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**Desenvolvimento de um Modelo Experimental de
Hidrocefalia por Neurocisticercose**

Dissertação apresentada à
Faculdade de Medicina,
Universidade Estadual Paulista
“Júlio de Mesquita Filho”, Câmpus
de Botucatu, para obtenção do título
de Mestre em Bases Gerais da
Cirurgia

Orientador: Prof. Dr. Marco Antônio Zanini

**Botucatu
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Resumo

Desenvolvimento de um modelo experimental de hidrocefalia por neurocisticercose

Neurocisticercose é a doença parasitária mais comum do sistema nervoso central. É causada pela ingestão de ovos de *Taenia solium*, cujas larvas se instalam nos diversos espaços intracranianos e no parênquima encefálico. Uma de suas principais complicações é a hidrocefalia, que pode estar presente em até 30% dos casos. O tratamento da hidrocefalia na neurocisticercose é mais complexo que nas outras formas de hidrocefalia. As taxas de disfunção de derivação ventricular para o peritônio são altas, bem como as taxas de morbidade e mortalidade associadas. Recentes modelos experimentais de neurocisticercose têm sido estudados com foco na relação entre parasita e hospedeiro. Por outro lado, modelos experimentais de hidrocefalia existem há muito tempo e têm contribuído para o entendimento das lesões no tecido encefálico causadas pelo desbalanço entre produção, circulação e absorção do líquido. O objetivo deste trabalho foi desenvolver um modelo experimental de hidrocefalia induzida por neurocisticercose. Inoculamos cistos de *Taenia crassiceps* (cestodo análogo ao *T. solium*) no espaço subaracnóideo de ratos através de punção suboccipital da cisterna magna. Por ressonância magnética observamos dilatação do sistema ventricular e das cisternas basais. Exame microscópico demonstrou lesões estruturais no encéfalo induzidas pela hidrocefalia, bem como infiltrados inflamatórios nos compartimentos líquóricos. Assim, desenvolvemos um modelo que reproduz a hidrocefalia por neurocisticercose humana, em que há um componente mecânico de obstrução da circulação do líquido pelo parasita, bem como por inflamação desencadeada pela resposta do hospedeiro contra o parasita.

Palavras-chave: hidrocefalia; neurocisticercose; modelo experimental

Abstract

Development of an experimental model neurocysticercosis-induced hydrocephalus

Neurocysticercosis is the most common parasitic disease of the central nervous system. It is caused by the ingestion of eggs from *Taenia solium*, whose larvae lodge in the brain and intracranial compartments. One of the main complications of neurocysticercosis is hydrocephalus, which may be present in up to 30% of patients. Treatment of hydrocephalus in neurocysticercosis is more difficult than in other forms of hydrocephalus. There are high rates of ventricle-peritoneal shunt malfunction, morbidity and death. Recent experimental models of neurocysticercosis have focused on the parasite-host interaction. On the other hand, experimental models of hydrocephalus accomplished for the understanding of brains lesions determined by the imbalance of production, circulation and absorption of cerebro-spinal fluid. We aimed to develop an experimental model of neurocysticercosis-induced hydrocephalus. We inoculated cysts from *Taenia crassiceps* (a cestode similar to *T. solium*) in the subarachnoid space of rats through a suboccipital puncture of the cisterna magna. Magnetic resonance imaging showed ventricle and basal cisterns enlargement. Microscopic assessment demonstrated brain lesions induced by hydrocephalus and inflammatory infiltrates to the cerebro-spinal fluid compartments. These findings reproduced human neurocysticercosis-induced hydrocephalus: mechanical obstruction of the cerebro-spinal fluid circulation and inflammatory infiltrates from the host immune response against the parasite.

Keywords: hydrocephalus; neurocysticercosis; experimental model

Abreviaturas e Siglas

AQP-4 – aquaporina-4

CNS – central nervous system

CSF – cerebrospinal fluid

CT – computerized tomography

EUA – Estados Unidos da América

G – Gauge

HE – hematoxilina e eosina

IV – quarto (ventrículo)

kg – quilograma

ml – mililitro

mm – milímetro

mm³ – milímetro cúbico

MRI – magnetic resonance imaging

ms – milissegundo

°C – graus Celsius

RM – ressonância magnética

SNC – sistema nervosa central

T – tesla

Th – T helper cell

UNESP – Universidade Estadual Paulista

USA – United States of America

VP – ventricle-peritoneal (*shunt*)

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Introdução

Introdução

Neurocisticercose é a principal doença parasitária do sistema nervosa central (SNC) em todo o mundo (Flisser et al., 2003). É também uma causa importante de epilepsia adquirida na idade adulta em regiões endêmicas (Coyle, 2014). Apesar de seu potencial de erradicação através de medidas sanitárias, a neurocisticercose é uma doença negligenciada e permanece endêmica na América Latina, África Sub-Saariana e em algumas regiões da Ásia. Fluxos migratórios são responsáveis pelo aparecimento de novos casos nos Estados Unidos e Europa (Wallin e Kurtzke, 2004; Coyle et al., 2012; O'Neal e Flecker, 2015).

Neurocisticercose é uma doença pleomórfica (Carpio et al., 2013). Cistos de *Taenia solium* podem se alojar em qualquer espaço do sistema nervoso central. Na forma mais comum, os cistos são encontrados no parênquima cerebral e podem causar crises epiléticas, cefaleia e déficits focais (Garcia et al., 2014). Na forma extraparenquimatosa, os cistos podem se localizar na medula espinhal, no globo ocular ou nos compartimentos liquóricos. Cistos em forma de cachos de uva no espaço subaracnóideo causam a chamada neurocisticercose racemosa. Na forma extraparenquimatosa há várias complicações como vasculite (com consequentes acidentes vasculares cerebrais), neuropatia de nervos cranianos, meningite cisticercótica, hidrocefalia, hipertensão intracraniana e morte (Estañol et al., 1986; Mahale et al., 2015).

Cistos nos espaços liquóricos evocam respostas inflamatórias que causam endimetite, ventriculite e aracnoidite. Como consequência, há um desequilíbrio na livre circulação de líquido. Cistos intraventriculares também podem causar obstrução mecânica ao fluxo líquido. O resultado destes mecanismos é a hidrocefalia (Matushita et al., 2010).

Hidrocefalia na neurocisticercose é um problema desafiador para neurocirurgiões. Diferentemente de outras causas de hidrocefalia, o manejo clínico e cirúrgico é mais complexo (Colli et al., 2002). Drogas antiparasitárias rotineiramente utilizadas na forma parenquimatosa não são tão efetivas contra cistos nos compartimentos liquóricos e podem, inclusive, piorar a resposta inflamatória (García et al., 2002). Por outro lado, as derivações ventriculares utilizadas para hidrocefalia têm risco considerável de disfunções e infecções, demandando revisões cirúrgicas frequentes (Kelley et al., 2002). A neuroendoscopia é uma técnica que muito contribuiu no tratamento da hidrocefalia por neurocisticercose porém não é eficaz em todos os casos (Rangel-Castilla et al., 2009).

O tratamento da neurocisticercose permanece em discussão e, apesar dos avanços e evidências, ainda tem muitas questões a serem respondidas. Se por um lado, mais ensaios clínicos são necessários, por outro lado, a compreensão completa de mecanismos fisiopatológicos em humanos é limitada pela escassez de tecidos disponíveis e pela interferência do tratamento com corticoterapia. Assim, estudos com pesquisa básica são também necessários para determinar a natureza do processo inflamatório que ocorre na neurocisticercose (Nash et al., 2006). Estes estudos poderão habilitar a comunidade científica a compreender melhor os mecanismos e moduladores da inflamação e assim propor novos alvos terapêuticos.

Estudos com neurocisticercose experimental têm sido publicados, porém com ênfase na forma parenquimatosa da doença. Contribuições especiais vieram do grupo de Alvarez e Teale. Este grupo criou um modelo de inoculação do cestodo *Mesocestoides corti* na convexidade cerebral de camundongos. Seus resultados mostraram as vias de migração leucocitária dos vasos para o tecido cerebral, as proteínas do complexo juncional envolvidas no aumento de permeabilidade vascular para células inflamatórias

e o padrão celular recrutado na resposta contra o parasita (Cardona et al., 1999; Alvarez e Teale, 2007; Alvarez e Teale, 2008). No entanto, este cestodo tem a capacidade de penetrar e invadir o parênquima cerebral, o que não corresponde ao comportamento biológico da *T. solium* no sistema nervoso central.

Em revisão sobre modelos experimentais de neurocisticercose, este grupo de autores aponta um outro cestodo similar à *T. solium* que poderia ser utilizado: trata-se da *Taenia crassiceps*. Porém este parasita distorce as estruturas intracranianas, o que seria uma limitação de seu uso como modelo experimental (Alvarez et al., 2010). Por outro lado, a distorção do tecido nervoso é uma das características da forma extraparenquimatosa da neurocisticercose.

T. crassiceps é um platelminto cujos hospedeiros intermediários são roedores silvestres e cujos hospedeiros definitivos são lobos, raposas e cães. Este parasita tem uma grande capacidade proliferativa: os cisticercos podem ser extraídos de roedores e mantidos indefinidamente na cavidade peritoneal de camundongos, onde se reproduzem de forma assexuada por gemulação (Willms e Zarubian, 2010).

Matos-Silva e colaboradores (2012) inocularam *T. crassiceps* no parênquima cerebral de camundongos e observaram diferentes padrões de inflamação de acordo com a linhagem dos animais. Por acaso, observaram ventriculomegalia em alguns animais, porém este não foi o objetivo do estudo e nem recebeu maior detalhamento.

