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Case Report

# Diagnostic and therapeutic management of Cryptococcosis in a kitten with practical considerations to veterinary pediatric therapeutic approach

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ABSTRACT

A 3-months-old male domestic kitten was referred for repeated seizures. Analysis revealed *Cryptococcus neoformans*. Levetiracetam and fluconazole were administered without significant clinical improvements and without negativization. Hypothesizing resistance, therapy was switched to amphotericin B. Seizure disappeared. Haematological controls highlighted transitory increasing of CK, BUN, ALP and cholesterol. PCR repeated two weeks after the treatment was negative for *Cryptococcus neoformans*. Nowadays the cat is 5 years old, and no seizures occurred since the age of 5 months.

## 1. Introduction

Cryptococcosis is a non-contagious systemic fungal disease, occurring worldwide and is observed more commonly in cats than in dogs [1]. The etiological agent is *Cryptococcus neoformans,* an airborne pathogen, and cats might acquire the infection by inhalating basidiospores in a contaminated environment. The incubation period is variable and when the invasion of nasal mucosal layer occurs, disease develops locally and/or systemically, from the upper respiratory tract to the central nervous system (CNS) [2,3]. CNS involvement becomes clinically evident with sudden blindness due to optical neuritis, seizures, or behavioral changes due to the development of granulomatous encephalo-myelitis with solitary or multiple lesions and it is usually associated with poor therapy response and survival outcome [4,5].

To diagnose *Cryptococcus neoformans*, it is possible to perform cytology, histology, antibody detection and culture, but the most reliable test is PCR. Advanced diagnostic imaging techniques (i.e. MRI) are recommended to investigate CNS lesions in case of symptomatic patients [1].

The prognosis is favorable in most cases if the diagnosis is made early (before dissemination or prior to development of irreversible lesions) and patients and owners comply with a long course of treatment (months) and follow-up (years) [5].

According to the literature, it is not possible to determine a predisposition of age and breed, even if it has been reported that Cryptococcosis is more frequently diagnosed in adults [6].

Considering all the aforementioned factors, the present case report should be worthy of reader's attention due to the fact it deals with the diagnostic and therapeutic process of a very young subject with a positive PCR for *Cryptococcus neoformans*, presenting critical CNS symptoms and that was unresponsive to first line specific treatment for Cryptococcosis.

## 2. Case

During winter 2015, a 2-month male owned kitten was referred to veterinarians for repeated seizure. Owners declared that the kitten was maintained in a household environment, that no trauma occurred, and that seizure started few days after adoption from a stray cat recovery association. According to the instruction given by the Veterinarian of the association, a first therapeutic attempt was tried to control seizure by oral (PO) administration of phenobarbital (2.5 mg q12h). The therapy was maintained for more than one month, without appreciable improvement.

At the age of 3-months, the kitten was referred for consultation to other Veterinarians (considered day 0). The general clinical examination was normal, except for a very low body condition score (BCS=1, the cat weighted 900grams), bristling hairs and depression. Owners showed

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several videos of seizure episodes. Several differentials were considered, mostly related to infectious etiologies and central nervous system malformation (i.e.: hydrocephalous). The kitten was presented few days later to a Veterinary Hospital for a further diagnostic work-up. Blood samples were collected to perform blood cell count and biochemistry. BCC was within the physiological range while among biochemical parameters the creatin phosphokinase (CK) was increased (626 U/L). Blood aliquots were also used for *Toxoplasma gondii* IgG/IgM detection, and *Cryptococcus neoformans* identification using real time PCR technique. For template DNA preparation, a blood sample was processed with the QIAamp tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The real time PCR was performed with oligonucleotide primers: Cryn-F and Cryn-R (5' GTTAAAAAGCTCGTAGTTG 3' and 5' TCCCTAGTCGGCATAGTTTA 3') complementary to highly conserved regions within the nuclear gene coding for a small subunit of rRNA (18S rDNA) of *C. neoformans* and generate a 429-bp amplicon [7]. Amplification was performed in a StepOne™ Real-Time PCR System (Applied Byosistems, ThermoFisher, UK).

A complete neurological examination and an MRI in presence of a paramagnetic intravenous marker were performed by a specialist. Hyperintensity in olfactory lobes, nostrils and olfactory sinus was highlighted in SeT2 sequences with moderate enhancement after the marker administration. An ophthalmologic exam revealed the presence of pathognomonic chorionic lesions attributable to cryptococcosis.

Given the severity and the frequency of seizure, levetiracetam was initiated at 20 mg/kg PO q8h. Real time PCR demonstrated the positivity for *C. neoformans*. According to this finding, blood sample and nasal swabs were seeded in specific culture media to isolate *C. neoformans* and fluconazole was initiated at 10 mg/kg PO q12h. Culture results were negative. Hematological controls were performed every 15 days. Several days (33) after the beginning of the therapy, seizures began once again, becoming more and more frequent in the further two weeks. PCR was repeated and demonstrated a persistent positivity. Veterinarians hypothesized a lack of efficacy of fluconazole and decided to hospitalize the patient. Seizures were treated by administering benzodiazepines rectally and it was chosen to administer amphotericin B at the dose of 1 mg/kg, q48h for three consecutive times. Fluid therapy was constantly maintained, and blood biochemistry was checked daily to evaluate possible renal toxicity. Seizure disappeared at the end of the administration protocol and the kitten was discharged. Following hematological controls highlighted a transitory increasing of creatine kinase, BUN, alkaline phosphatase, and cholesterol. The clinical examination was good, and the PCR repeated two weeks after the end of the treatment was negative for *C. neoformans*.

