

DISTRIBUTION OF PATHOGENIC *NAEGLERIA* SPP IN THAILAND

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Abstract. Research concerning the distribution, isolation, viability, ultrastructure, morphology and immunogenicity of *Naegleria fowleri* has been increasing in Thailand during 1988-2000. The distribution of the organism was carried out from 1985 to 1987 in Si Sa Ket and Ubol Rachathani Provinces, after the first fatal case was reported in Si Sa Ket. Since then in a 1998 survey of *N. fowleri* in stagnant water around industrial areas was carried out in Pathum Thani, Samut Prakan and Lopburi provinces. The results showed that 10% of pathogenic *Naegleria* belonged to species *fowleri* as characterized by morphology and the occurrence of pathogenesis in mice after nasal inoculation. In the same year, Nacapunchai *et al* (1999) determined the prevalence of amebae in aquatic habitat of human environments in five parts of Thailand during the summer. Fourteen percent of free living *Naegleria* spp were found in both soil and water resources. Recent studies of the ultrastructure, factors affecting the viability and SDS-PAGE electrophoretic patterns of 3 Thai strains of pathogenic *Naegleria* spp indicated their similarities in morphological characteristics of pathogenic reference control, *Naegleria fowleri* CDC VO 3081. Additional study using a genetic approach to species criteria using allozyme electrophoresis had been conducted.

INTRODUCTION

The ameboflagellate *Naegleria fowleri* is a highly virulent organism. The first isolations of the organism were from patients with meningoencephalitis from several countries and continents. Many early attempts to isolate *N. fowleri* from environmental source failed. In the present survey *Naegleria fowleri* from water resources were successfully recovered and selective isolation showed the high temperature tolerance of the amebae. The viability on temperature, pH, chlorination, salinity, formalin, ultraviolet, drainage, rainfall, solar heat, etc, are important factors to explain the distribution of the organism in environmental and polluted resources. The information supported by SDS-PAGE electrophoresis and isoenzyme electrophoresis analysis aided in the identification of *Naegleria* spp in Thailand.

Survey (Figs 1-4)

The *Naegleria* is under the family Vahlkampfiidae. The classification of free-living amebae by Chang represents a continuation of the ideas of both Singh and Hanumiah (1979) and Page (1981) and is based on phylogenetic principles. *Naegleria* spp are distributed world-wide but the true incidence of human infection and prevalence is unknown. Primary amebic meningoencephalitis (PAM) due to *N. fowleri* has existed for a number of years and most cases were diagnosed retrospectively (Beaver *et al*, 1984). Victims for the most part are healthy young individuals with a recent history of contacting water with this amebae (Callicott, 1968). The disease affected males about three times more often than in females. This may be

indicative of greater participation by male in water-related sports rather than sexual predisposition. About 130 cases of PAM have been reported world-wide due to *N. fowleri* (Abraham and Lawande, 1982; Lawande *et al*, 1979; Sugita *et al*, 1999). Of these 50 PAM cases were from the USA (Anderson *et al*, 1972), 16 cases reported from Czechoslovakia between 1962 to 1965 (Cerva *et al*, 1968) and 17 cases reported in Australia (Dorsch *et al*, 1983). The first case of *Naegleria* infection in Thailand was reported by Jariya *et al* in 1983 from Si Sa Ket Province. The second case was from Bangkok (Poungvarin and Jariya, 1991), the third and fourth case were from Trad Province (Somboonyosdech *et al*, 1987) and the fifth case was from Nakhon Pathom Province (Sirinavin *et al*, 1989). All of these patients died soon after infection. Luckily, the sixth case from Samut Prakan survived after treatment with amphotericin B and ketoconazole. (Poungvarin and Jariya, 1991). A few survivors were reported during 1970-1992. The last case was a 38-year-old lorry driver with a history of auditory hallucination and impaired concentration associated with early morning headache, visual disturbance, seizure and signs of raised intracranial pressure. After he had swum in several of the hot springs which existed along his route especially in the area around Zhongshan, a town 100 km north-west of Hong Kong. The first intervention slowed a right frontal abscess and pus drainage showed living *N. fowleri*. He was treated with amphotericin B 60 mg/day and rifampicin 460 mg/day, intravenous chloramphenicol 1 g qid and surgery with decompressive occipital lobectomy (Angela *et al*, 1993). The outcome of psychological assessment revealed moderate disability. Since then, a

