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Coccidian parasites from birds at rehabilitation centers in Portugal, with notes on *Avispora bubonis* in Old World

Coccídios parasitas de aves em centros de reabilitação em Portugal, com notas sobre Avispora bubonis no Velho Mundo

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Abstract

Portugal has some rehabilitation centers for wild animals, which are responsible for the rehabilitation and reintroduction of birds, among other animals, into the wild. Coccidian parasites of these wild birds in rehabilitation centers are especially important because these centers can introduce coccidian species into new environments through the reintroduction of their respective hosts. In this context, the current study aimed to identify intestinal coccidia from wild birds at two rehabilitation centers for wild animals located in two municipalities of Portugal. Eighty-nine wild birds of 9 orders and 11 families were sampled, of which 22 (25%) were positive for Coccidia. *Avispora* spp. were found in raptors. Sporocysts of Sarcocystinae subfamily were recovered from owls. An *Isospora* sp. was found in *Turdus merula* Linnaeus, 1758, and an *Eimeria* sp. was found in *Fulica atra* Linnaeus, 1758. Among the coccidian species, *Avispora bubonis* (Cawthorn, Stockdale, 1981) can be highlighted. The finding of this species indicates that transmission of coccidians from the New World to the Old World may be occurring, potentially through dispersion by *Bubo scandiacus* (Linnaeus, 1758) through Arctic regions or by means of anthropic activities, and/or through other unknown mechanisms.

Keywords: Morphology, taxonomy, ecology, Coccidia, oocysts, raptors.

Resumo

Portugal possui alguns centros de reabilitação de animais silvestres, responsáveis pela reabilitação e reintrodução de aves, entre outros animais, na natureza. Os coccídios parasitas dessas aves silvestres em centros de reabilitação são especialmente importantes porque esses centros podem introduzir espécies de coccídios em novos ambientes através da reintrodução de seus respectivos hospedeiros. Neste contexto, o presente estudo visou identificar coccídios intestinais de aves silvestres em dois centros de reabilitação de animais silvestres localizados em dois municípios de Portugal. Oitenta e nove aves silvestres de 9 ordens e 11 famílias foram amostradas, das quais 22 (25%) foram positivas para coccídios. *Avispora* spp. foram encontradas em aves de rapina. Esporocistos de coccídios da subfamilia Sarcocystinae

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foram encontrados em corujas. Uma *Isospora* sp. foi encontrada em *Turdus merula* Linnaeus, 1758 e uma *Eimeria* sp. foi encontrada em *Fulica atra* Linnaeus, 1758. Entre as espécies de coccídios, *Avispora bubonis* (Cawthorn, Stockdale, 1981) pode ser destacada. O encontro dessa espécie indica que a transmissão de coccídios do Novo Mundo para o Velho Mundo pode estar ocorrendo, potencialmente através da dispersão por *Bubo scandiacus* (Linnaeus, 1758) pelas regiões árticas ou por meio de atividades antrópicas, e/ou através de outros mecanismos desconhecidos.

Palavras-chave: Morfologia, taxonomia, ecologia, Coccidia, oocistos, aves de rapina.

Introduction

Avian coccidiosis is a predominantly intestinal disease caused by obligate intracellular parasitic protozoa belonging to the subclass Coccidia (BERTO et al., 2014a; RUGGIERO et al., 2015). Over recent years, reports and descriptions of species of Coccidia have become relatively frequent. Duszynski et al. (2004) validated and grouped hundreds of species that have been found in several families and orders of the subclass Aves. However, many of these species need to be redescribed or better characterized in order to provide efficient and reliable identification in other hosts (DUSZYNSKI et al., 2004; BERTO et al., 2011). Another difficulty associated with identification of these species is host specificity. According to Duszynski et al. (2004), Berto et al. (2011) and the publications validated by these authors, Coccidia are specific at the family level of the host bird. However, new classifications in the subclass Aves are often suggested, especially in the order Passeriformes, because their families have been regrouped (CBRO, 2014; DEL HOYO et al., 2016; BRANDS, 2018).

