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# Persistence of *Trichinella spiralis* muscle larvae in natural decaying mice

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**Abstract** The influence of natural weather conditions on the viability and reproductive capability of *Trichinella spiralis* muscle larvae in mouse corpses exposed to summer and winter conditions in the Buenos Aires Province, Argentina, was studied. For this purpose, a total of 49 mouse corpses harbouring muscle larvae of *T. spiralis* were exposed for a period of 1, 2, 4 and 6 weeks in each of the seasons. Control corpses maintained at 8°C were also included. In summer, *T. spiralis* muscle larvae were recovered from corpses exposed up to 1 week only. The viability of these larvae was 54.2%, and the reproductive capability index in mice (RCI) was 13.1 and significantly lower than the control ($p<0.0005$). Morphologic deterioration and reduction in the glycogen content of cysts and larvae were observed at the second week of exposition. By week 4, larval stages of *Dermestes maculatus* were observed inside corpses, and 22 live muscle larvae of *T. spiralis* were obtained by artificial digestion of their bodies. In winter, *T. spiralis* muscle larvae were always recovered, the viability being almost 100% except for a significant reduction by week 6 of exposition ($p<0.0001$). For this season, the RCI were 50.5, 46.9, 59.7 and 45.2 for the periods of 1, 2, 4 and 6 weeks of exposition, respectively. The morphology of cysts and larvae did not show alterations, and no variations were observed as well in glycogen reserves during the 6-week period of exposition. RCI of non-exposed muscle larvae were always significantly higher that any of those recorded from muscle larvae that belonged to exposed corpses ($p=0.0005$). The present results demonstrate that muscle larvae of *T. spiralis* are able to survive in nature and keep infective for a 1-week period in summer and at least for 6 weeks in winter, becoming an important source of infection for scavengers. In summer, larvae stages of *D. maculatus*, and probably other insects, may play an important role in the survival and transmission of *T. spiralis* in the sylvatic cycle.

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## Introduction

*Trichinella spiralis* is a nematode that infects a variety of mammalian hosts including humans (Murrell et al. 2000). The newly born larvae arrive and penetrate the striated muscle tissue and become infective from day 17 onwards since the host became infected. Once the nurse cell that protects the parasite from the immune host response has completed its development by day 30 post infection (p.i.), the larvae can survive for a long time (Campbell 1983; Sacchi et al. 2001), maintaining the capacity to infect new hosts after the parasitized animal dies (Despommier 1998).

In nature, the complete decomposition of muscle tissues occurs at different intervals and depends strongly on weather conditions and on the fauna succession that feed upon the corpses (Oliva 1997). Thus, regional and seasonal weather

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conditions may have a great influence on the decay of animal tissues and consequently on the period in which encysted muscle larvae of *T. spiralis* can remain alive and infective for a new host.

*T. spiralis* is the dominant *Trichinella* species circulating in Argentina which is also associated to high prevalence in pigs, rats, armadillos and wild boars (Ribicich et al. 2010).

Studies testing the persistence and infectivity of *Trichinella* sp. larvae in muscle tissue have included laboratory assessments of parasite tolerance to cold and freezing temperatures, "wet-curing" and seasoning (Theodoropoulos et al. 2000; Malakauskas and Kapel 2003; Hill et al. 2007; Medina-Lerena et al. 2009). Other authors have also evaluated the same parameters during tissue exposition to a constant temperature and humidity (Von Köller et al. 2001), and studies on the tolerance to freezing temperature (−5°C) of different isolates of *Trichinella nativa* from fox tissues were made by Davidson et al. (2008).

However, researches on the longevity of *T. spiralis* muscle larvae in corpses naturally exposed to weather conditions are very scarce in the literature (Oivanen et al. 2002), and no reports could be found where the influence of the fauna related to corpse decomposition have also been involved in the studies.

The aims of the present study were to evaluate the viability and infective capability of *T. spiralis* muscle larvae in decaying mice exposed to the natural environmental conditions of summer and winter and to determine the potential role of the fauna associated to muscle decomposition on the survival and transmission of *T. spiralis* in nature.