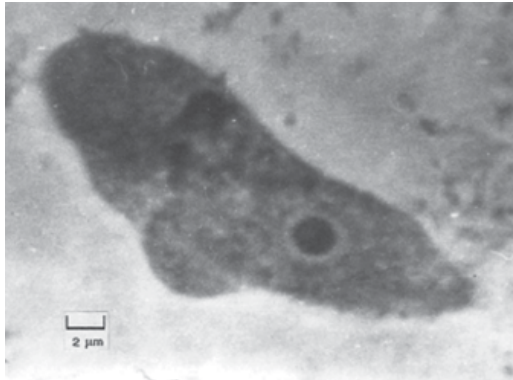


Fig 1- Trophozoite form in iron hematoxylin; Cytoplasm appears fine granular clear nucleus, dense central nucleolus and halo. (Mag 1,250)

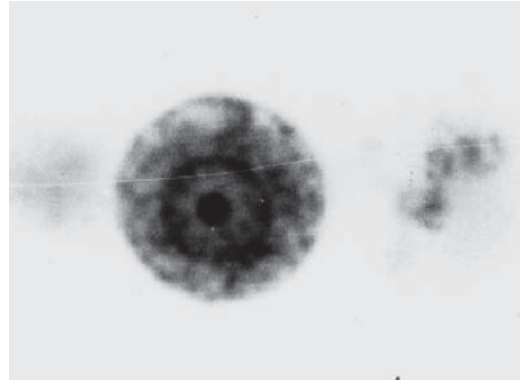


Fig 3- Cyst form in iron hematoxylin; Single cyst wall and typical nucleus of amoeba were demonstrated. (Mag 1,250)



Fig 2- Trophozoite form in iron hematoxylin; Living flagellate of *Naegleria* spp showing flagellum. (Mag 1,250)

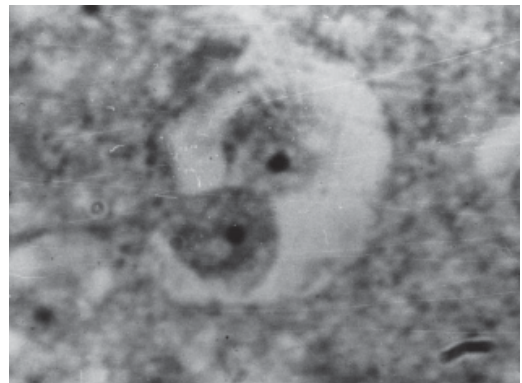


Fig 4- The histological studies revealed the large central karyosome and halo around the nucleus of the organism. The amount of amoebae were accumulated around perivascular space. (Mag 1,250)

number of studies concerning the epidemiology were carried out during 1988-2000.

The distribution of *Naegleria* spp in Thailand both in tropical and subtropical areas have been studied. A survey for *N. fowleri* was carried out from 1985 to 1987 in Si Sa Ket and Ubon Rachathani Province (Jariya *et al*, 1988), after the first fatal cases was reported in Si Sa Ket. In 1998, a survey of *N. fowleri* in stagnant water around industrial area was done in Pathum Thani, Samut Prakan and Lopburi Province in order to detect the prevalence of *Naegleria* and the conditions which was most likely to be found. This research revealed that 10% of the *Naegleria* spp belonged to species *fowleri* as characterized by characteristic morphology and the occurrence of pathology in mice (*Ratus ralus*) after nasal inoculation (Jariya *et al*, 1997). This means that *N. fowleri* not only survives in natural waters but can also proliferate

in thermal polluted water. (De Jonckheere *et al*, 1977). Nacapunchai *et al* (1999) determined the prevalence of amoebae in aquatic habitat of human environments in five parts of Thailand during the summer of 1998, and reported that 14 % of free living *Naegleri* spp were found in both soil and water resources (Mascaro *et al*, 1989). Due to the fact that it can proliferate in surface water, local people can be easily infected just by swimming (Tyndall *et al*, 1989).