Other factors that need to be considered in the specific identification of Coccidia of birds include: (1) the geographic ranges of the wild birds; (2) breeding of exotic birds near wild areas; (3) the legal trade and, especially, the illegal trade of wild birds, which, in addition to transporting birds and their coccidians, favors transmission due to the large quantities of oocysts that are shed from stressed or immunosuppressed birds that are victims of maltreatment; and (4) practices at rehabilitation centers for wild animals, which may fail to identify or quantify coccidians and may reintroduce birds that are shedding large quantities of oocysts, into environments other than their original ones (BERTO & LOPES, 2013).

Portugal is a potentially favorable country for a study model in this regard, since it has birds that have previously been described as hosts of several coccidian species, both in the wild and in captivity/breeding (BERTO et al., 2014b; CARDOZO et al., 2015, 2016, 2017). In this context, the aim of the current study was to identify coccidian species that were recovered from wild birds at two rehabilitation centers for wild animals located in two municipalities of Portugal. The study also aimed to address the possibilities and potentialities of transmission and dispersion of coccidians between hosts of the same family and/or hosts that are phylogenetically and ecologically close.

Materials and Methods

Fecal samples were collected from 80 birds that were kept in individual cages for rehabilitation and reintroduction into the wild at the Lisbon Center for Wild Animal Recovery (Centro de Recuperação de Animais Silvestres de Lisboa, LxCRAS), in Monsanto Forest Park, Lisbon, Portugal. In addition, samples were also collected from nine birds that were kept under these same conditions at the Wildlife Rehabilitation and Investigation Centre of Ria Formosa (Centro de Recuperação e Investigação de Animais Selvagens - Associação ALDEIA, RIAS-ALDEIA), Ria Formosa Natural Park, Olhão, Portugal. The samples were collected immediately after defecation and were placed in plastic vials containing 2.5% potassium dichromate (K₂Cr₂O₇) solution at 1:6 (v/v). In the laboratory, the samples were incubated at room temperature for 10 days. The oocysts were recovered by means of flotation in Sheather's sugar solution (specific gravity: 1.20). Morphological observations, photomicrographs and measurements were made using an Olympus BX40 microscope equipped with a digital camera (Olympus DP10). All measurements were made in micrometers and are given as the mean followed by the range in parentheses. The descriptions of oocysts and sporocysts followed the guidelines of Duszynski & Wilber (1997) and Berto et al. (2014a).

Results and Discussion

Distribution of Coccidia into orders, families and species

Birds of 9 orders and 11 distinct families were sampled (Table 1). Raptors (orders Accipitriformes, Falconiformes and Strigiformes) were the most representative in terms of both numbers of species (61%; 11/18) and specimens (78%; 69/89). The order with the greatest diversity of species sampled was Accipitriformes (28%; 5/18), followed by the orders Strigiformes (22%; 4/18) and Falconiformes (11%; 2/18). Falconiformes (31%; 28/89) and Strigiformes (31%; 28/89) were the most representative orders in terms of the numbers of specimens sampled, followed by the order Accipitriformes (15%; 13/89).

The current study emphasizes the finding that raptors predominate among the birds held at rehabilitation centers in Portugal, given that Tomás et al. (2017) also predominantly sampled raptors for a parasitic helminth survey. In contrast, in the New World, especially in the Neotropical region, Psittaciformes and Passeriformes are the predominant birds held at rehabilitation centers because of their beauty and vocalization and, consequently, the frequent seizures of these birds from illegal trading (BERTO & LOPES, 2013).

The total numbers of birds sampled, along with their prevalences according to order, family and species, are shown in Table 1. Among the 11 different families of birds to which the specimens that were sampled belonged, only those in the families Falconidae (Falconiformes), Rallidae (Gruiformes), Turdidae (Passeriformes), Strigidae and Tytonidae (Strigiformes) shed oocysts of Coccidia. These oocysts were initially non-sporulated but sporulated within Table 1. Prevalence of coccidian parasites among birds at two rehabilitation centers for wild animals in mainland Portugal, organized by order, family and species.