Finalmente, modelos experimentais de hidrocefalia têm sido estudados há muito tempo. De fato, há mais de cem anos, os estudos de Dandy foram fundamentais para a compreensão da fisiologia normal da circulação liquórica (Dandy e Blackfan, 1914). Desde então, diferentes substâncias foram empregadas na tentativa de induzir hidrocefalia experimentalmente (Hochwald, 1985). O agente mais comumente utilizado hoje é o silicato hidratado de alumínio, o caulim. A injeção cisternal de caulim é fácil,

barata e efetiva para induzir hidrocefalia em animais. Este modelo tem sido muito útil para a compreensão de alterações estruturais, comportamentais e radiológicas causadas pela hidrocefalia (Del Bigio, 1993; Lopes et al., 2009; Skjolding et al., 2010; Olopade et al., 2012). Porém a hidrocefalia induzida pelo caulim geralmente é rapidamente progressiva e causa adoecimento importante dos animais, o que limita análises a longo prazo e não reproduz com exatidão o que ocorre na hidrocefalia por neurocisticercose.

Assim, um modelo experimental de hidrocefalia por neurocisticercose é necessário para melhor compreensão dos mecanismos fisiopatológicos envolvidos nessa condição tão complexa da prática clínica.

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Hydrocephalus in neurocysticercosis: challenges for clinical practice and research needs

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Abstract

Neurocysticercosis is the leading parasitic disease of the central nervous system in the world. The clinical manifestations of neurocysticercosis are pleomorphic and depend on the location, number and size of the parasites in the nervous system. Cysts in the cerebrospinal fluid compartments cause the so-called racemose type of neurocysticercosis. Hydrocephalus is the most devastating complication of racemose neurocysticercosis. In this paper, we review relevant data on the epidemiology of hydrocephalus in neurocysticercosis, the pathophysiologic mechanisms involved, and the controversies regarding clinical and surgical management. The need for further research to better understand the disease is highlighted.

Keywords

Neurocysticercosis; hydrocephalus; cerebrospinal fluid; medical therapy; ventricle-peritoneal shunts; endoscopic neurosurgery

Introduction

Neurocysticercosis is the leading parasitic disease of the central nervous system in the world. Despite a significant reduction in its prevalence in developed countries, migratory flows have reintroduced the disease in Europe and in the United States ([Wallin and Kurtzke, 2004](#)). In developing countries, neurocysticercosis remains endemic, especially in Latin America, India and sub-Saharan Africa ([Flisser et al., 2003](#)). The disease is a major cause of acquired epilepsy of adulthood in endemic regions ([Coyle, 2014](#)).

Despite its potential for eradication, neurocysticercosis is a neglected disease, remains endemic, and can result in severe medical problems that do not receive

adequate attention ([Coyle et al., 2012](#)). Recent data from the United States show that neurocysticercosis is responsible for a large number of imaging exams, surgical procedures and hospitalizations, increasing public health expenditure ([O'Neal and Flecker, 2015](#)).

The clinical manifestations of neurocysticercosis are pleomorphic and depend on the location, number and size of the parasites in the nervous system. In the most common form, i.e., parenchymal neurocysticercosis, cysts lodge within the brain parenchyma and cause seizures, focal deficits and headache ([Garcia et al., 2014](#)). In the extraparenchymal form, cysts can be found in the orbit, in the cerebrospinal fluid (CSF) compartments, and in the spine ([Mahale et al., 2015](#)). Cysts in the CSF compartments cause the so-called racemose type of neurocysticercosis. In this form, cysts are found in the subarachnoid space, in the basal cisterns, and inside the ventricles. Degenerating cysts in the CSF space elicit an inflammatory response that can cause vasculitis (followed by stroke) and meningitis (cysticercal meningitis), which will lead to arachnoiditis characterized by reduced CSF absorption and obstruction of CSF flow ([Sinha and Sharma, 2009](#)). Furthermore, the cysts themselves can cause mechanical obstruction of CSF flow ([Matushita et al., 2011](#)). In these situations, the impaired CSF absorption and obstruction of CSF flow can cause hydrocephalus.

Hydrocephalus

Hydrocephalus is caused by an imbalance in the production, absorption and circulation of CSF. Different types of damage to the central nervous system (CNS), such as trauma, infections, vascular injuries and neoplasms, can trigger acquired

hydrocephalus. Congenital hydrocephalus is also common and is related to CNS malformations and intrauterine injuries.

The standard treatment of hydrocephalus consists of the diversion of CSF flow either to other body compartments, such as the peritoneal cavity, or to the CNS itself by means of third ventriculostomy. Ventriculoperitoneal (VP) shunts are the most common tool to treat hydrocephalus. However, patients with VP shunts are at increased risk of shunt malfunction and infection ([Bayston, 1994](#)).

Treatment of hydrocephalus in neurocysticercosis is more complex due to the pleomorphism of the disease ([Carpio, 2013](#)). Hydrocephalus is an important cause of intracranial hypertension symptoms in patients with neurocysticercosis and results in higher rates of morbidity and associated death ([Sotelo and Marin, 1987](#); [Cuetter et al., 2002](#); [Sotelo, 2011](#)). Racemose neurocysticercosis is therefore called the malignant form of the disease ([Estañol et al., 1986](#)). Associated hydrocephalus makes the disease even more aggressive. Treatment of hydrocephalus in neurocysticercosis lacks optimal results and continues to be a matter of debate. Hydrocephalic patients with neurocysticercosis are at higher risk of shunt malfunction and infection and therefore require a larger number of revision surgeries. The more surgeries a patient undergoes, the higher is the mortality ([Agapejev et al., 2007](#)).

Neuroendoscopy has become a useful tool in the management of neurocysticercosis-associated hydrocephalus ([Zymberg, 2013](#)). This surgical procedure permits the removal of cysts in the ventricles and fenestration of the third ventricle, creating a new pathway for CSF flow. However, as stated earlier, the pleomorphism of the disease characterized by the presence of cysts in any CSF compartment renders hydrocephalus in neurocysticercosis a major challenge to neurologists and neurosurgeons.

Epidemiology of hydrocephalus in neurocysticercosis

Hydrocephalus occurs in about 30% of patients with neurocysticercosis ([Estanõl et al., 1986](#)). In 16% of these patients, the symptoms of hydrocephalus are responsible for the onset of the disease ([Wallin and Kurtzke, 2004](#)). This proportion of hydrocephalus increases to 64% among patients with the extraparenchymal form of the disease ([Figueroa et al., 2011](#)).

Ventriculoperitoneal shunt is the most common surgical procedure for the management of hydrocephalus in neurocysticercosis. However, the procedure is associated with a high risk of malfunction and infection ([Kelley et al., 2002](#)). Hydrocephalus and shunt-related complications increase the overall mortality of patients with neurocysticercosis ([DeGiorgio et al., 2002](#)). The first experience from [Loyo et al. \(1980\)](#) and [McCormick et al. \(1982\)](#) showed 26% and 20% mortality, respectively, in patients with VP-shunted hydrocephalus. [Kelley et al. \(2002\)](#) reported a rate of shunt failure of 57%, most of them occurring during the first 6 months after surgery. [Colli et al. \(2002\)](#) found a rate of 25.2% of bacterial shunt infection and 18.1% mortality in patients with VP shunts during the follow-up period. In the series of [Agapejev et al. \(2007\)](#), 53.6% of the patients required at least one revision and up to seven revisions were necessary in some cases. The authors observed a correlation between the number of shunt revisions and overall mortality.

Recent series continue to show high rates of shunt failure. [Torres-Corzo et al. \(2010\)](#) found a rate of shunt malfunction of 27.2%, but the combination with endoscopic third ventriculostomy decreased this rate to 6.6%.

Mechanisms of hydrocephalus in neurocysticercosis

There are two main pathophysiologic mechanisms underlying hydrocephalus in neurocysticercosis: mechanical obstruction and inflammation ([Estañol et al., 1983](#)). It is generally accepted that the cysts enter the ventricular cavity through the choroid plexus ([Proaño et al., 2009](#)) and then migrate into the ventricles. Once they reach a larger size, the cysts can obstruct smaller sites of the ventricular system: the foramina of Monro, the cerebral aqueduct, and the fourth ventricle and its exit into the subarachnoid space, including the foramina of Luschka and Magendie ([McCormick et al., 1982](#)). This obstruction can be slow or acute so that symptoms of hydrocephalus are acute or progress chronically. The free movement of cysts in the ventricles can cause transient CSF obstruction and transient symptoms of hydrocephalus ([Shandera and Kass, 2006](#)).

On the other hand, the cysts can elicit inflammatory reactions when in contact with the ependymal surface inside the ventricles. The fibrotic scars secondary to the inflammatory reaction obstruct the cerebral aqueduct and the foramina of Luschka and Magendie ([Lobato et al., 1981](#)). This hypothesis is supported by ancient autopsy data and by indirect findings of inflammation on contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) scans and intraoperative visualization of an abnormal ventricular surface ([Kimura-Hayama et al., 2010](#)).

In the subarachnoid space of the basal cisterns, inflammation is also responsible for disturbances of CSF circulation. Inflammation of the basal cisterns is also seen by contrast enhancement on MRI and during surgical exposure. The inflammatory process in the subarachnoid space triggers vasculitis which, in turn, can cause stroke ([Garcia and Del Brutto, 2003](#)). Yet another inflammatory mechanism could be explained by the high CSF protein level that increases CSF viscosity ([Amelot and Faillot, 2014](#)) and diminishes its free flow throughout the subarachnoid compartments.

In the brain parenchyma, cysts relapse different antigen complexes during different stages of development ([Alvarez et al., 2008](#)). This is a mechanism of the parasite to evade the host immune response. In the CSF compartments, the death of the parasite also relapses many different antigen complexes that cause intense inflammatory reactions ([Matushita et al., 2011](#)). Additionally, circulating parasite antigens may be detectable in patients with hydrocephalus secondary to neurocysticercosis even in the absence of parasites on radiologic imaging ([Garcia et al., 2002](#)).

In fact, the pathophysiologic mechanisms underlying neurocysticercosis-associated hydrocephalus, including inflammatory mediators, chemotactic factors and specific antigenic fractions, are not fully understood. One reason is the interference of drugs administered to patients, such as corticosteroids and cysticidal therapy, and the second the lack of available tissues for biopsy and histologic assessment. Basic and experimental research is needed to clarify these issues.