Ultrastructures (Figs 5-10)

The ultrastructure of pathogenic Thai strain was carried out (Mankalanond *et al*, 1999). The results revealed that the ultrastructure morphology from scanning electron microscope showed non-distinguish irregular outer surface and wrinkled membrane of *Naegleria* spp compared with reference control *N. fowleri* CDC VO3081. It was also reported by transmission electron microscope that rough

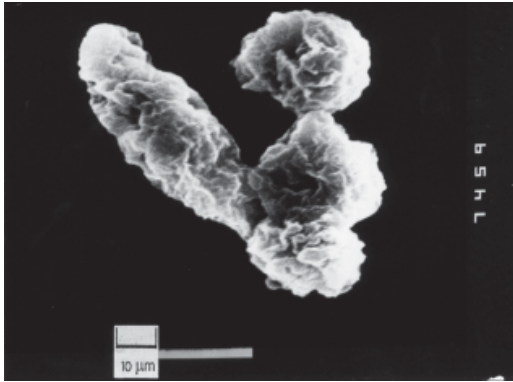


Fig 5- Trophozoite form (SEM); Irregular outer surface and wrinkled membrane were demonstrated.(Mag 3,000)

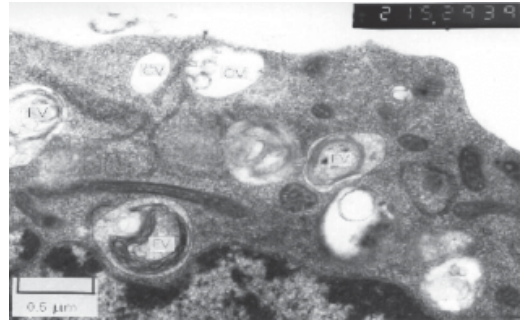


Fig 8- Trophozoite form (TEM); Vacuole (Unit membrane and contain electron-dense materials surrounded by whorl-like formations). Food and contractile vacuole were demonstrated. (Mag 30,300)



Fig 6- Trophozoite form (TEM); Rough endoplasmic reticulum appear as elongated tubular vesicles cover with ribosomes, nucleus, nucleolus. (Mag 10,100)

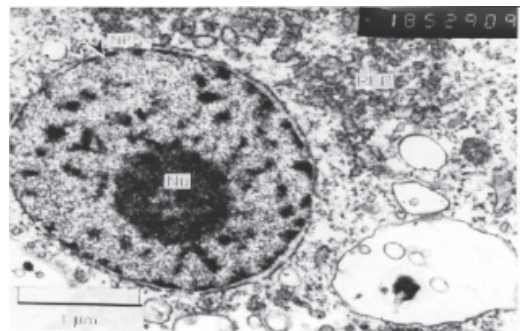


Fig 9- Trophozoite form (TEM); Nucleus, nuclear membrane was bounded by two clear electron dense. A few pores could be seen within the nuclear envelope. Nucleolus at the central portion of the nucleus appears dense. (Mag 26,000)

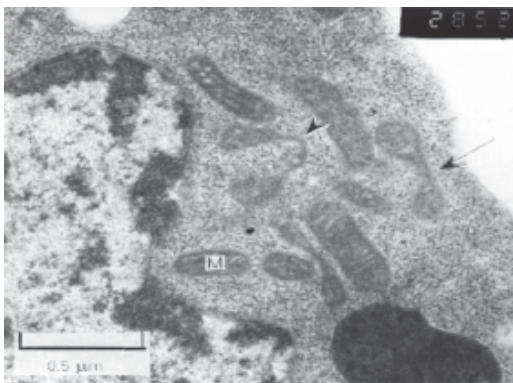


Fig 7- Trophozoite form (TEM); Dumb-bell shaped, cup-shape and oval-shape mitochondria were demonstrated. (Mag 40,500)



Fig 10- Flagellate form (SEM); Pear shape and flagellum were seen. (Mag 30,000)

endoplasmic reticulum appeared as elongated tubular vesicles covered with ribosome and closely associate to mitochondria. Most impressive was the presence of many dum-bell shaped mitochondria. These were bound by two membranes separated by clear space, the outer membrane being thinner than the inner one. The cristae were thick, prominent and irregularly arranged. Cup shape and oval shape mitochondria were also found. Most numerous vacuoles were demonstrated, food and contractile vacuoles which were bound by a unit membrane and contains electron-dense material. The nuclear membrane was bounded by two clear electron dense. A few pores could be seen within the nuclear envelope and nucleolus at the central portion of the nucleus appeared dense.