## Materials and methods

### Animals and *T. spiralis* infections

A total of 49 male 8-week-old CF1 mice were kept according to the PAHO/WHO rules and with controlled temperature and air conditions. Food and water were administered ad libitum. Lighting was provided on a 12-h light/dark cycle (Cuba Caparó 1982).

Each animal was inoculated with 300 decapsulated muscle larva (ML) of *T. spiralis* by means of a gastric canule adapted to a disposable syringe to supply a volume of 0.5 ml/animal. The *T. spiralis* strain used in this study was obtained from a natural case of pig trichinellosis in Argentina and maintained in CF1 mice by serial passages. For the inoculums, ML were recovered by enzymatic digestion (Gamble 1996), resuspended in distilled water and counted in a stereomicroscope to prepare each of the infective doses.

Uses and practices with laboratory animals were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Sciences, UNCPBA, Tandil, Argentina (http://www.vet.unicen.edu.ar).

### Experimental groups and exposition to environmental conditions

At 5 weeks post inoculation (p.i.), animals were sacrificed by cervical dislocation according to bioethics rules (AVMA 2001). The corpses were weighed and separated into four comparable groups named as G0, G1, G2 and G3. The G0 was the non-exposed control group. The corpses of G1, G2 and G3 were exposed to natural environmental summer and winter and to controlled conditions at 8°C respectively for a period of 1, 2, 4 and 6 weeks. The environmental experiments were performed on a specially conditioned plot belonging to the UNCPBA University, located in Tandil, Buenos Aires Province, Argentina (37°32′S; 59°15′W). The study comprises the period from January 1 up to February 11 in summer and from July 1 up to August 12 of 2009 in winter, respectively. The experimental design is shown in Table 1.

Corpses exposed to environmental conditions were deposited in metallic cages containing at the floor level a thin soil–pasture layer—white clover (*Trifolium repens*), red clover (*Trifolium pratense*), ryegrass (*Rye grass*) and orchard grass (*Dactylis glomerata*). As an additional biosecurity means, the cage was covered with a wire mesh 0.5 cm wide and was located in a fenced area restricted to small and large animals.

### Parasitological examinations

After each period of exposition, the corpses were removed and weighed to calculate the reduction from the initial

**Table 1** Experimental design

| Groups of exposition | Time of exposition (weeks) | Animals/group (*n*) |
|---|---|---|
| Control, non-exposed (G0) | 0 | 5 |
| Summer (G1) | 1 | 4 |
| | 2 | 4 |
| | 4 | 4 |
| | 6 | 4 |
| Winter (G2) | 1 | 4 |
| | 2 | 4 |
| | 4 | 4 |
| | 6 | 4 |
| Controlled (G3) | 1 | 3 |
| | 2 | 3 |
| | 4 | 3 |
| | 6 | 3 |

weight as an indicator of the decomposition status; the presence of necrophagous and necrophyllic insects was also registered and identified based on the descriptions of Oliva (1997). Corpses were dissected to obtain the carcass as well as muscle tissue samples for morphologic studies and glycogen cyst/larvae contents by haematoxylin–eosin (HE) and Periodic Acid Schiff's (PAS) stains, respectively. Afterwards, carcasses were weighed again, grinded and individually processed by enzymatic digestion (Gamble 1996) for the recovery of *T. spiralis* larvae. The viability (percent) of ML was determined throughout motility and the typical coiled disposition at the microscope view. When a comma appearance or internal anatomic damages were observed, the larvae were considerate dead. Live larvae were counted to prepare individual inoculums of 300 ML each to infect CF1 mice to establish the reproduction capability index—RCI: recovered larvae/inoculated larvae.

### Meteorological data

Temperature and relative humidity data were recorded daily by a meteorological station (Cavadevices, FCV) located nearly to the place where the studies were performed.

### Statistical analysis

The statistics was performed with a factorial ANOVA to analyse the differences between survival rate and RCI of *T. spiralis* ML at different periods and conditions of corpse exposition. Analyses were made with Infostat Statistical Software 2008.

## Results

### Decomposition status of corpses

The mean weight of corpses before and after exposition is shown in Fig. 1. Results showed a great reduction in the weight of corpses exposed to summer environmental conditions.