Controversies regarding the medical treatment of racemose neurocysticercosis and implications for hydrocephalus

It is well accepted that the use of anthelmintic drugs improves the overall prognosis of patients with neurocysticercosis ([García et al., 2002](#)). The first studies on the use of Praziquantel and Albendazole for the treatment of neurocysticercosis were concerned about the risk of symptom worsening since acceleration of the parasite's death could exacerbate local inflammation ([Coyle, 2014](#)). Hence, the concomitant use of corticosteroids was proposed. Today, except for the risk of intense brain edema in patients with a high parasite burden, specialist consensus recommends the medical

treatment of viable parenchymal cysts. High-level evidence indicates better seizure control if patients are treated ([Garcia et al., 2004](#)).

On the other hand, medical treatment of racemose neurocysticercosis has been controversial for a long time. The risk of intense inflammatory reactions in the CSF space and acute hydrocephalus has cautioned physicians ([García et al., 2002](#)). There are reports of cases in which simple treatment of intestinal teniasis has triggered neurologic symptoms ([Ramos-Zúñiga et al., 2013](#)). Patients with hydrocephalus and elevated intracranial pressure are at high risk of death if exposed to anthelmintic drugs without previous shunt ([DeGiorgio et al., 2002](#)).

Disappointingly, the bioavailability of these drugs in the CSF is low. Consequently, they may not prevent parasite growth, but only induce the release of antigens that perpetuate chronic inflammation ([Takayanagui, 2004](#)). Many authors therefore advocate the surgical excision of large cysts in the basal cisterns or in the sylvian fissure prior to medical treatment. However, new studies using higher doses of Albendazole combined with Praziquantel and corticosteroids have shown a reduction and even the disappearance of cysts in the CSF compartments ([Proaño et al., 2001](#)).

When hydrocephalus is present and cysts can be excised surgically, current guidelines recommend the need for cyst removal prior to medical therapy. The death of cysts inside the ventricles can cause ependymitis, which creates strong adhesion of the cyst to the ventricular wall. This adhesion makes surgical excision difficult and more risky, limiting the possibility of mechanical desobstruction of CSF pathways. After surgery, medical therapy can be initiated to treat remaining cysts ([García et al., 2002](#)).

Contrast enhancement of ependymal or arachnoid cysts in the presence of hydrocephalus at diagnosis and prior to any treatment should be done with caution. Surgical excision may be difficult and medical therapy can worsen symptoms. In these

cases, hydrocephalus should be treated first in order to avoid an acute increase in intracranial pressure ([García et al., 2002](#)) and anthelmintic treatment can be initiated thereafter. [Kelley et al. \(2002\)](#) found that post-shunt anthelmintic treatment improves shunt longevity. Little evidence is available to agree or disagree with their proposal and further basic and clinical studies are therefore needed

Surgical management of hydrocephalus in neurocysticercosis

For a long time, VP shunts have played a key role in the surgical management of hydrocephalus. In neurocysticercosis-associated hydrocephalus, VP shunts somehow guaranteed the control of intracranial pressure, especially if anthelmintic drugs were to be administered to patients at risk of an intense inflammatory response ([García et al., 2002](#)).

In addition to the risk of infection itself, VP shunts are associated with other complications in the management of hydrocephalus in neurocysticercosis. Cysts can be sucked into the proximal catheter, leading to obstruction of the drainage system. Additionally, mucoid material and inflammation-related debris can obstruct the VP catheters ([Rangel-Castilla et al., 2009](#)). In fact, protein content is higher in the CSF of patients with racemose neurocysticercosis, a fact predisposing to shunt obstruction ([Bazan et al., 2015](#)). Many patients with neurocysticercosis-induced hydrocephalus therefore require frequent shunt revisions ([Apuzzo et al., 1984](#)). Additionally, VP shunts change the hydrodynamics of CSF, i.e., CSF flow through the ventricles is different in the presence of a VP shunt. In this respect, cysts in the posterior fossa may be drawn into the supratentorial compartment ([Rangel-Castilla et al., 2009](#)).

The introduction of endoscopy in neurosurgery changed the management of hydrocephalus ([Di Rocco et al., 2006](#)). Today, non-communicating hydrocephalus, i.e., hydrocephalus due to mechanical obstruction of CSF pathways, can be managed with ventricular endoscopy that permits visualization of the ventricular cavities, biopsy procedures, removal of intraventricular lesions, and fenestration of the third ventricle directly into the subarachnoid basal cisterns. This procedure has the advantage that it does not require permanent devices, decreasing the risk of shunt-associated infection. Advances in the technique using flexible endoscopes permitted access to the occipital horn, posterior portion of the third ventricle, and cerebral aqueduct. Neurosurgeons are now able to remove cysts that could only be accessed by craniotomy, which is technically demanding and associated with major complications ([Rangel-Castilla et al., 2009](#)).

Although endoscopy means an advance in the management of hydrocephalus in neurocysticercosis, it does not solve all associated complications. First, endoscopic surgeries have their own risks, mainly the difficulty of managing intraoperative bleeding. Second, the removal of cysts attached to the ependyma or vascular structures is dangerous, irrespective of the surgical technique ([Goetl et al., 2008](#)). Finally, hydrocephalus in neurocysticercosis may also be communicating; in these cases, the opening of the third ventricle to the subarachnoid space is not effective.

All these points could be summarized in one reason: the multifactorial nature of hydrocephalus in neurocysticercosis.

Future research needs

The laboratory understanding of a disease is a pivotal step in the clinical management of patients. Experimental models provide new insights and permit the development of new therapeutic strategies. Today we understand better the mechanisms underlying the parasite-host interactions in parenchymal neurocysticercosis, as well as the parasite's strategies to evade from inflammation and the routes of leukocyte migration from the CNS vasculature to the site of disease ([Terrazas et al., 1998](#); [Alvarez and Teale, 2007](#); [Alvarez et al., 2008](#)).

Further human studies and new animal models are needed to determine the characteristics of inflammation in the extraparenchymal form of neurocysticercosis. The results of such studies could contribute to the identification of agents that are able to control inflammation and, finally, to prevent hydrocephalus or at least to manage it properly ([Nash et al., 2006](#)). The malignant form of neurocysticercosis requires a better understanding and more effective treatment.

Conclusions

Neurocysticercosis remains endemic in developing countries and has once again emerged as a public health concern in developed countries. Its main devastating complication is hydrocephalus, which is responsible for high rates of hospitalization, disability, and death. Neurocysticercosis is a pleomorphic disease and hydrocephalus in neurocysticercosis is also pleomorphic and multifactorial. Mechanical obstruction and inflammation play a key role in the pathophysiology of the disease. Anthelmintic drugs and surgical options have limitations since they are unable to solve all problems satisfactorily. Reproducing extraparenchymal neurocysticercosis and hydrocephalus in

the laboratory may contribute to a better understanding of the condition and to the development of new therapeutic approaches.

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Justificativa

Justificativa

Neurocisticercose é uma doença comum e a hidrocefalia é uma complicação frequentemente associada. Embora haja na literatura trabalhos publicados sobre neurocisticercose experimental e hidrocefalia experimental, não há um modelo que combine as duas condições. A simulação laboratorial da hidrocefalia induzida por neurocisticercose será útil para melhor compreender os mecanismos fisiopatológicos envolvidos, como também para testar novos alvos de terapia.

Objetivos

Objetivos

- ✓ Desenvolver um modelo experimental de hidrocefalia induzida pela inoculação cisternal de *T. crassiceps*;
- ✓ Avaliar o tempo e o mecanismo de desenvolvimento de hidrocefalia em ratos após a inoculação cisternal de cistos de *T. crassiceps*.

Materiais e Métodos

Materiais e Métodos

O projeto de pesquisa foi aprovado pela Comissão de Ética no Uso de Animais, sob o protocolo 1028/2013, seguindo as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal.

Foram utilizados 23 ratos Wistar machos pesando entre 150 e 250g. Os animais foram mantidos em condições sanitárias adequadas, em ambiente com controle de temperatura (21.°C) e alternância de luz-escuro. Os animais receberam ração e água *ad libitum*.

Delineamento Experimental

O estudo foi dividido em duas etapas. Na primeira, testamos a ocorrência de hidrocefalia comparando o resultado da inoculação dos parasitas com a clássica injeção cisternal de caulim. Na segunda, estudamos detalhes das alterações induzidas pela inoculação dos parasitas nos animais.

Assim, na primeira etapa utilizamos 17 animais: 10 receberam inoculação de cistos, 5 receberam injeção de caulim e 2, de soro fisiológico. Após um mês de inoculação, foi realizada ressonância magnética em 5 dos 10 animais do grupo cistos e em todos os animais do grupo caulim e salina. A seguir, estes doze animais foram eutanasiados. Os 5 animais restantes foram submetidos a ressonância magnética após 3 meses.

Para a segunda etapa, utilizamos 11 animais (5 da etapa anterior e outros 6 novos animais). Todos eles foram submetidos a estudo de ressonância magnética com 4 meses após a inoculação. A eutanásia foi realizada a seguir e os encéfalos foram retirados para exame microscópico.

Parasitas e Inoculações

Cistos de *T. crassiceps* foram cedidos pela Faculdade de Medicina Veterinária e Zootecnia da UNESP, *campus* de Botucatu. Os cistos foram mantidos na cavidade peritoneal de camundongos (*Mus musculus*) (figura 1). Após retirada asséptica dos cistos, eles foram selecionados de acordo com sua viabilidade (figura 2), sendo selecionados para inoculação aqueles com até 3mm.

Os ratos foram anestesiados com solução de xilazina (20mg/ml) e cetamina (100mg/ml) por via intraperitoneal na dose de 0,1ml/kg. A seguir, foram feitas as inoculações. Cada rato foi inoculado com 50 cistos em meio de salina fisiológica num volume total de 0,1ml, através de punção cisternal suboccipital com agulha de 25G (figura 3). Para injeção de caulim, utilizou-se 0,1ml de solução de silicato de alumínio hidratado a 25%. Os animais foram submetidos ao mesmo protocolo de anestesia. Para a injeção de soro fisiológico, utilizou-se 0,1ml de solução a 0,9%.