Nowadays the cat is 5 years old, and seizures disappeared at the age of 5 months.

## 3. Discussion

This is the first case report describing a possible drug resistance phenomenon in a 3 months-old kitten affected by *C. neoformans* with good outcome even with a critical involvement of CNS.

No prospective controlled studies exist on the treatment of feline cryptococcosis, and all data are based on retrospective studies and case reports. Treatment guidelines have been delineated and the choice of appropriate antifungal drug depends on many factors.[1] Differences in the susceptibility of the various *Cryptococcus* spp. and genotypes to antimycotic drugs, in general and against fluconazole (FCZ) in particular, have been reported [6]. Consequently, the molecular identification of the isolates is important in defining the treatment protocol. Moreover, isolates with different geographical origins may show different susceptibilities [8]. According to these considerations, some drugs are considered as a milestone in Cryptococcosis therapy, but information for paediatric subjects is anecdotical.

Azoles encompass fungistatic drugs, that can inhibit the synthesis of ergosterol, a primary component of fungal membranes [9]. Fluconazole seems to be more effective than itraconazole for infections involving the CNS, eye, and urinary tract, and is also better tolerated.[4] Fluconazole and itraconazole are both less toxic and present better therapeutic and pharmacodynamic/pharmacokinetic characteristics than ketoconazole. The most significant advantage of fluconazole is its ability (shared neither with ketoconazole nor itraconazole) to pass the blood-brain barrier and the blood-ocular barrier, thus making it suitable to treat mycotic infections of these organs. Side effects of fluconazole may include gastrointestinal disturbances and hepatotoxicity and it is considered teratogenic [10]. Fluconazole should be administered in adults at the dose of 50 mg PO q12h, for 1- 2 months after the resolution of clinical signs [11]. Itraconazole may be used in kittens at the dose of 5 to 10 mg/kg q24h [12].

Amphotericin B is an old polyene antimycotic drug, fungistatic or fungicidal, depending on concentration [13]. It acts by binding sterols in the cell membrane and altering the permeability [11]. Alone or in combination with 5-flucytosine it may be the first choice or can be the right alternative after a first treatment with fluconazole or itraconazole.[4] It is not well absorbed by oral route, thus it must be administrated intravenously (IV), with subsequent problems in restrained patients. Novel approaches aimed at improving the oral availability of amphotericin B by using various excipients have been published [14]. Side effects are mainly represented by nephrotoxicity. To avoid this effect, liposomal preparations have been developed, with a lipid complex and a colloidal dispersion in cholesterol sulphate. Although significantly less toxic, these preparations are more expensive, thus limiting the use in veterinary medicine. Consequently, they are used mostly in cases in which other drugs fail [15]. Doses varies according to the diagnosis and the formulation.

In the present case, Veterinarians decided not to administer glucocorticoids even if this class of drugs is suggested to be used in adult cats affected by this pathology [12]. Glucocorticoids should be considered if signs of brain oedema are present or in case of significant neurologic deterioration in MRI. Dexamethasone sodium phosphate 0.1 mg/kg IV or subcutaneously q12h followed by prednisone 0.5 mg/kg PO q12h is used. Prednisone should be tapered over 1 to 2 weeks if possible, to avoid immunosuppression especially in young subjects [12].

The clinical course of the kitten presented in this case report, could indicate a resistance to azoles: *C. neoformans* resistance to azole compounds has been frequently reported in humans presenting AIDS [16]. Relating to veterinary medicine, the molecular mechanisms behind azoles resistance in *C. neoformans* have been demonstrated in the increased expression of ERG11 and efflux pumps [17]. Moreover, Kano and colleagues reported an *in vitro* resistance to fluconazole in a *C. neoformans* var. *grubii* from a cat but the microorganisms remained susceptible to amphotericin B and itraconazole [18]. These results are in accordance with Lester and co-workers who reported resistance to fluconazole in some *C. neoformans* isolates that remain susceptible to other azoles [19]. It is important to underline that all information related to amphotericin effects and azole resistance are all related to adult subjects and that it is necessary to collect specific data about pediatric ones due to the fact that drug disposition, distribution, metabolism and excretion are extremely different between the two aforementioned categories of patients. Till the age of 6 months, puppies and kittens have a higher percentage of body water than adults, demonstrating a variable binding of the drug with plasma proteins, showing a deficiency of phase I and II enzymes and a reduced renal excretion [20]. According to this, it is correct to hypothesize the administration of lower doses and to prolong intervals of administration to avoid side effects but, it has to be considered also a poor therapeutic response that may result from the limited administration.

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## Consent

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

## Declaration of competing interest

There are none.

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