Weight reductions were 16.7 g±2.9, 24.3 g±2.5 and 27.9 g ±0.8 at 1, 2 and 4 weeks of exposition, respectively, although the higher reduction was registered after 6 weeks p.e. (33.2 g± 2.4) when only desiccated skin and bones were recovered.

The initial weight of corpses was maintained throughout the exposition in winter or controlled conditions. A light decrease of the initial weight was only observed at 6 week p.e. with a reduction of 13.9 g±1.6 and 8.1 g±4.1 in corpses exposed to winter or controlled conditions, respectively.

### Fauna associated to decomposition of corpses

Adult stages of *Dermestes maculatus* and *Sarcophaga* spp. were collected at 1 week p.e. under summer conditions although at 2 weeks p.e., either necrophagous or necrophilic insects were not observed in the cage or corpses. Larval stages of *D. maculatus* were observed in corpses at 4 and 6 weeks p.e. No insects of interest were observed in corpses exposed to winter conditions at any time of the study.

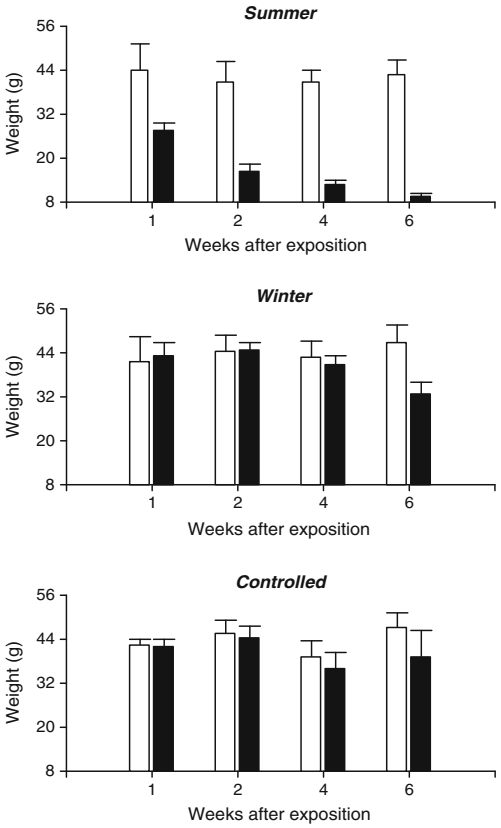


**Fig. 1** Initial (*white column*) and final (*black column*) mean weight of corpses exposed for 1, 2, 4 and 6 weeks to summer, winter and controlled conditions

### Parasitological examinations

In summer (G1), *T. spiralis* ML were recovered alive from corpses exposed up to 1 week only. The viability and RCI were 54.2% and 13.1, respectively, being both significantly lower than G0 and G3 at the same time of exposition ($p=0.026$; $p<0.0001$). The viability was also lower than G1 at the same time of exposition.

Larval stages of *D. maculatus* observed at 4 and 6 weeks p.e. were collected, pooled (40 larvae ±5) and processed by enzymatic digestion for the recovery of *T. spiralis* ML. At 4 weeks of exposition, 22 live muscle larvae were recovered whilst no ML were found in larval stages of *D. maculatus* collected at 6 weeks p.e.

From corpses exposed to winter conditions (G2), live ML were recovered throughout the 6 weeks of exposition, although a significant reduction ($p<0.0001$) in rate survival was observed towards 6 weeks p.e.

**Table 2** Number of *T. spiralis* larvae (mean group), proportions of live larvae (percent) and ratio of RCI from mouse corpses

| Groups of exposition | Time of exposition (weeks) | Larvae recovery (mean±SD) | % of live larvae (mean±SD) | RCI (mean±SD)[a] |
|---|---|---|---|---|
| G0 | 0 | 25,000±9,411 | 98.5±3.3a | 174.4±46.2a |
| G1 | 1 | 11,327±4,546 | 54.2±21.9b | 13.1±11.6b |
| | 2 | 5,681±5,936 | 0 | – |
| | 4 | 185±220 | 0 | – |
| | 6 | 330±469 | 0 | – |
| G2 | 1 | 17,085±14,250 | 97.8±3.7a | 50.5±14.9b, c |
| | 2 | 10,084±8,488 | 97.1±4.3 | 46.9±16 |
| | 4 | 18,624±11,036 | 98.6±1.8 | 59.7±16.9 |
| | 6 | 14,291±7,179 | 24.6±16.3 | 45.2±51.1 |
| G3 | 1 | 19,443±11,175 | 100a | 90.3±14.6c |
| | 2 | 23,664±10,139 | 100 | 116.2±110.7 |
| | 4 | 47,663±17,156 | 100 | 79.2±19.6 |
| | 6 | 24,997±6,695 | 100 | 56.1±2.4 |