Orders/ Families/ Species	Samples		- Considius analise	т 1ч.ч ж
	Positive	Total	Coccidian species	Localities
Accipitriformes: Accipitridae				
Buteo buteo (Linnaeus, 1758)	0	6	_	LxCRAS
	0	1	-	RIAS
Circaetus gallicus (Gmelin, 1788)	0	1	_	LxCRAS
8	0	1	_	RIAS
<i>Elanus caeruleus</i> (Desfontaines, 1789)	0	2	_	LxCRAS
Hieranetus pennatus (Greelin 1788)	0	1	_	LxCRAS
Milnus migrans (Boddaert, 1783)	0	1	_	RIAS
Subtotale:	0	13		10110
Canzimulaiformos: Canzimulaidao	0	15		
Caprimulgue auropage Lippoous 1758	0	1		L CDAS
Caprimulgus europaeus Linnaeus, 1738	0	1	—	LXCNAS
	0	1		
Charadriiformes: Laridae	0	10		LCDAC
Larus sp.	0	13	-	LxCRAS
	0	1	-	RIAS
Subtotals:	0	14		
Ciconiiformes: Ciconiidae				
Ciconia ciconia (Linnaeus, 1758)	0	1	-	LxCRAS
Subtotals:	0	1		
Falconiformes: Falconidae				
Falco naumanni Fleischer, 1818	0	1	_	RIAS
Falco tinnunculus Linnaeus, 1758	5	27	<i>Avispora peneireiroi</i> (Cardozo, Berto, Caetano, Maniero, Fonseca, Lopes, 2016)	LxCRAS
Subtotals:	5 (18%)	28		
Gruiformes: Rallidae				
Fulica atra Linnaeus, 1758	1	1	Eimeria paludosa (Leger, Hesse, 1922)	LxCRAS
Subtotals:	1 (100%)	1		
Passeriformes: Passeridae	(
Passer domesticus (Linnaeus, 1758)	0	1	_	RIAS
Subtotals:	0	1		
Passeriformes: Turdidae	Ū	-		
Turdus merula Linnaeus, 1758	1	1	<i>Isospora lusitanensis</i> Cardozo, Berto, Fonseca, Tomás, Thada Lapas, 2015	RIAS
Subsector	1 (1000/-)	1	mode, Lopes, 2013	
	1 (100%)	1		
relecaniformes: Ardeidae	0	1		
<i>Nycticorax nycticorax</i> (Linnaeus, 1/58)	0	1	—	LXCRAS
Subtotals:	0	1		
Strigiformes: Strigidae				
Athene noctua (Scopoli, 1769)	12	18	<i>Avispora mochogalegoi</i> Cardozo, Berto, Caetano, Maniero, Santos, Fonseca, Lopes, 2017	LxCRAS
Bubo bubo (Linnaeus, 1758)	1	1	Avispora bubonis (Cawthorn, Stockdale, 1981)	LxCRAS
	0	1		RIAS
Strix aluco Linnaeus, 1758	0	1	_	LxCRAS
Subtotals:	13 (62%)	21		
Strigiformes: Tytonidae				
Tyto alba (Scopoli, 1769)	2	6	Frenkelia sp. or Sarcocystis sp.	LxCRAS
	0	1	1 / 1	RIAS
Subtotals:	2 (29%)	7		
Total:	22 (25%)	89		

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10 days after collection, with the exception of the oocysts/sporocysts of Sarcocystidae which were shed sporulated. No birds of the orders Accipitriformes, Caprimulgiformes, Charadriiformes, Ciconiiformes or Pelecaniformes were positive for Coccidia. The highest prevalences of Coccidia were observed in the families Strigidae and Tytonidae of the order Strigiformes (54%; 15/28), after disregarding those families in which only one bird was sampled, and this was positive.

As expected, the genera *Avispora* Schuster, Woo, Poon, Lau, Sivakumar, Kinne, 2016, *Frenkelia* Biocca, 1968, and *Sarcocystis* Lankester, 1882, presented the highest prevalence, since the birds sampled were predominantly raptors. Transmission of these coccidian genera depends on predation (*Frenkelia* or *Sarcocystis*) or is facilitated by it (*Avispora*). Thus, the predominance of these coccidians can be correlated with the predominance of the

raptors (birds of prey). In contrast, *Eimeria* Schneider, 1875, and *Isospora* Schneider, 1881, which are coccidians with direct transmission (fecal-oral), were less frequent because of the small samples of Passeriformes and Gruiformes (BERTO et al., 2014a; SCHUSTER et al., 2016).