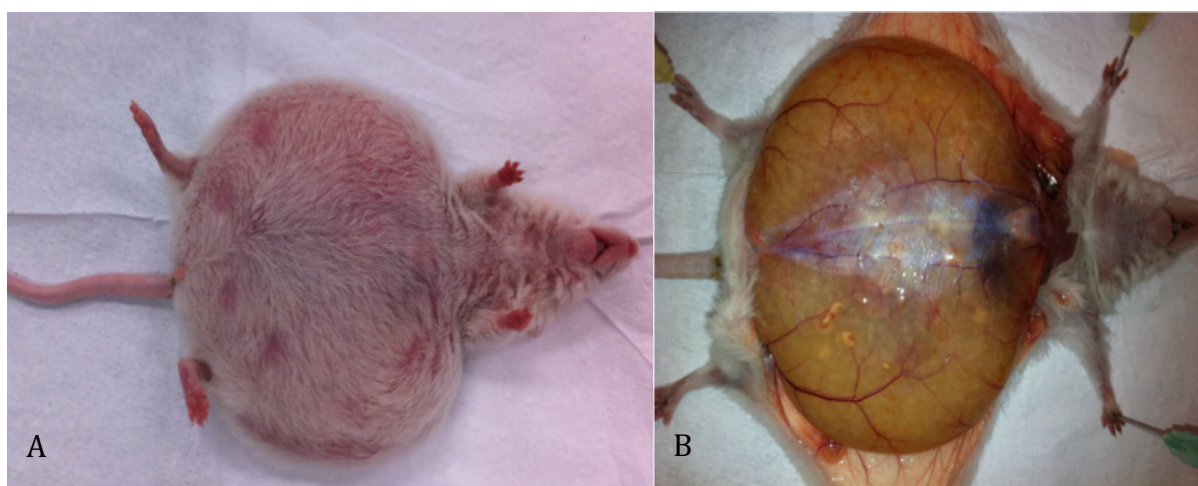


Figura 1: Camundongo (*M. musculus*) com cistos de *T. crassiceps* mantidos em sua cavidade peritoneal. Em A, observa-se o aumento do abdome. Em B, após a abertura de pele e tecido subcutâneo, é evidente a distensão da cavidade peritoneal.

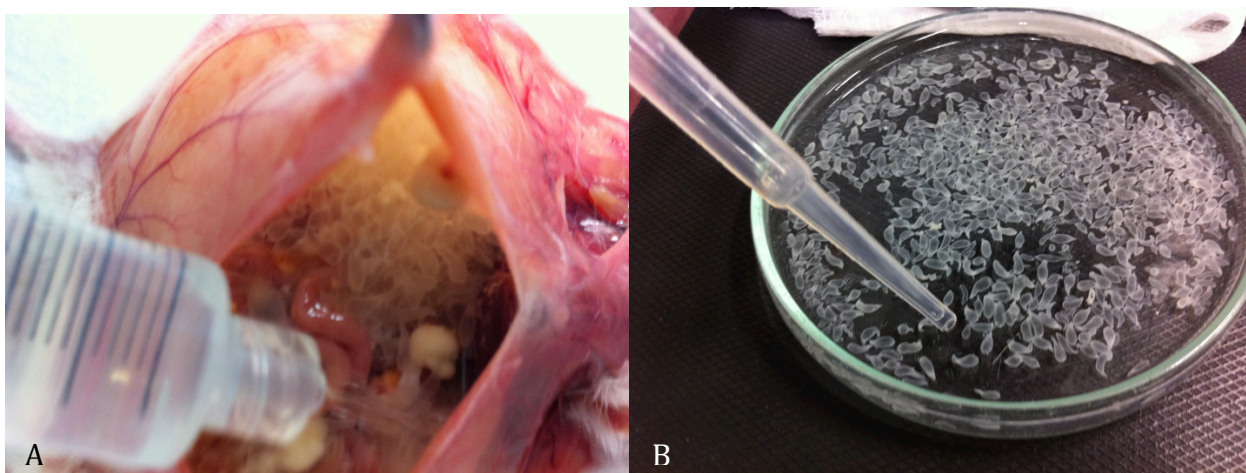


Figura 2: Após abertura do peritônio, os cistos foram retirados de forma asséptica (A) e, então, selecionados manualmente de acordo com sua viabilidade e tamanho (B).

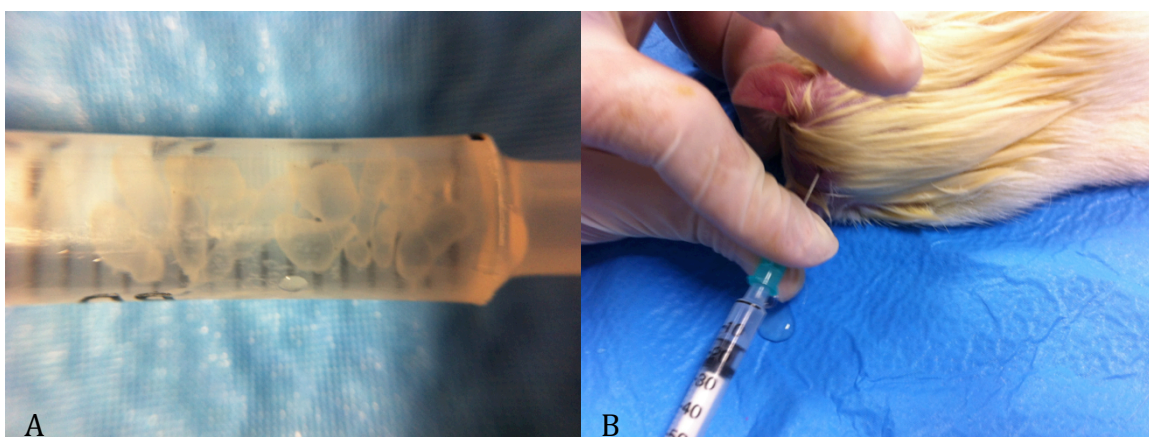


Figura 3: Os 50 cistos selecionados em solução fisiológica (A) foram então, inoculados em cada rato a partir de punção cisternal suboccipital (B).

Análise Radiológica

Para avaliação da ocorrência de hidrocefalia os animais foram submetidos a ressonância magnética (RM). Foi utilizado aparelho Esaote modelo Vet-MR de 0,25T. Para o protocolo de aquisição das imagens, foi utilizado T2 eco-gradiente com espessura de 0,6mm, tempo eco de 5ms e tempo repetição de 10ms. Para o exame, os animais foram submetidos ao mesmo protocolo de anestesia previamente descrito.

Para quantificação do volume ventricular, foi utilizado o software ITK-SNAP (versão 3.0.0, EUA). A partir das imagens de ressonância magnética, foi feita segmentação manual da área ventricular em cada uma das imagens, pixel a pixel, nos três eixos: transversal, sagital e longitudinal. A partir da soma das áreas foi determinado o volume ventricular, criando-se também, uma representação tridimensional do sistema ventricular dos animais.

Análise Histológica

Após o estudo de ressonância magnética, os animais foram eutanasiados com *overdose* de pentobarbital. Foi, então, realizada toracotomia para exposição cardíaca e dos vasos da base para realização de perfusão cardíaca com solução fisiológica seguida de formalina tamponada. Após a remoção dos encéfalos, eles foram fotografados e fixados em solução tampão com formol a 10% durante 24h. A seguir, os encéfalos foram seccionados ao nível do quiasma óptico e do IV ventrículo. As secções foram desidratadas em banhos de álcool e parafinizadas. Os cortes histológicos foram feitos com espessura de 3 μ m e corados com hematoxilina e eosina (HE).

Para a análise histológica, utilizou-se método estereológico de Mouton (2014), focando na dilatação ventricular, alterações do epêndima, plexo coróide e do parênquima.

Análise Estatística

A análise estatística comparou o volume ventricular entre os grupos de animais com cistos, caulim e salina. Para esta comparação, foi utilizado o teste não paramétrico de Kruskal-Wallis seguido do teste de Dunn para comparações múltiplas. As diferenças

foram consideradas estatisticamente significante se $p < 0,05$. Foram utilizados os *softwares* SPSS (versão 21.0), R (versão 2.11.0) e GraphPad (versão 5.0).

Resultados

Apresentados na forma de artigos científicos

Paper 2 – Artigo com os resultados da primeira etapa do trabalho

Artigo submetido à revista Acta Cirúrgica Brasileira (FI 0,661)

Development of an experimental model of neurocysticercosis-induced hydrocephalus

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Abstract

Purpose: To develop an experimental model of neurocysticercosis-induced hydrocephalus

Methods: There were used 17 rats. Ten animals were inoculated with *Taenia crassiceps* cysts into the subarachnoid space. Five animals were injected with 0.1ml of 25% kaolin (a standard solution for the development of experimental hydrocephalus) and two animals were injected with saline. Magnetic resonance imaging (MRI) was used to evaluate enlargement of the ventricles after one or three months of inoculation. Volumetric study was used to quantify the ventricle enlargement.

Results: Seven of the 10 animals in the cyst group developed hydrocephalus, two of them within 1 month and five within 3 months after inoculation. Three of the five animals in the kaolin group had hydrocephalus and none in the saline group. Ventricle volumes were significantly higher in the 3-months MRI cyst subgroup than in the 1-month cyst subgroup. Differences between cyst subgroups and kaolin group did not reach statistical significance.

Conclusions: Our findings suggest that the developed model may reproduce the human condition of neurocysticercosis-related hydrocephalus, which exhibits a slowly progressive chronic course.

Keywords

Neurocysticercosis; hydrocephalus; magnetic resonance imaging; cerebrospinal fluid

Introduction

Neurocysticercosis is the most common parasitic disease of the central nervous system. The clinical manifestations of neurocysticercosis are pleomorphic and depend on the number and developmental stage of the parasite and site affected, as well as on the immune response of the patient.^{1,2} Cysts located in the basal cisterns and ventricles are associated with a more aggressive course. This condition is called extraparenchymal neurocysticercosis and can manifest as vasculitis, cysticercal meningitis, hydrocephalus and intracranial hypertension.^{3,4}

Hydrocephalus is the result of chronic inflammatory arachnoiditis or mechanical obstruction of cerebrospinal fluid (CSF) flow.⁴⁻⁶ The condition occurs in about 30% of patients with neurocysticercosis and is associated with high rates of mortality.^{4,7} The treatment of choice in these cases is surgery consisting of the microsurgical removal of cysts, when possible, or CSF shunts.⁴ Although CSF shunts are the most widely employed procedure, they are associated with several complications such as infection and dysfunction, which require reoperations.^{8,9} Thus, hydrocephalus is the malignant form of neurocysticercosis *par excellence*. Several studies of experimental neurocysticercosis focusing on immune responses and on parasite-host interactions have been published^{10,11} but none of them focused on hydrocephalus.