Different letters by column (percent of live larvae, RCI) indicate significant differences ($p<0.05$)

[a]Two mice were used

The ML that belonged to the corpses of G2 and G3 maintained a comparable infective capability throughout the study but lower than G0 ($p=0.0005$) (Table 2). The ML of *T. spiralis* recovered from corpses of G3 were 100% alive through the study.

## Histochemical studies

Muscle samples from corpses of G0 showed a chronic and granulomatous inflammation involving cellular infiltration with eosinophils, macrophages, lymphocytes and plasmatic cells. Tissue samples of corpses exposed to summer, winter or controlled refrigerator conditions showed a high level of autolysis which precluded further studies.

Either nurse cells or ML of muscle samples of G2 and G3 maintained their typical morphology and disposition throughout the study (Fig. 2a and c), and no differences in glycogen contents were observed either in cysts or larvae (Table 3).

However, alterations were recognized in nurse cells of muscle samples from corpses of G1 showing cysts with irregular shape at 2 weeks p.e. (Fig. 2b) and also a light decrease in glycogen contents towards 1 week p.e. being still lower at 2 weeks p.e. (Table 3). Muscle samples from G1 at 4 and 6 weeks of exposition could not be processed.

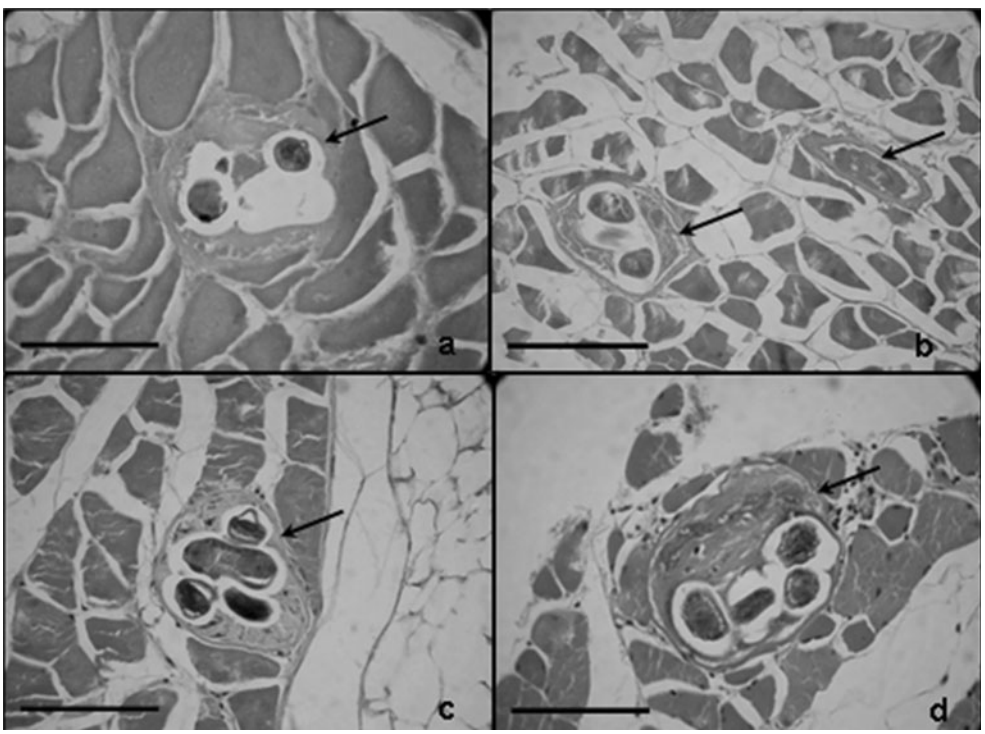


**Fig. 2** Histology of muscle tissue of mouse corpses infected with *T. spiralis* and exposed to different conditions. Nurse cells (*arrows*) look morphologically whole after 6 weeks p.e. to winter (**a**) and controlled (**b**) conditions, comparable as observed in the control group (**d**). After 2 weeks of exposition to summer conditions (**c**), nurse cells were noticed to be contracted as muscular bundles. HE staining was used on all sections. The *bar* indicates 10 μm (×400)

