other more important factors. Factors that had a positive influence included initial preparation before stocking and pond management during the maintenance period. The success of shrimp farming was determined by good preparation, covering the management of organic sludge that was generally done by drying, the reversal of the land base for a process of mineralization well. Good preparation will provide a land base is under aerobic conditions so as to provide good conditions during maintenance shrimp (Corsin e*t al.,* 2001; Tendencia *et al.,* 2011).

CONCLUSION

The results showed that the prevalence of WSSV infected pond was 65.6%, considered as hyperendemic. Factors affecting the WSSV prevalence were selection of seed, pond's size, and drying ponds. Shrimp production were influenced by stocked seed stadia, water depth, and negatively affected by WSSV-infection of shrimp.

SUGGESTION

The findings suggest that more research in order to explore more factors in relation to WSSV infection and risk factors that may influenced on shrimp production in wider scale of research. Futher research in order control measure in shrimp farming in relation to WSSV infection is still necessary as shrimp is potensial as main commodity for fisheries.

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