The complexity of the relation between host and parasite, modulated by use of corticoids in clinical practice, make it difficult to fully understand the pathophysiologic mechanisms responsible for determining hydrocephalus in neurocysticercosis. This way, an animal model to help understand the inflammatory response is critical.

The aim of the present study was to develop an experimental model of neurocysticercosis-induced hydrocephalus in an attempt to reproduce a common and challenging clinical problem in the laboratory.

Materials and methods

The animals were handled according to ethical guidelines and current legislation. The board of the local Ethics Committee on Animal Use approved the project.

Seventeen adult Wistar rats (*Rattus norvegicus*) weighing 150 to 250 g were used. The animals were kept under adequate sanitary conditions in a temperature-controlled room (21°C) under light-dark control. Food and water were available *ad libitum*.

Parasites and inoculation

Taenia crassiceps cysts were maintained in the peritoneal cavity of mice (*Mus musculus*). After aseptic removal, the cysts were injected into the cisterns of rats by suboccipital puncture with a 25G needle. All animals were submitted to general anesthesia by intraperitoneal injection of a mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml) at a dose of 0.1 ml/kg.

Experimental groups

The animals were divided into three groups: cyst group (subarachnoid injection of 50 cysts, n = 10), kaolin group (subarachnoid injection of 0.1 ml of 25% kaolin – hydrated aluminum silicate, a standard solution to induce experimental hydrocephalus^{12, 13}; n = 5), and sham group (subarachnoid injection of 0.1 ml of 0.9% saline, n = 2).

Radiologic assessment

Magnetic resonance imaging (MRI) using a 0.25 T equipment (Esaote model Vet-MR) was performed to verify the development of hydrocephalus. The protocol consisted

of T2-weighted gradient echo acquisitions (0.6-mm thickness; T_{eco} = 5 ms; T_{repetition} = 10 ms). MRI was performed within one month after the procedure in the kaolin and sham groups and also in 5 animals of the cyst group. The other 5 animals of the cyst group were submitted to MRI 3 months after inoculation. The animals were euthanized after MRI. Ventricle volume was assessed using the ITK-SNAP software (version 3.0.0, USA) to determine the severity of hydrocephalus.

Statistical analysis

The statistical analysis was performed using the softwares SPSS version 21.0, R version 2.11.0 and GraphPad version 5.0. For comparison of ventricle volume we used the Kruskal-Wallis nonparametric test followed by the Dunn test for multiple comparisons. Differences were considered statistically significant if $p < 0.05$.

Results

In the kaolin group, 3 of the 5 animals developed hydrocephalus (Figure 1A). Volumetric analysis in this group showed a median ventricle volume of 24.4 mm³. In the cyst group, 7 of the 10 animals developed hydrocephalus (Figure 1B and 1C), which was more evident in the subgroup of animals that underwent MRI 3 months after inoculation (5/5 animals, median ventricle volume = 120 mm³) than in the subgroup submitted to MRI 1 month after inoculation (2/5 animals, median ventricle volume = 4.8 mm³). No animal of the sham group had hydrocephalus (Figure 1D; mean ventricle volume = 3.0 mm³). Ventricle volumes were significantly higher in the 3-months MRI cyst subgroup than in the 1-month cyst subgroup. Differences between cyst subgroups and kaolin

group did not reach statistical significance ($p > 0.05$). The data are summarized in Table 1. We did not observe any changes on neurobehavioural patterns of cyst-inoculated animals through the experimental course period.

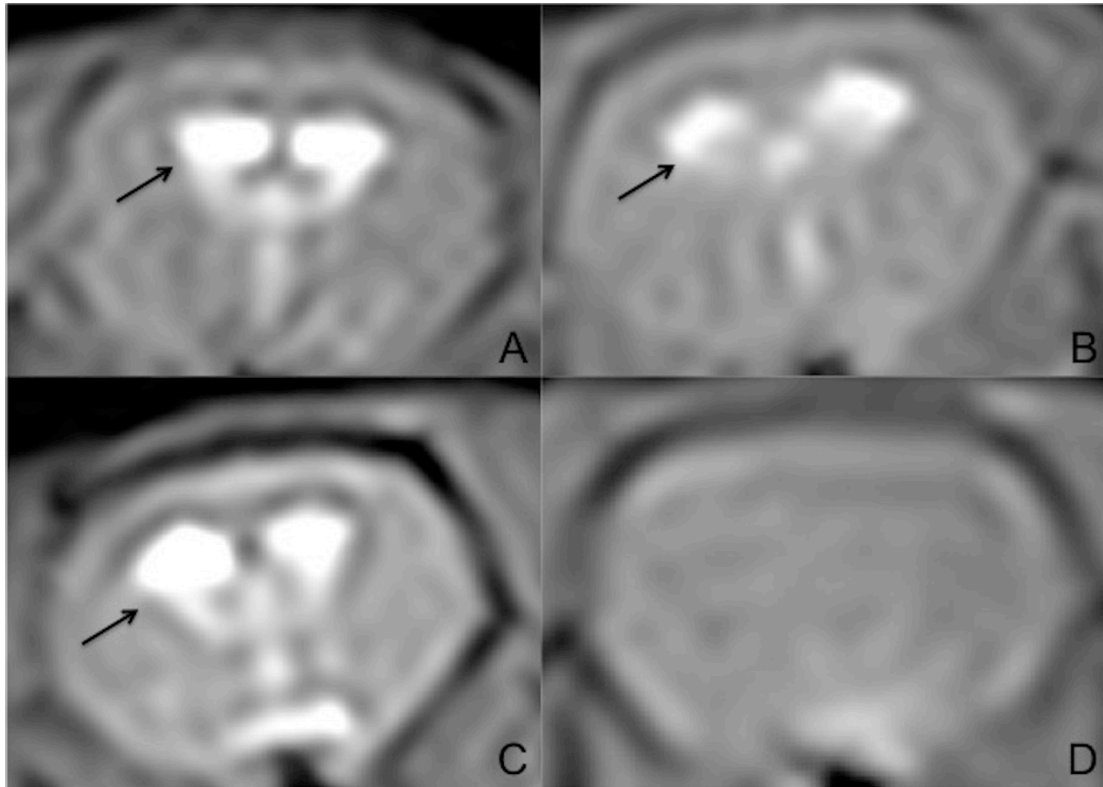


Figure 1 – T2-weighted gradient echo MRI scans obtained for each experimental group. A: kaolin group; B: cyst group, MRI performed 1 month after inoculation; C: cyst group, MRI performed 3 months after inoculation; D: sham group. The selected images are from equivalent head positions. Enlarged ventricles are pointed with arrows. A and C show definite ventricle enlargement, whereas B shows mild ventricle enlargement. Ventricle is not visible on D, as the animal is not hydrocephalic.

Table 1 – Number of successful inductions of hydrocephalus and median ventricle volume in the experimental groups.

	Kaolin group	Cyst group		Sham group
	(n = 5)	1 month MRI (n = 5)	3 months MRI (n = 5)	(n = 2)
Number of hydrocephalic animals	3	2	5	0
Median ventricle volume (mm ³)	24.4	4.8*	120.0*	Mean: 3.0
Range (mm ³)	(1.0 – 692.0)	(0.4 – 40.2)	(49.0 – 437.0)	(1.68 – 4.35)

*Ventricle volumes were significantly higher in the 3-months MRI subgroup than in the 1-month MRI subgroup ($p < 0.05$). The cutoff value of 5.0mm³ was set to consider a normal ventricle volume.¹⁴

Discussion

Human studies on the inflammatory response to neurocysticercosis are very limited because of the chronic progression of the disease, its different developmental stages, and the influence of administered corticosteroids.¹⁰

Since *Taenia solium*, the tapeworm that causes human neurocysticercosis, does not infect rodents in experimental assays,¹¹ alternative parasites have been used. *Taenia crassiceps* is a tapeworm related to *T. solium*. Its definitive hosts are foxes, wolves and dogs, and its intermediate hosts are wild rodents.¹⁵ The biological cycle of *T. crassiceps* resembles that of *T. solium*, a fact rendering it a useful alternative. Another parasite widely used as a model of experimental neurocysticercosis is *Mesocestoides corti*; however, this parasite tends to invade the brain parenchyma and to replicate in the CNS, so it does not exactly reproduce the biological behavior of *T. solium* in the CNS.¹¹

With respect to experimental neurocysticercosis, studies from the group of Teale^{10, 16} have focused on the type of cell-mediated immune response and on the mechanisms responsible for leukocyte migration across the blood-brain barrier and

ependyma. The authors found that the immune response of mice to neurocysticercosis is mediated by gamma delta T lymphocytes guided by a Th1 pattern of response.¹⁰ Furthermore, junctional complex proteins and matrix metalloproteinases were found to be directly involved in leukocyte infiltration.¹⁶

Matos-Silva et al. analyzed general inflammatory responses in a mouse model of encephalitis after inoculation of *T. crassiceps*. The authors observed the occurrence of ventriculomegaly, but this finding was casual and was probably due to mechanical obstruction of the lateral ventricle since the ventriculomegaly observed was asymmetrical, shifting the midline.¹⁷

Hydrocephalus in experimental neurocysticercosis has so far not been well documented. This may be due to the fact that all previously published experimental studies of neurocysticercosis injected cysts into the convex brain surface through a burr hole, which tends to mimic human parenchymal neurocysticercosis. We believed that it was necessary to inject the cysts into the CSF compartments in order to reproduce human extraparenchymal neurocysticercosis.