**Table 3** PAS staining intensity observed in muscle tissue samples of mice infected with *T. spiralis* and exposed for 1, 2, 4 and 6 weeks to different conditions

| Groups of exposition | Time of exposition (weeks) | PAS staining reaction |
|---|---|---|
| G0 | 0 | ++++ |
| G1 | 1 | ++ |
| | 2 | + |
| | 4 | n/d |
| | 6 | n/d |
| G2 | 1 | ++++ |
| | 2 | +++ |
| | 4 | +++ |
| | 6 | +++ |
| G3 | 1 | ++++ |
| | 2 | ++++ |
| | 4 | ++++ |
| | 6 | ++++ |

*n/d* no sample tissue was obtained

## Meteorological data

Daily climatic records for each period of the study are shown in Fig. 3. The summer period was characterized by high temperatures (media, 23.4°C; min, 14.6°C; máx, 29.5°C), a low relative humidity (media, 54.7%; HR min, 33%; HR máx, 83%) and scarce rainfalls. In the winter period, the media temperature was 6.1°C (min, −2.4°C; máx, 20°C), and the HR media was 75.1% (HR min, 25%; HR max, 100%).

## Discussion

Corpses of domestic and wild animals with *Trichinella* represent a continuous hazard because a significant component of the transmission involves scavenger animals. The protective collagen capsule of the nurse cell and the endogenous carbohydrates which act as energy source when the host is dead (Campbell 1983) are features of the encysted *T. spiralis* larvae that influence their transformation into an infection source for healthy animals. It is important to determine under field conditions which other factors can influence the persistence of *T. spiralis* ML in nature.

Experimental studies have examined the infectivity of *Trichinella* sp. recovered from decaying carcasses exposed to laboratory conditions (Malakauskas and Kapel 2003; Hill et al. 2007; Medina-Lerena et al. 2009; Von Köller et al. 2001), but the persistence of *T. spiralis* in rat carcasses exposed to environmental conditions has only recently been described (Oivanen et al. 2002). In this study, ML of *T. spiralis* recovered from rat carcass exposed in boxes to summer conditions of Finland survived only 2 weeks and showed a low infective capability.

In the present study, under summer conditions, a decrease in larvae and nurse cell glycogen content was observed at the first week of exposition, although structural alteration of muscle tissue due to desiccation and fast decomposition of corpses may account for the low survival and reproductive capability observed in recovered larvae.

Furthermore, populations of insects associated to cadaveric decomposition were also evaluated here. The