Factors affecting viability (Figs 11-16)

Due to the fact that the amebae appears to multiply better during the hotter months of the year suggesting a seasonal pattern (Griffin, 1983; Nerad *et al*, 1983). These strains are rare and transient and there are many factors that stimulates growth. Supporting this knowledge, the viability of *Naegleria* spp was carried out in the same year (Tiewcharoen and Junnu, 1999a). We can concluded that *Naegleria* survives as the amebae form at -40°C but optimal condition were 28°C. It does not survive if the pH was less than 5 or more than 12 and preferred fresh water to brackish in salty water higher than 2%. In addition, they were killed by chlorine concentration greater than 0.75 ppm and also in formalin at a concentration of greater than

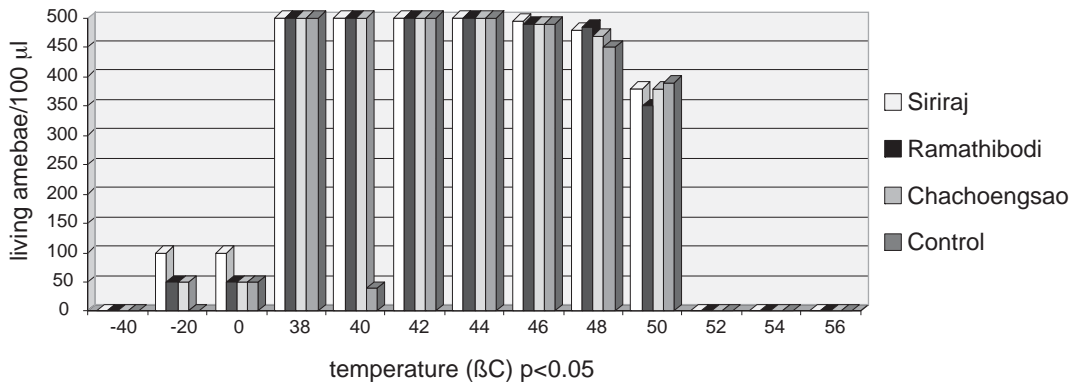


Fig 11- The effect of temperature on *Naegleria fowleri* and pathogenic *Naegleria* spp. Trophozoites degenerated in hours at temperature -40°C. Pathogenic *Naegleria* spp were killed at temperature greater than 50°C for 24 hours.

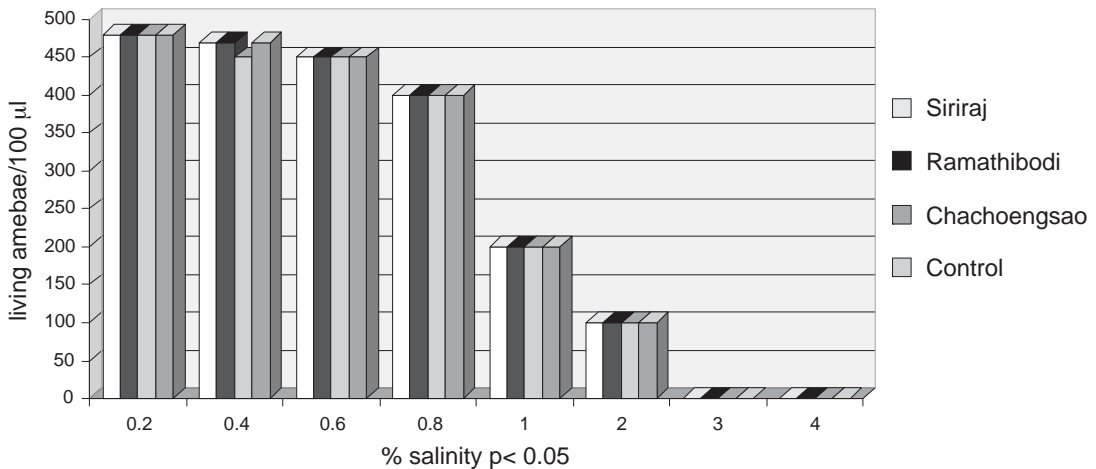


Fig 12- The effect of salinity of *Naegleria fowleri* and pathogenic *Naegleria* spp. Trophozoites were destroyed in saline concentrations higher than 2%.