One may argue that our hydrocephalus results were due to mechanical obstruction of CSF outflow from the fourth ventricle to the subarachnoid space – it is, an obstructive hydrocephalus. We cannot rule out this possibility. However the fact that hydrocephalus was observed in only 2/5 animals within 1 month and in all animals after 3 months points to the possibility of a slowly progressing inflammatory reaction resulting in elevated resistance to CSF absorption, which favors the occurrence of arachnoiditis leading to hydrocephalus. This may reproduce the human condition of neurocysticercosis-related hydrocephalus, which exhibits an “arrested” course (a hydrocephalus that progresses very gradually and seems to be compensated).¹⁸

The kaolin model has permitted significant advances in the understanding of the pathophysiology of hydrocephalus: ependymal disruption, periventricular edema, reactive astrogliosis, axon damage, corpus callosum flattening, myelin disintegration, and neuronal degeneration.^{13, 19, 20} Recently, aquaporin-4 and its upregulation in hydrocephalus have been suggested as an alternative pathway for CSF absorption and partial compensation of CSF imbalance.²¹⁻²³ However in the kaolin-induced hydrocephalus model it is difficult to achieve control of the severity of hydrocephalus²⁴, as we have observed in our results from the kaolin group. Additionally, the classic kaolin model induces rapid enlargement of the brain ventricles, which differs from the “arrested” course of many patients seen in clinical practice. In this classic model, the animals have severe clinical manifestations such as loss of weight and severe neurobehavioural changes.²⁵ To study an arrested course of hydrocephalus, Del Bigio et al proposed a model of low-dose kaolin injection into immature rats. The authors achieved slow ventricle enlargement associated with pathological alterations similar to those seen in humans.¹⁸ However, another model of adult-onset slow progressive hydrocephalus is needed. Our model may have accomplished this goal, with the advantage of keeping animals healthy without noticeable neurobehavioural changes. Weekly repeated MRI scans of animals with neurocysticercosis-induced hydrocephalus could confirm if our model reproduces the arrested course of many patients. Also the use of contrast enhancement on MRI could highlight inflammatory reactions within CSF compartments. In the same way, analysis of CSF contents could demonstrate inflammatory changes.

Future investigation of our model will differentiate whether hydrocephalus is due to mechanical obstruction or to inflammatory responses in the basal cisterns,

although both events occur in clinical practice. Finally, we will study histological and immunological features elicited by the presence of cysts in the CSF compartments.

Conclusions

We developed an experimental model of hydrocephalus induced by neurocysticercosis that may mimic human hydrocephalus secondary to neurocysticercosis.

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Declaration

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Paper 3 – Artigo com os resultados da segunda etapa do trabalho

Artigo submetido à revista Acta Tropica (FI 2,27)

***Taenia crassiceps* injection into the subarachnoid space of rats simulates racemose neurocysticercosis**

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Abstract

Neurocysticercosis is a major public health concern. Despite its potential for eradication, the disease remains endemic in developing countries and has risen again in Europe and USA. Basic studies on neurocysticercosis are needed to better understand the pathophysiologic mechanisms in order to improve perspectives of therapy. Much has

been published on experimental parenchymal neurocysticercosis, but experimental models of racemose neurocysticercosis have not yet been studied. We developed a simple model by the injection of *Taenia crassiceps* into the subarachnoid space of rats. Magnetic resonance imaging and histologic assessment confirmed similar findings from human racemose neurocysticercosis: basal cisterns enlargement, hydrocephalus and inflammatory infiltration through the ependyma and choroid plexus to the cerebrospinal fluid spaces.

1. Introduction

Neurocysticercosis is the most common parasitic disease of the central nervous system (CNS). It remains endemic in developing countries and migratory flows reintroduced the disease in Europe and USA ([Flisser et al., 2003](#) and [O'Neal and Flecker, 2015](#)).

Clinical presentation of neurocysticercosis is variable for it depends on the number, location, size and stage of development of the parasite within the CNS. In the most common form, parenchymal cysts are responsible for seizures and focal neurologic deficits ([Sotelo, 2011](#)). On the other hand, cysts may be lodged in the cerebrospinal (CSF) compartments such as subarachnoid cisterns and brain ventricles. This is called racemose neurocysticercosis, also known as the malignant form of the disease, for its higher rates of incapacity, hospitalization and deaths ([Estañol et al., 1986](#)).

Cysts within the subarachnoid space and ventricles invoke important inflammatory response that may determine arachnoiditis, ependymitis, vasculitis and cranial nerve entrapment. As a result, patients may have hydrocephalus, stroke, neuropathies and intracranial hypertension ([Proaño et al., 2009](#)).

Due to the pleomorphism of the disease, the complex host-parasite interaction and the effects of corticosteroid immune suppression, it is difficult to fully understand the pathophysiology mechanism underlying racemose neurocysticercosis. This is the reason why basic studies are needed (Nash et al., 2006). Actually, researches have been published on experimental neurocysticercosis, but most of them focus on the host-parasite interaction within the brain parenchyma (Alvarez et al., 2010). We aimed to create an experimental model of racemose neurocysticercosis through the direct injection of cestodes in the subarachnoid space of rats.

2. Materials and methods

The animals were handled according to ethical guidelines and current legislation. The board of the local Ethics Committee on Animal Use approved the project.

Eleven adult Wistar rats (*Rattus norvegicus*) weighing 200 to 250 g were used. The animals were kept under adequate sanitary conditions in a temperature-controlled room (21°C) under light-dark control. Food and water were available *ad libitum*.

2.1. Parasites and inoculation

Taenia crassiceps cysts were maintained in the peritoneal cavity of mice (*Mus musculus*). After aseptic removal, 50 cysts were injected into the cisterns of each rat by suboccipital puncture with a 25G needle. All animals were submitted to general anesthesia by intraperitoneal injection of a mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml) at a dose of 0.1 ml/kg.

2.2. Radiologic assessment

Four months after inoculation, magnetic resonance imaging (MRI) using a 0.25 T equipment (Esaote model Vet-MR) was performed to verify the occurrence of ventricular dilatation and distribution of cysts within the ventricles and basal cisterns. The protocol consisted of T2-weighted gradient echo acquisitions (0.6-mm thickness; T_{eco} = 5 ms; T_{repetition} = 10 ms).

2.3. Histologic assessment

Soon after MRI the animals were euthanized with overdose of pentobarbital. Cardiac perfusion was performed with buffered formalin and the encephala were removed. After removal of the encephala, they were photographed and then fixed overnight with formalin solution, neutral buffered, 10%. Slices were done at the level of lateral and fourth ventricles and then dehydrated with alcohols and blocked in paraffin. Sections were performed at 3 micrometres and stained with hematoxylin and eosin (HE).

Ventricle dilatation, changes in the ependymal epithelium, choroid plexus and the parenchyma were analysed by stereologic methods ([Mouton, 2014](#)).

3. Results and Discussion

MRI showed that all animals had ventricle enlargement. Nine animals presented enlargement of the basal cisterns. A cyst could be detected inside the ventricle of one animal (figure 1). Mesoscopic view confirmed cysts juxtaposed to brainstem (figure 2).

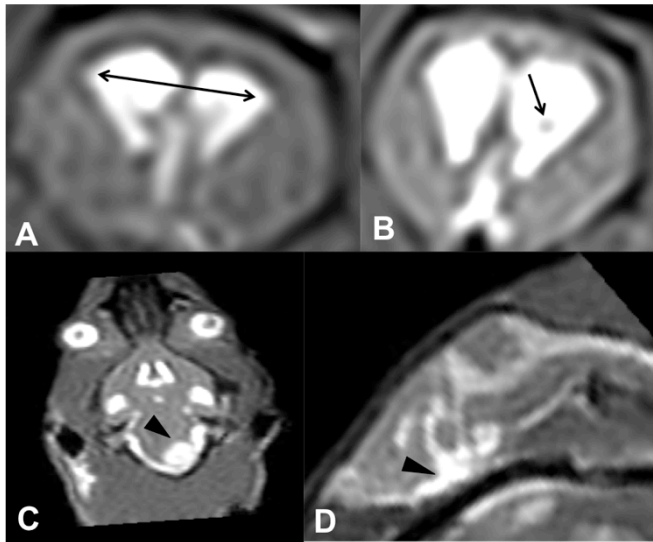


Figure 1 – MRI findings through T2 echo gradient. A: ventricle enlargement is obvious, double-arrow points the maximal distance between frontal horns. B: the arrow shows a hypo signal inside the left lateral ventricle corresponding to a cyst. C and D: arrowheads show basal cisterns enlargement.

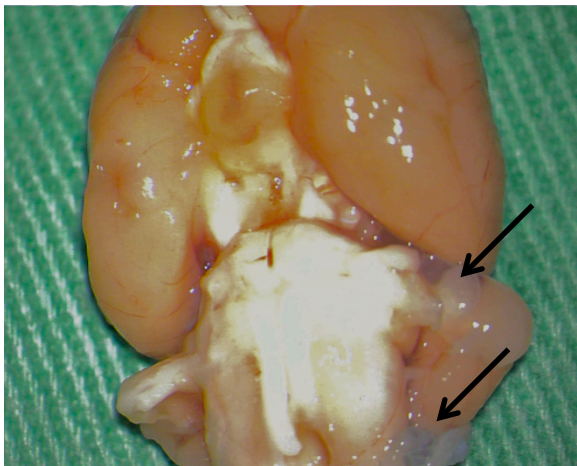


Figure 2 – Mesoscopic view shows cysts juxtaposed to the brainstem (arrows)

In human neurocysticercosis these findings are typical and useful for diagnosis purposes, i.e., the identification of racemose cysts in the CSF compartments: basal subarachnoid cisterns and ventricles ([García and Del Brutto, 2003](#)). Hydrocephalus occurs in up to 30% of patients with neurocysticercosis ([Matushita et al., 2011](#)). Despite advances of experimental studies of neurocysticercosis, ventricle enlargement has not

been systematically found. The article of [Mattos-Silva et al. \(2012\)](#) showed asymmetrical ventriculomegaly as an anecdotal finding. Our model may be useful for the understanding of hydrocephalus in neurocysticercosis, which has a different evolution from other forms of hydrocephalus

On histologic slices, we observed cysts inside the ventricles. Inflammatory cells were found in the choroid plexus in the fourth ventricle and in the ependyma. There was an increase on the cellularity of the brain parenchyma near the ventricle cavity that corresponds to reactive astrogliosis. Choroid plexus became thick and edematous. Finally, the ependyma became thin, flattened and without cilia (figure 3).