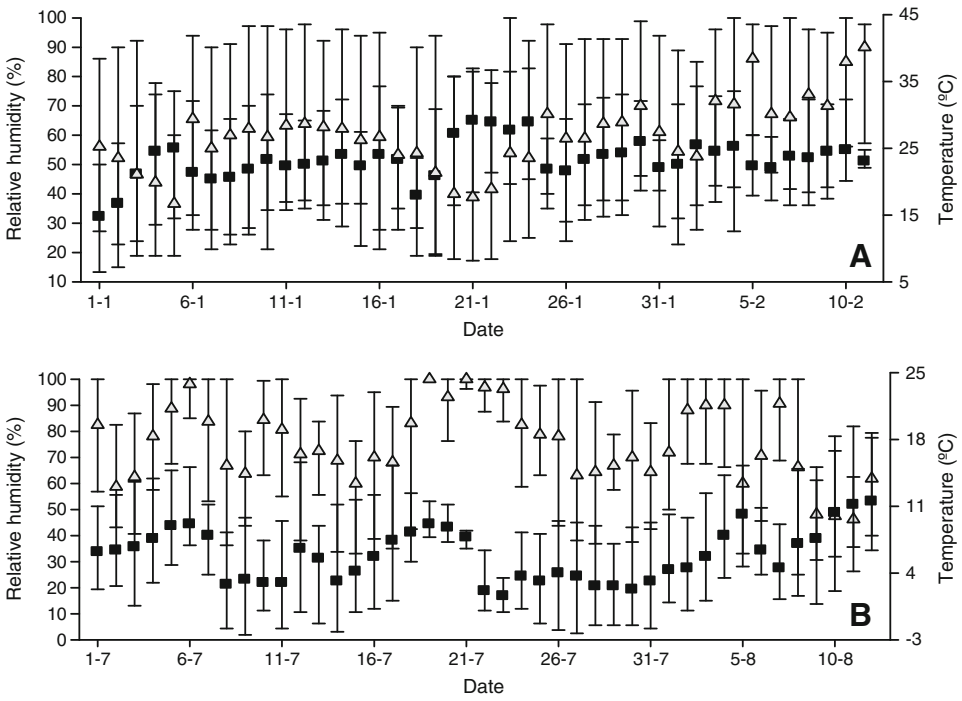


**Fig. 3** Temperature (*black square*) and relative humidity (*white triangle*)—range and mean registered on summer (**a**) and winter (**b**)

new and most striking finding in this study was that larval stages of the necrophagous insect *D. maculatus* were able to ingest *T. spiralis* ML which were afterward recovered alive by enzymatic digestion of the insect larval bodies. Further studies showed that these ML were fully infective for a new host.

Mechanical transmission of *T. spiralis* ML by dipterous and crustaceous organisms has been studied under laboratory conditions (Maroli and Pozio 2000; Hulebak 1980), but in the present study, the potential role of those insects as paratenic hosts was demonstrated. Thus, muscle larvae of *T. spiralis* that could survive for 1 week in corpses and for a period of 4 weeks in *D. maculatus* larvae might be taken into account as a potential source of infection.

In winter, the survival and RCI of recovered muscle larvae were minimally affected along the period of exposition. The low temperatures recorded in this season may account for the conservation of corpses (Centeno et al. 2002), but in an opposite way, the freezing action of extremely cold temperatures could affect ML survival as observed at 6 weeks of exposition.

Davidson et al. (2008) demonstrated that one of three isolates of *T. nativa* obtained from different latitudes near the arctic was more resistant when it was exposed in fox tissues to 7 weeks of freezing temperatures (−5°C) pointing out that intraspecific differences might explain variations in the tolerance to environmental stress. In this way, further studies on different isolates of *T. spiralis*, from wild or synanthropic animals, should be conducted to determine eventual differences in both survival and infection capability after exposition to environmental temperatures.

The significant reduction of the RCI of larvae compared to controls seems to indicate that direct transmission of the parasite is the most efficient way for the propagation of *T. spiralis* in nature. These data agree with Malakauskas and Kapel (2003) who found that the RCI of *T. spiralis* ML recovered from muscle rat tissue maintained at 5°C decreased from 168 up to 101 after 4 weeks of exposition. Similarly, Hill et al. (2007) reported a large reduction in the RCI (from 23, 5 to 8) of *T. spiralis* ML exposed for a period of 6 weeks in muscle tissue of a horse at 5°C. Theodoropoulos et al. (2000) have demonstrated that RCI of *T .spiralis* ML declined from 130.1 to 83.2 after an exposition period of 4 weeks in sheep muscle tissue to 5°C.

Probably and according to the low degradation of exposed muscle tissues observed here, the persistence of *T. spiralis* in muscle tissues might exceed the 6-week period of the winter considered in the present study. This is in consonance with the findings of Medina-Lerena et al. (2009) who conserved muscle-infected tissue in cold conditions and recovered live ML of *T. spiralis* up to 250 days of storage. *T. spiralis* ML has demonstrated to tolerate environmental stress until consumed by a new host which represents a high risk for scavengers as well as for pigs that are raised outdoors (Ribicich et al. 2010).

In conclusion, the present study demonstrated that encysted *T. spiralis* muscle larvae in corpses exposed to natural decomposition can survive and remain infective after the exposition of 1 week in summer and at least 6 weeks in winter. These data suggest that the environmental temperature and humidity, and the fauna associated to corpse decomposition, play an important role in the persistence of ML and therefore in the transmission of *T. spiralis* in nature.

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