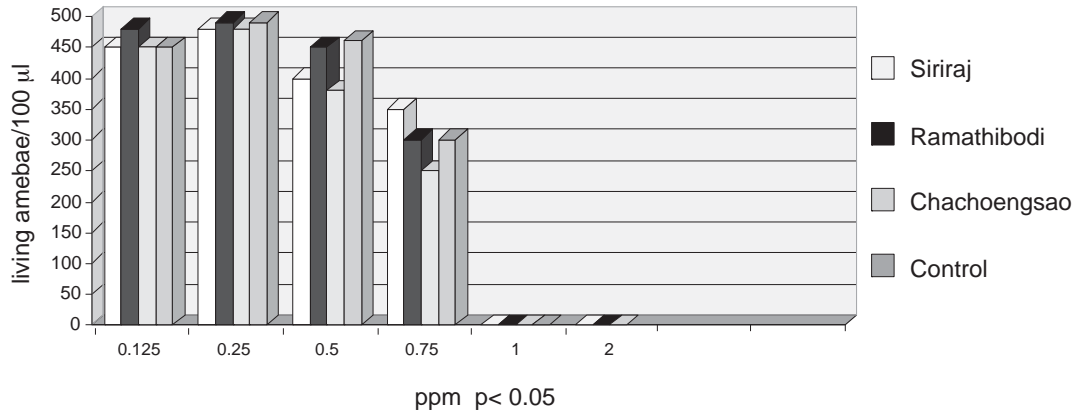


Fig 13- The effect of chlorine on *Naegleria fowleri* and pathogenic *Naegleria* spp. Chlorine concentrations greater than 0.75 ppm killed the amebae.

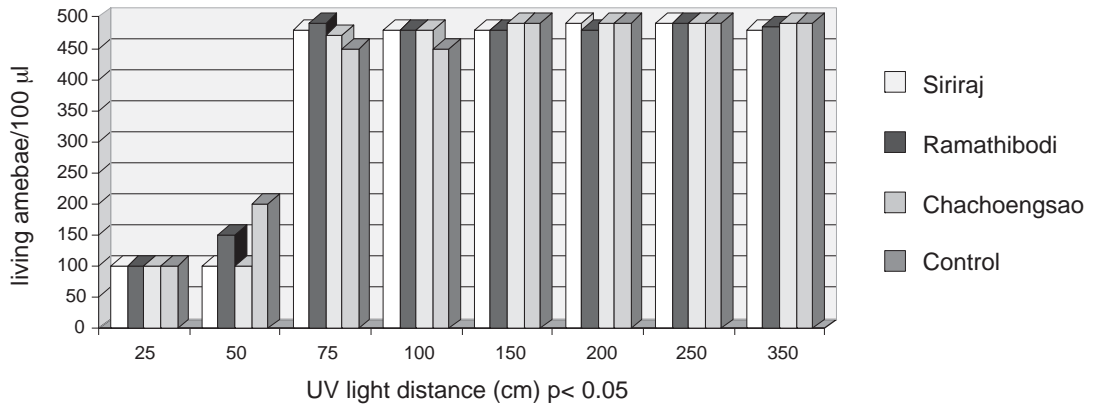


Fig 14- The effect of UV light distance on *Naegleria fowleri* and pathogenic *Naegleria* spp. The amebae were resistance to ultraviolet light.

