



Case Report

Feline neonatal toxoplasmosis and cystoisospora coinfection - Case Report

Felipe Jacques Sanches^{1*}, Suene Andrielle da Silva Queiroz¹, Ana Paula Lourenção de Albuquerque¹, Priscila da Silva Queiroz¹, Michele Ferreira de Assis¹, Paulo Fernandes Marcusso²

¹Universidade Estadual de Maringá (UEM), Campus Regional de Umuarama - PR.

²Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Campus de Unaí - MG.

ARTICLE INFO

Article history

Received 28 January 2018

Received in revised form 12 May 2018

Accepted 14 May 2018

Keywords:

Cat

Kitten

Acute

Protozoan

Zoonosis

ABSTRACT

Toxoplasma gondii and *Cystoisospora* spp. are coccidian protozoa and compulsory intracellular parasites. These parasites present an enteric cycle phase, wild and domestic felines are definitive hosts capable of eliminating non-sporulated oocysts by faeces. Furthermore, they share the same transmission path, through the ingestion of infectious oocysts or the ingestion of cysts present on tissues. Most cat infections occur subclinically. Commonly, clinical and severe disease develops more often in kittens and immunocompromised animals. The present paper reports a case of coinfection of *T. gondii* and *Cystoisospora* spp in a feline of approximately 20 days old, weighing 260 grams, assisted at the Veterinary Hospital - UEM. The patient presented acute signs of limited mobility, remaining only in sternal position, cervical ventroflexion, dyspnea, lethargy and anorexia. *T. gondii* was identified through Polymerase Chain Reaction (PCR) test using a blood sample, whereas the co-analysis by flotation in saturated saline solution (Willis technique) was able to verify the presence of oocysts of *Cystoisospora* spp. After negative result from PCR test using stool sample, the presence of *Cystoisospora* spp. was confirmed, differentiating it from oocysts of *T. gondii*. The treatment based on sulfamethoxazole and trimethoprim was able to control the infection and decrease disease symptoms, proving to be effective and showing significant clinical improvement within 3 days after starting the treatment.

INTRODUCTION

Toxoplasma gondii is the most common parasitic zoonosis in humans (ELMORE et al., 2010; VIDOTTO et al., 2015). Also capable of infecting a large number of animals, such as birds and reptiles, and mammals as well. However, only felids are considered definitive hosts, able of completing the life cycle of the coccidia by eliminating non-sporulated oocysts from the faeces, which contaminate the environment and, after sporulation, become infectious for animals and humans. (MUNHOZ et al., 2017; ZULPO et al., 2018). The infection occurs after the ingestion of any of the three stages of life of the protozoa: oocysts sporulated by the ingestion of feces, tachyzoites via transplacental and / or transmamial or bradyzoites by carnivorous diet (LAPPIN, 2010).

Cystoisospora spp. (or *Isoospora*), as well as *T. gondii*, is a coccidia that belongs to the phylum Apicomplexa. These two protozoa present similar characteristics, such as: both are compulsory intracellular parasites; present an enteric cycle phase; have the cat as the definitive host; a common transmission path, through the ingestion of infectious oocysts or the ingestion of cysts present on tissues (BRESCHIANI; COELHO; PAIVA, 2015; CRYSTAL, 2009).

The clinical changes observed in feline toxoplasmosis are nonspecific, including gastroenteric, respiratory, hemolympathic, ocular, muscular and / or neurological disorders (STRITAL et al., 2016). The clinical manifestations commonly reported are: anorexia, lethargy and dyspnea, also jaundice, ascites, fever, abdominal pain, diarrhea, emesis, pneumonia, hepatitis, pancreatitis, encephalitis and neurological deficits,

* Corresponding author: felipejaques@gmail.com

muscle hyperesthesia, gait stiffness and ocular lesions. (BRESCIANI et al., 2016; COSTA et al., 2015). As well as toxoplasmosis, most infections with *Cystoisospora spp.* are subclinical, and more susceptible and severe in kittens and immunosuppressed cats, causing symptoms such as severe diarrhea, hematochezia, loss of weight, anorexia, dehydration and death (BRESCIANI; COELHO; PAIVA, 2015; CRYSTAL, 2009).

Assist in the diagnosis the clinical manifestation, hematological and biochemical exams, as well as radiographic, cytological, serological, coproparasitological and molecular exams (DUBEY; LINDSAY; LAPPIN, 2009; VIDOTTO et al., 2015).