Falconidae

Two different species from the family Falconidae were sampled: the lesser kestrel *Falco naumanni* Fleischer, 1818 and the common kestrel *Falco tinnunculus* Linnaeus, 1758. Only the common kestrels were positive for a coccidian species, which was identified as *Avispora peneireiroi* (Cardozo, Berto, Caetano, Maniero, Fonseca, Lopes, 2016). Its oocysts (Figure 1A) were described by Cardozo et al. (2016) as ellipsoidal with a bilayered



Figure 1. Photomicrographs of sporulated oocysts and sporocysts of *Avispora peneireiroi* from *Falco tinnunculus* (A), *Eimeria paludosa* from *Fulica atra* (B-C), *Isospora lusitanensis* from *Turdus merula* (D), Sarcocystid sporocyst from *Tyto alba* (E), *Avispora mochogalegoi* from *Athene noctua* (F) and *Avispora bubonis* from *Bubo bubo* (G-I). Sheather's sugar solution. Note the conoid (con), micropyle (m), polar granule (pg), refractile body (rb), Stieda body (sb), sub-Stieda body (ssb), sporocyst residuum (sr) and sporozoite (sz). Bars: 10 µm.

wall. They measured (n = 20) 47.1 (42-49) × 37.6 (34-40) μ m, with a shape index of 1.25 (1.2-1.4). No micropyle, oocyst residuum or polar granule was present. The sporocysts were subspherical, measuring (n = 20) 25.1 (24-27) × 24.3 (24-25) μ m. Stieda, sub-Stieda and para-Stieda bodies were absent. The sporocyst residuum was composed of many homogenous globules scattered throughout the periphery of the sporocyst. Sporozoites without striations discernible, but with one spherical refractile body and a nucleus.

This species was originally described in the genus *Caryospora* Leger, 1904 (CARDOZO et al., 2016). However, after the work of Schuster et al. (2016), all species of *Caryospora* that had been recorded in raptors were taxonomically transferred to the genus *Avispora*, based on the morphological, biological and molecular differences in *Caryospora* spp. between raptors and reptiles (UPTON et al., 1990; BERTO et al., 2014a; SCHUSTER et al., 2016).

Rallidae

From the family Rallidae, only one common coot (*Fulica atra* Linnaeus, 1758), was sampled, but it was positive for a coccidian species identified as *Eimeria paludosa* (Leger, Hesse, 1922). This coccidian was originally described in France, from this same host species (*F. atra*) and from common moorhens (*Gallinula chloropus* Linnaeus, 1758) (LEGER & HESSE, 1922). After a few decades, McAllister & Upton (1990) redescribed this same species from American coots (*Fulica americana* Gmelin, 1789). Recently, Yang et al. (2014) identified it genotypically from dusky moorhens (*Gallinula tenebrosa* Gould, 1846) in Australia.

In addition to this species, five other Eimeria spp. were recorded from Rallidae: (1) Eimeria bragini Dzerzhinskii & Kairooaev, 1989; (2) Eimeria mongolica Matschoulsky, 1941; (3) Eimeria porphyrulae Lainson, 1994; (4) Eimeria crecis Jeanes, Vaughan-Higgins, Green, Sainsbury, Marshall, Blake, 2013; and (5) Eimeria nenei (DUSZYNSKI et al., 2004; JEANES et al., 2013). However, E. paludosa has striking characteristic features that easily enabled its identification in the current work. Its oocysts (Figure 1B, C) were ovoidal with a bilayered wall and measured $(n = 15) 16.1 (15-20) \times 11.5 (10-14) \mu m$ with a shape index of 1.4 (1.3-1.6). A micropyle was present and prominent. Oocyst residuum was absent, but a large polar granule was present, usually located below the micropyle. The sporocysts were ovoidal, measuring (n = 15) 8.1 (6-10) \times 5.8 (5-8) µm. Stieda and sub-Stieda bodies were present. Para-Stieda bodies were absent. The sporocyst residuum was composed of fine granules scattered between sporozoites. The sporozoites had one spherical refractile body and a nucleus. In this regard, this present study provides the first records of E. paludosa from F. atra since the time of its original description in 1922 (LEGER & HESSE, 1922).