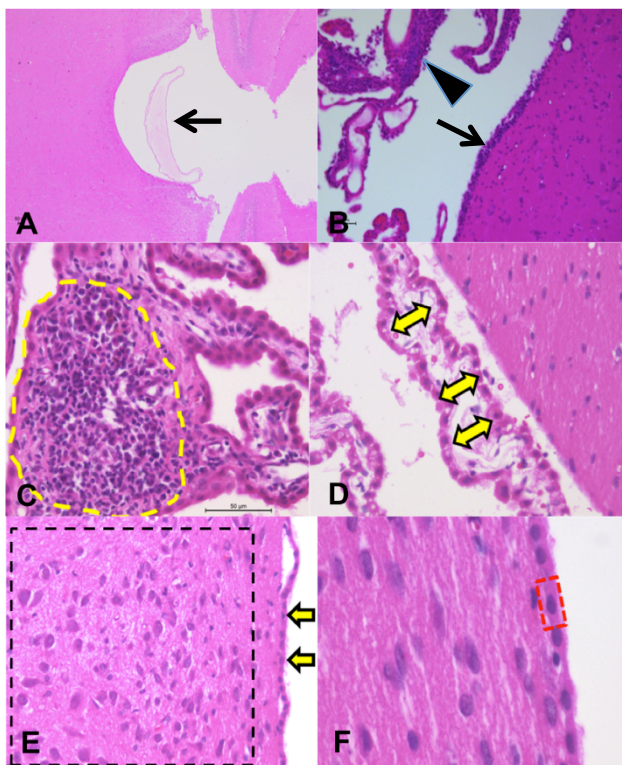


Figure 3 – A cyst can be seen inside the ventricle (A, arrow). There is inflammatory cells in the ependyma (B, arrow) and in the choroid plexus (arrowhead). The choroid plexus has lymphoplasmocytic infiltration (C, dotted line) and became thick and edematous (D). There is astroglial proliferation in the periventricular region (E, dotted line) and ependymal epithelium became flattened (F) with loss of normal cuboid pattern and cilia (dotted line).

Ependymal disruption, choroid plexus edema and reactive astrogliosis are commonly seen on experimental models of hydrocephalus ([Del Bigio, 1993](#)). In fact, the elevated pressure in the ventricles disrupts the ependyma. The CSF extravasates through the brain parenchyma in attempt to be absorbed in water-channel molecules in the basal membrane of astrocytes feet to the vessels ([Skjolding et al., 2010](#)).

Hydrocephalus could be a simple consequence of mechanical obstruction of parasites in the normal pathways of CSF circulation. But the identification of inflammatory infiltrates in the choroid plexus points to the occurrence of an inflammatory response in the CSF compartments. Inflammatory cells in the ependyma simulate the ependymitis that occurs in human neurocysticercosis. This way, we experimentally reproduced inflammatory patterns of the disease. In addition, the chronic physiopathologic process is established with dynamic and adaptive morphologic evidences.

In human racemose neurocysticercosis, cysts in the CSF compartments have a different behaviour from the parenchymal form: membranes proliferate through the subarachnoid space and may achieve large sizes. They may obstruct the CSF circulation and distort the adjacent brain parenchyma, leading to different symptoms ([Mahale et al., 2015](#)). Degenerating cysts and the cysts themselves evoke inflammatory response in the subarachnoid space, within the ventricle and on the ependyma surface. These inflammatory processes may determine headache and vasculitis. The consequent scars in the subarachnoid space disturb the CSF absorption as also its circulation, leading to hydrocephalus ([Sotelo and Marin, 1987](#)).

Experimental parenchymal neurocysticercosis has been well studied by the group of Teale ([Cardona et al., 1999](#), [Alvarez and Teale, 2007](#), and [Alvarez and Teale, 2008](#)). In general, they inoculate the cestode *Mesocostoides corti* through a burr hole in

the parietal bone. Even though the cysts are not directly introduced in the brain parenchyma, as this cestode has the capacity of invasion, they soon achieve the parenchyma, where determine inflammatory process well documented by the authors. Though these studies have important contributions to the understanding of neurocysticercosis, they do not explain all the pathophysiologic changes from the racemose form.

In fact, intracranial injection of *Taenia crassiceps* had been previously attempted, but the fact that this metacestode displaces the nervous tissue was faced as a disadvantage for a model of experimental neurocysticercosis ([Alvarez et al., 2010](#)). On the other hand, displacement of CNS structures is a real concern in the racemose form of neurocysticercosis. In the current proposed model, we observed common findings of racemose neurocysticercosis.

Conclusions

Through a simple subarachnoid injection of *Taenia crassiceps* we could simulate racemose neurocysticercosis: cysts throughout the CSF compartments, hydrocephalus, basal cisterns enlargement, ependymitis and inflammatory infiltrates in the CSF space. This model may be useful for the understanding of racemose neurocysticercosis and allows for new researches perspectives.

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Discussão

Discussão

Estudos experimentais são úteis e necessários para o entendimento dos mecanismos fisiopatológicos envolvidos nas doenças, especialmente para aquelas cujo estudo detalhado de mecanismos seja dificultado pela variabilidade de apresentação clínica e pela influência do próprio tratamento, como é o caso da neurocisticercose.

Diversos estudos experimentais têm sido publicados com foco na forma parenquimatosa da neurocisticercose. Porém na prática clínica a forma extraparenquimatosa é de manejo mais complexo, especialmente quando há hidrocefalia associada. Por outro lado, os estudos experimentais de hidrocefalia não reproduzem com exatidão o que ocorre na hidrocefalia da neurocisticercose – em geral, uma evolução mais insidiosa.

Assim, um modelo experimental de hidrocefalia por neurocisticercose pode ser útil na compreensão da fisiopatologia da doença, especialmente com relação às interações entre parasita e hospedeiro no espaço liquórico.

Estudos experimentais com *T. solium* teriam o potencial de fornecer resultados mais próximos do que ocorre na doença em humanos, porém sempre se acreditou que *T. solium* não infecta ratos experimental (Alvarez et al., 2010). Estudo recente conseguiu induzir neurocisticercose em ratos com cepas de *T. solium* (Verastegui et al., 2015). Para isso, os autores retiraram ovos de proglótides do verme adulto. A seguir, induziram a maturação de oncosferas dos ovos e as injetaram em ratos. Após 4 meses, conseguiram demonstrar alterações patológicas encefálicas. Conquanto seja promissora, a técnica tem grande complexidade metodológica para execução. Assim, o uso de cestodos análogos e de fácil manutenção, como *M. corti* e *T. crassiceps*, é também útil.

A capacidade de distorção de estruturas encefálicas por *T. crassiceps* pode ser interessante para simular a neurocisticercose racemosa, em que vesículas sem escólex

se proliferam como cachos de uva no espaço subaracnóideo ou ventricular. Na verdade, o estudo de Matos-Silva e colaboradores (2012) verificou a ocorrência de ventriculomegalia assimétrica em alguns de seus animais.

Os resultados da primeira etapa de nosso trabalho mostraram ventriculomegalia com a inoculação cisternal de *T. crassiceps*. Porém a dilatação do sistema ventricular poderia ser simplesmente resultado da obstrução mecânica das saídas de líquido pelo IV ventrículo ou ainda no aqueduto cerebral ou forame de Monro. Uma obstrução intermitente e progressiva poderia justificar o maior número de animais hidrocefálicos aos 3 meses que no primeiro mês pós-inoculação, bem como um volume ventricular significativamente maior aos 3 meses. Por outro lado, esta evolução mais arrastada também poderia se dever a um componente inflamatório.

Na segunda etapa do trabalho, verificamos infiltrados inflamatórios no epêndima e no plexo coróide. A ependimite é um achado intraoperatório bem conhecido na hidrocefalia por neurocisticercose (Proaño et al., 2009). Os estudos de Alvarez e Teale também mostraram o epêndima como uma via importante de infiltração leucocitária para os espaços liquóricos (Alvarez e Teale, 2007). Em nosso modelo, a ruptura do epêndima pela própria hidrocefalia poderia facilitar a infiltração de leucócitos para a cavidade ventricular. Por outro lado, no modelo experimental de neurocisticercose em camundongos com *M. corti*, o plexo coróide não é um sítio importante de infiltração de leucócito, conquanto o seja em cérebros normais (Alvarez e Teale, 2006) e no que observamos no modelo ora desenvolvido.

Assim acreditamos que nossos resultados se devam a uma combinação de obstrução mecânica com inflamação, o que, na verdade, ocorre na hidrocefalia na neurocisticercose.

As alterações patológicas observadas em nosso modelo estão de acordo com o que se observa na hidrocefalia experimental em geral, ou seja, ruptura e adelgaçamento do epitélio endimário, edema do plexo coróide e proliferação astrogial principalmente na região periventricular (Del Bigio, 1993).

Alterações comportamentais dos animais são bem documentadas nos modelos experimentais de hidrocefalia (Lopes et al., 2009; Olopade et al., 2012). Em nosso modelo, embora não tenhamos feito uma análise sistemática dos animais, não notamos adoecimento nem alterações significativas seu comportamento. Isso pode ser explicado pela evolução lenta da dilatação ventricular, o que permitiria aos animais a adoção de mecanismos compensatórios.

Recentemente tem-se estudado o papel da aquaporina-4 (AQP-4), um canal de água, como via alternativa para absorção de líquido. Em animais com hidrocefalia há aumento da expressão da AQP-4 na membrana basolateral de células do epêndima e nos pés vasculares dos astrócitos (Owler et al., 2010). Essa expressão aumentada se correlaciona com áreas de alteração do coeficiente de difusão aparente de água em estudos com ressonância magnética (Skjolding et al., 2010). Quando submetidos à injeção cisternal de caulim, animais com deficiência na expressão de AQP-4 têm hidrocefalia de instalação mais rápida (Bloch et al., 2006). Questionamos se em nosso modelo, com uma hidrocefalia de evolução mais lenta, a AQP-4 pode ter um padrão de expressão diferente do que se tem reportado na literatura.