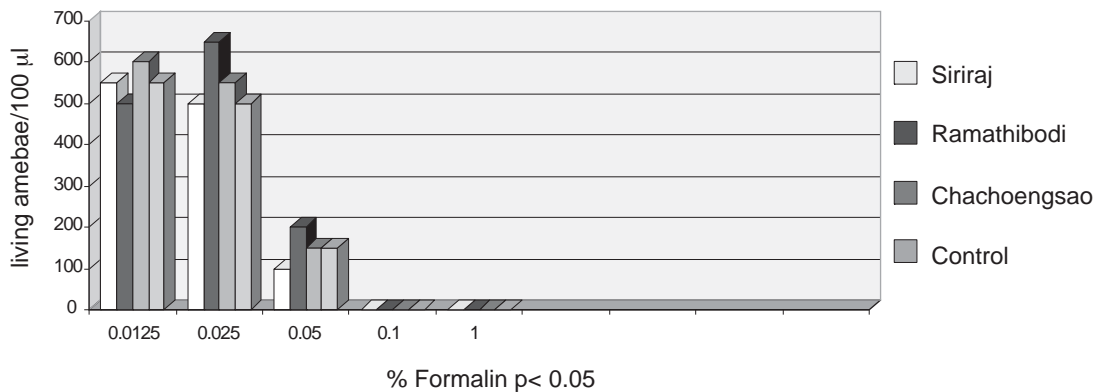


Fig 15- The effect of formatlin on *Naegleria fowleri* and pathogenic *Naegleria* spp. All *Naegleria* spp were sensitive to formalin at concentrations greater than 0.05%.

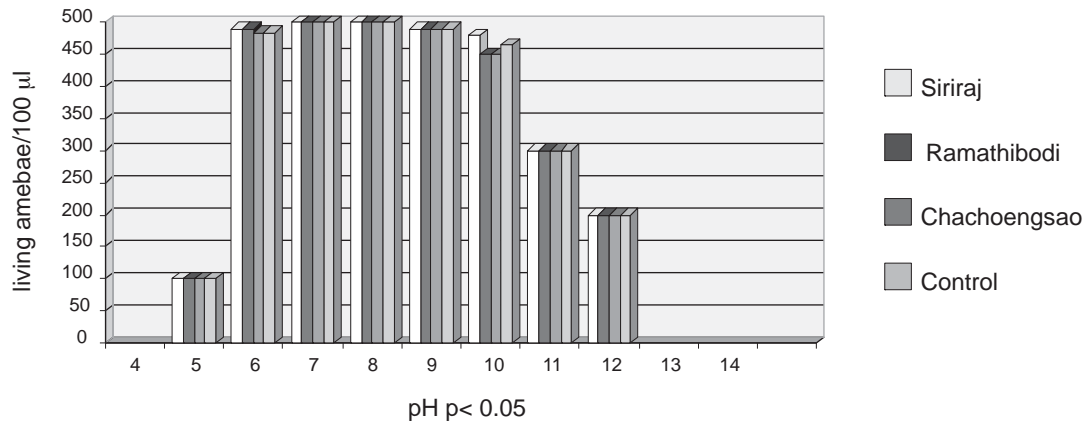


Fig 16- The effect of pH on *Naegleria fowleri* and pathogenic *Naegleria* spp. The amoebae could not survive at a pH of less than 5 or more than 12.

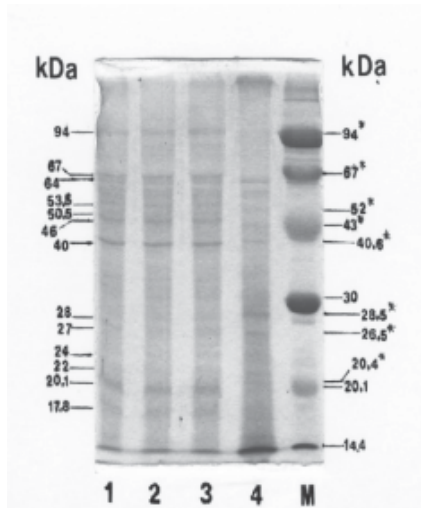


Fig 17- Representative of proteins profiled extracted of *Naegleria fowleri*, pathogenic *Naegleria* spp; Siriraj strain (Lane 1), Ramathibodi strain (Lane 2), Chachoengsao strain (Lane 3), CDC VO3081 strain (Lane 4), M=Molecular weight markers.

0.05%. However, all of the amoebae were resistant to ultraviolet light.

Immunological studies (Fig 17)

Recent studies carried out in 1999 on the molecular weight of pathogenic Thai strain *Naegleria* and the reference control strain *N. fowleri* by SDS-PAGE electrophoresis. The strains of pathogenic *Naegleria* isolated from fatal Thai patients were similar to *N. fowleri* and differed in a few distinct protein compounds (Tiewcharoen *et al*, 1999b). A genetic approach to species criteria using allozyme electrophoresis has also been conducted. The isoenzyme patterns were studied by using vertical slab gel electrophoresis on 7.5 % separating polyacrylamide

gel. All specific and non specific allozyme of pathogenic *Naegleria* Thai strain will be reported in next year.

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