Turdidae

A single specimen of Eurasian blackbird (*Turdus merula* Linnaeus, 1758) was sampled and this was positive for *Isospora lusitanensis* Cardozo, Berto, Fonseca, Tomás, Thode, Lopes, 2015 (CARDOZO et al., 2015). Its oocysts (Figure 1D) were described by Cardozo et al. (2015) as subspherical to ovoidal with a smooth, bi-layered wall. They measured (n = 62) 26.4 (22-30) × 23.4 (19-27) μ m, with a shape index of 1.1 (1.0-1.3). Micropyles and oocyst residuum were absent, but a polar granule was present. The sporocysts were ellipsoidal, measuring (n = 13) on average 16.0 (15-18) × 10.9 (10-12) μ m. Stieda bodies were knob-like and sub-Stieda bodies were prominent and rounded. The sporocyst residuum was composed of scattered spherules. The sporozoites had one refractile body and a nucleus. Although isosporans from *T. merula* have been researched in several scientific studies in Europe, they have always been identified as *Isospora turdi* Schwalbach, 1959. However, in Cardozo et al. (2015), *I. lusitanensis* was described as a new species by accurately comparing its morphometric and morphological characteristics.

Strigidae

Three different owl species in Strigidae were sampled: (1) the little owl Athene noctua (Scopoli, 1769); (2) the Eurasian eagle-owl Bubo bubo (Linnaeus, 1758); and (3) the tawny owl Strix aluco Linnaeus, 1758. The specimen of S. aluco that was sampled was negative for Coccidia; however, 12 of the 18 little owls (67%) that were sampled were positive for Avispora mochogalegoi Cardozo, Berto, Caetano, Maniero, Santos, Fonseca, Lopes, 2017. The oocysts of this species (Figure 1F) were described by Cardozo et al. (2017) as ellipsoidal with a bilayered wall. They measured (n = 15) 38.9 (37-43) \times 32.9 (31-37) µm, with a shape index of 1.2 (1.1-1.2). There was no micropyle, oocyst residuum or polar granule. The sporocysts were subspherical, measuring (n = 15) 21.1 (20-24) × 20.1 (19-23) µm. Stieda, sub-Stieda and para-Stieda bodies were absent. The sporocyst residuum was composed of a compact subspherical mass of granules. The sporozoites had striations, one spherical refractile body and a robust nucleus. This was the first species originally described in the newly created genus Avispora.

The Eurasian eagle-owl sampled at the LxCRAS shed oocysts that were very similar to those of Avispora bubonis (Cawthorn, Stockdale, 1981). The incompatibility of this identification lies in the fact that A. bubonis was originally described from the great horned owl Bubo virginianus (Gmelin, 1788) in Canada, and thus from a New World owl (UPTON et al., 1986, 1990). Its oocysts (Figure 1G-I) were subspherical to ellipsoidal and measured $(n = 15) 45.4 (42-49) \times 37.4 (34-40) \mu m$, with a shape index of 1.2 (1.1-1.4). The wall was bi-layered and delicate, with a thickness of ~1.1 µm. The outer layer was thicker and clearer and the inner layer was thinner and darker. There was no micropyle, oocyst residuum or polar granule. The sporocysts were subspherical, measuring (n = 15) 25.1 (24-27) × 24.2 (23-25) μ m, with a shape index of 1.04 (1.0-1.1). Stieda, sub-Stieda and para-Stieda bodies were absent. The sporocyst residuum was granular and diffuse. The sporozoites had a prominent conoid at the anterior end, one refractile body and a nucleus.

The morphological and morphometric characteristic features of these oocysts were absolutely compatible with the description of *A. bubonis*, even in the smallest details such as the prominent conoid at the anterior end of the sporozoite (Figure 1H), which was highlighted by Upton et al. (1986, 1990). Thus, it becomes impracticable to identify these oocysts as another species or as a new species. At the same time, it would be unlikely that transmission occurred directly or in the same environment between these hosts, since they are not sympatric and inhabit distinct and distant continents. Hence, two hypotheses can be formulated to explain this transmission: (1) through anthropic activities, such as legal or illegal trading or breeding of exotic raptors in zoos or for falconry, etc., which could bring these naturally separated hosts closer together; and (2) through the geographic range of the snowy owl Bubo scandiacus (Linnaeus, 1758), which has a huge range, predominantly across Arctic regions, from western Scandinavia through northern Russia to Alaska, northern Canada and Greenland. This owl has also bred occasionally in Iceland and the UK and, in winter, they move further south into the USA, northern Europe and northern Asia (BIRDLIFE INTERNATIONAL, 2016). Thus, B. scandiacus is an owl potentially occurring in the New and Old World and which is sympatric with *B. bubo* and *B. virginianus*, and could have maintained and transmitted A. bubonis.