Outrossim nosso modelo abre perspectivas para o estudo da hidrocefalia crônica. No clássico modelo com caulim, a hidrocefalia se instala rapidamente, o que não ocorre em todas as formas de hidrocefalia em humanos. A evolução rápida da hidrocefalia por caulim limita a possibilidade de estudá-la a longo prazo, haja vista o adoecimento dos animais. De fato, há poucos estudos com análise após mais de 16 semanas de inoculação.

Para contornar este problema, Del Bigio produziu um modelo de hidrocefalia de evolução arrastada a partir de pequenas doses de caulim em ratos com 3 semanas de vida e que foram mantidos vivos por 9 meses após a inoculação. Seus resultados foram semelhantes ao que ocorre em humanos em termos de lesão de substância branca (Del Bigio et al., 2003). Há que se notar, porém, que a indução da hidrocefalia ocorreu em animais jovens. Nesse sentido, justifica-se um modelo experimental de hidrocefalia em animais adultos.

Limitações do trabalho

O número de animais foi pequeno para estabelecer mais comparações entre grupos, especialmente com a injeção de caulim. Porém o número de animais com inoculação de *T. crassiceps* foi suficiente para observar as alterações desejadas, principalmente a dilatação ventricular.

Não realizamos uma avaliação sistemática do comportamento dos animais. A observação de que não sofreram alterações significativas é subjetiva e deve ser comprovada em estudos posteriores

Questiona-se também o papel de componentes inflamatórios presentes na cavidade peritoneal dos camundongos onde se mantiveram os cistos de *T. crassiceps*. Eventualmente os debrís inflamatórios peritoneais de camundongos podem ter alguma influência na resposta inflamatória intracraniana dos ratos.

A variabilidade da hidrocefalia induzida pelo caulim em nossos animais também é digna de nota, embora alguns estudos mencionem que, de fato, a resposta ao caulim cisternal nem sempre seja homogênea (Shaolin et al., 2015).

As imagens de ressonância magnética foram prejudicadas pela capacidade do aparelho (0,25 T): com o aumento da magnificação há perda de resolução. No entanto, as

alterações detectadas foram suficientes para determinar a ocorrência de hidrocefalia e a mensuração do volume ventricular.

Com relação à análise histológica, não quantificamos a intensidade da resposta inflamatória em cada animal. A análise também se restringiu à coloração com hematoxilina e eosina. Outras colorações e técnicas, como imunoistoquímica, acrescentariam informações com relação às alterações inflamatórias, astrogliose reativa e perda de mielina na substância branca.

Também não estudamos alterações líquóricas. Sabe-se que na neurocisticercose extraparenquimatosa há alterações de líquido como hipoglicorraquia, hiperproteinorraquia, aumento de leucócitos e eosinofilia. O encontro dessas alterações em nosso modelo corroboraria a hipótese de haver um componente inflamatório como causa da hidrocefalia.

Finalmente, apesar das semelhanças filogenéticas entre *T. crassiceps* e *T. solium*, as reações imunológicas e inflamatórias desencadeadas por cada um devem ser diferentes. Além do mais, a inoculação dos cistos diretamente no sistema nervoso central não representa o mesmo processo de ativação dos cistos de *T. solium* em sua passagem pelo trato gastrointestinal.

Conclusões

Conclusões

- ✓ A inoculação cisternal de cistos de *T. crassiceps* induz hidrocefalia experimental em ratos;
- ✓ A hidrocefalia induzida pela inoculação de *T. crassiceps* em ratos desenvolve-se lenta e progressivamente;
- ✓ Neste modelo, a hidrocefalia parece ser devida a uma combinação de obstrução mecânica e inflamação.

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Referências

(exceto referências citadas apenas nos artigos enviados para publicação)

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Certificado

CERTIFICAMOS que o (Protocolo CEUA 1038-2013) "**Desenvolvimento de um modelo experimental de hidrocefalia por neurocisticercose**", a ser conduzido pelo Dr. Pedro Tadão Hamamoto Filho, orientado pelo Prof. Dr. Marco Antonio Zanini, com a colaboração de Germano Francisco Biondi e Apoio Técnico de Diego Generoso e José Antonio Germiniano, está de acordo com o Conselho Nacional de Controle de Experimentação Animal - CONCEA, com a ressalva de que os "**coelhos e ratos**" são provenientes de Biotério convencional, sem condições de atestar a Sanidade dos mesmos.

Projeto de Pesquisa aprovado em reunião da CEUA em 29/08/2013



Profª Adjunta Noeme Sousa Rocha
Presidente no exercício da CEUA



Alberto Santos Capelluppi
Secretário da CEUA

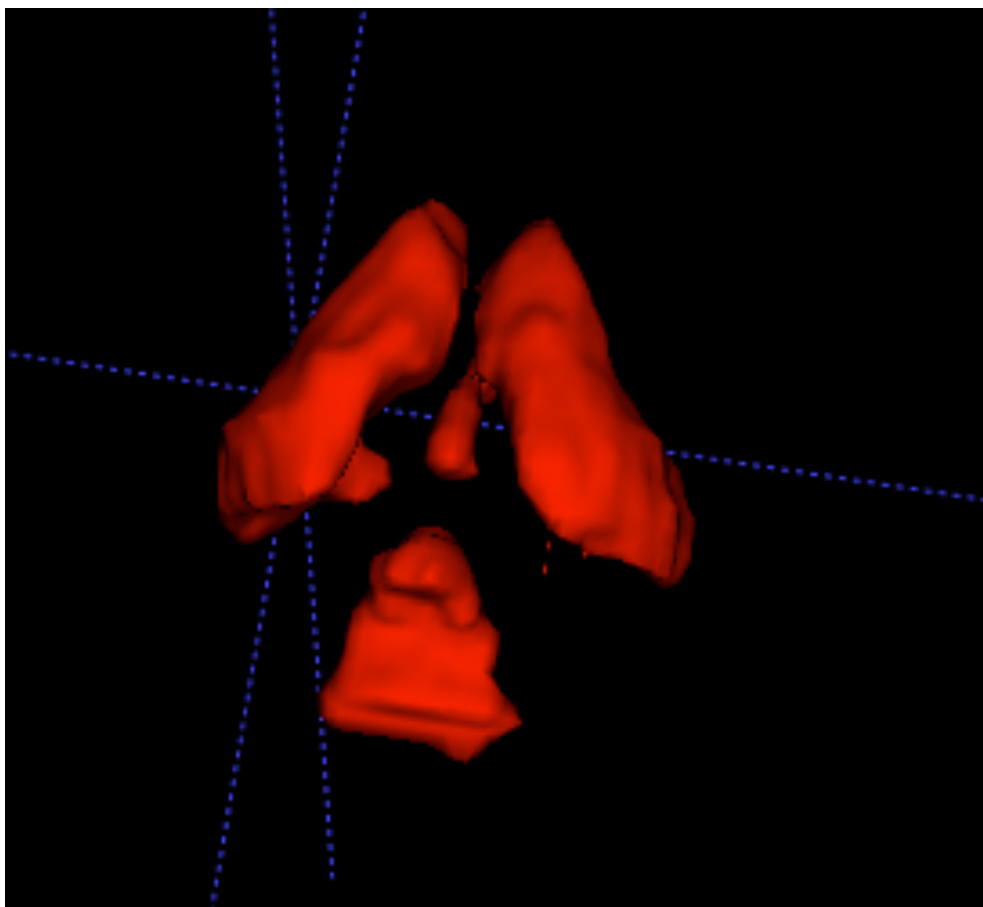
Anexos

Anexo 1 – Valores do volume ventricular dos animais da primeira etapa do estudo

Grupo	Animal	Volume ventricular (mm ³)
Cistos RM após 1 mês	1	0,67
	2	38,78*
	3	40,27*
	4	4,85
	5	0,39
Cistos RM após 3 meses	6	76,46*
	7	120,00*
	8	48,80*
	9	137,20*
	10	436,9*
Caulim 25%	11	64,89*
	12	692,60*
	13	24,42*
	14	2,27
	15	1,09
Salina 0,9%	16	4,35
	17	1,69

* Animais com hidrocefalia

Anexo 2 – Exemplo de reconstrução tridimensional do volume ventricular

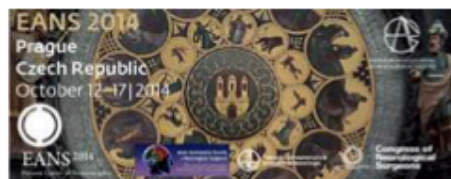


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Dear Dr Pedro Tadao Hamamoto Filho

On behalf of the Scientific Committee, we are very pleased to inform you that your abstract No. **Eans1-0357** entitled "DEVELOPMENT OF AN EXPERIMENTAL MODEL OF NEUROCYSTICERCOSIS-INDUCED HYDROCEPHALUS: A PILOT STUDY " has been accepted as part of the **Top 100 POSTER / E-POSTER PRESENTATION** at the 15th European Congress of Neurosurgery – EANS 2014, taking place in Prague, Czech Republic, October 12-17, 2014.

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Yours sincerely,

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TAENIA CRASSICEPS INJECTION INTO THE SUBARACHNOID SPACE OF RATS SIMULATES NEUROCYSTICERCOSIS-INDUCED HYDROCEPHALUS

Dos autores

Pedro Tadao Hamamoto Filho; Rodrigo Bazan; Luiz Carlos Vulcano; Germando Francisco Biondi; Marco Antônio Zanini;

Recebeu a ***Menção Honrosa de 2º Lugar*** pelo trabalho apresentado na *modalidade Oral* durante o “X NEURÃO – Congresso de Neurocirurgia do Estado de São Paulo”, ocorrido entre 04 e 06 de setembro de 2015, no Centro de Convenções Rebouças, em São Paulo – SP.

São Paulo, 06 de setembro de 2015.



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