A finding of *A. bubonis* from *B. scandiacus* could confirm this transmission across the Arctic regions. Moreover, this has becomes an extremely important matter, since *B. scandiacus* is categorized as 'vulnerable' by the International Union for Conservation of Nature and Natural Resources (IUCN) (BIRDLIFE INTERNATIONAL, 2016). However, it is noteworthy that the IUCN study does not state that *B. scandiacus* is a host for *A. bubonis*. Nonetheless, identification of *A. bubonis* from owls in both the New World and the Old World indicates that *B. scandiacus*, or some other mechanism, has driven its dispersion from the New World to the Old World. Thus, further studies on *A. bubonis* may or may not confirm this hypothesis.

Tytonidae

The only specimen of the common barn owl (*Tyto alba* Scopoli, 1769) that was sampled in the RIAS was negative for Coccidia. However, two of the six common barn owls (33%) sampled at the LxCRAS were positive for sporocysts of Sarcocystidae. These sporocysts (Figure 1E) were reported by Berto et al. (2014b) to be ellipsoidal, and they measured (n = 15) 12.2 (11-13) × 9.9 (9-11) μ m with a shape index of 1.2 (1.1-1.3). The sporocyst residuum was composed of scattered large granules. The sporozoites were vermiform, with a refractile body and a nucleus.

Sarcocystidae is a family of coccidians characterized by formation of tissue cysts in intermediate hosts, which infect the definitive host after predation and/or ingestion. This family is divided into the subfamilies Toxoplasmatinae and Sarcocystinae. Among the distinctive features of these sub-families, the sporogony and wall morphology of the oocysts are noteworthy. In Toxoplasmatinae, the sporogony is exogenous and the oocysts have a well-defined wall; whereas, in Sarcocystinae the sporogony is endogenous and the oocysts have a very thin and delicate wall that rapidly ruptures, thus releasing the sporocysts (TENTER et al., 2002; BERTO et al., 2014a).

Therefore, the finding of sporocysts and/or oocysts with thin wall in the feces of owls indicates that a species of Sarcocystinae can be identified. This brings together the genera *Frenkelia* and *Sarcocystis. Frenkelia* spp. have never been reported from owls and so far have only been described from hawks. Therefore, the sporocysts identified in the current study are potentially from *Sarcocystis* sp.

A review of the literature on *Sarcocystis* sporocysts reported from owls was conducted by Berto et al. (2014b). After morphological and morphometrical comparisons with the sporocysts recovered from *T. alba* at LxCRAS, these were considered quite similar to the sporocysts described as *Sarcocystis dispersa* Cerná, Kolarova, Sulc, 1977, for which northern long-eared owls (*Asio otus* Linnaeus, 1758) and common barn owls (*T. alba*) are the definitive hosts and house mice (*Mus musculus* Linnaeus, 1758) are the intermediate hosts (CERNÁ, 1976; CERNÁ et al., 1978). These same remarks were made by Upton et al. (1990), also for sporocysts recovered from common barn owls (*T. alba*).

Conclusion

In the current study were identified the coccidian species *A. peneireiroi*, *A. mochogalegoi*, *A. bubonis*, *E. paludosa*, *I. lusitanensis* and a sarcocystid species from birds of two rehabilitation centers in Portugal. The birds at the rehabilitation centers were predominantly raptors. These birds presented moderate prevalences of coccidians of the genera *Avispora* and *Sarcocystis*, which are genera associated with the habit of predation. Among these coccidian species identified, *A. bubonis* can be highlighted. Identification of this species indicates that transmission of coccidians from the New World to the Old World may be occurring, potentially through dispersion by *B. scandiacus* through Arctic regions or by means of anthropic activities, and/or through other unknown mechanisms.

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