It is known that there is no therapy to cure toxoplasmosis, but there are infection control and symptom reduction, and treatments that include anti-Toxoplasma drugs such as clindamycin; sulfonamide associated with trimethoprim; and azithromycin (BRESCIANI et al., 2016; ELMORE et al., 2010; JAVINSKY, 2016; LAPPIN, 2010). In a paper written by Barbosa et al. (2012), significant therapeutic efficacy was observed with the use of enrofloxacin *in vitro* and *in vivo* models, and it could also be considered a therapeutic option for the control of toxoplasmosis. However, recurrence of the disease is always possible, since *T. gondii* can not be eliminated by any drug protocol (JAVINSKY, 2016; LAPPIN, 2010; NORSWORTHY; GRACE, 2009). Sulfam-based therapies associated or not with trimethoprim have a good clinical response in cases of *Cystoisospora spp.* infections (BRESCIANI; COELHO; PAIVA, 2015; CRYSTAL, 2009; JAVINSKY, 2016).

As the therapeutic response is good in most cases of toxoplasmosis, the prognosis will be good to reserved. Poor prognosis is mainly for immunosuppressed cats with liver or lung disease (LAPPIN, 2010; VIDOTTO et al., 2015).

The aim of this paper is to report a case of *T. gondii* and *Cystoisospora spp.* coinfection in a newborn feline.

CASE REPORT

On October 18th, 2016, was assisted by the Veterinary Hospital – UEM, a feline, mongrel, approximately 20 to 25 days old, weighing 260 grams.

The owner reported acute musculoskeletal weakness, lethargy, and cervical ventroflexion for less than 6 to 8 hours. The kitten had been rescued 10 days ago, along with 8 other kittens from two different litters and had been in his care ever since. Nevertheless, four kittens came to death without apparent neurological signs.

Previously, the animal was in good health condition, presenting normorexia (fed with Pet Milk® - milk substitute for animal feeding), which intake of the

artificial milk was provided every 2 to 3 hours following label instructions and well received by the animal.

The clinical symptoms had sudden onset, including: locomotor difficulty, sternal position, cervical ventroflexion, dyspnea, lethargy and anorexia. Seizure, sialorrhea, emesis and ascites, among other non-specific clinical manifestations weren't observed. Seven days before, the animal presented soft to liquid stool of lumpy appearance, with presence of striae of blood, lasting 5 days. The vermifugation had not been performed yet.

On physical examination, the patient presented: rectal temperature 37.8 °C; lung fields clear to auscultation, but dyspneic breathing; Normorritimic and normophonetic heart sounds, with rhythmic and strong arterial pulse; Oral and ocular mucosa with normal color; Absence of pain to abdominal palpation, but with moderate abdominal distension; Normohydrate; and non-reactive submandibular and popliteal lymph nodes. During the appointment the feline remained in a sternal position, presenting cervical ventroflexion and muscular weakness. There were no relevant disorders during the neurological examination (present reflexes, cranial nerves intact), but the consciousness was decreased (depressed, drowsy).

Initially, glycemia was measured and was shown to be within normal reference values for the species; Hemogram and polymerase chain reaction (PCR) tests were requested for toxoplasmosis and neosporosis diagnosis; Also a coproparasitological exam. There were no significant alterations in the hemogram, and the coproparasitology was positive to *Cystoisospora spp.*

The treatment was based on sulfamethoxazole and trimethoprim at a dose of 15mg / kg / SID - once a day, until new recommendations, considering that it was a neonate. The supportive therapy was based on supplemental vitamin (Glutamina® - NutriSana) and Lactobac Cat® (probiotic and prebiotic), both enteral, instituted during the treatment.

Three days after the start of treatment, the owner reported total and progressive improvement in health status, and absence of the clinical disorders described previously.

The PCR result confirmed the suspected of toxoplasmosis and was negative for *Neospora spp.* Thus, the initial treatment was maintained and prolonged for 28 days. At the end of the treatment the animal was in excellent physical condition, alert and with no clinical alterations.

DISCUSSION

The present report presents a case of feline toxoplasmosis and cystoisosporosis diagnosed in a

neonate. Vidotto et al. (2015) and Elmore et al. (2010) reported that the higher prevalence of the disease in kittens with congenital infection, due to transplacental transmission or during lactation, are more likely to develop clinical signs.

The diarrhea reported by the owner may have resulted from the enteroepithelial replication of *T. gondii*, as discussed by Lappin (2010). Despite unspecific, clinical manifestations of anorexia, lethargy and dyspnea are described by Vidotto et al. (2015) and Dubey; Lindsay; Lappin (2009), and may appear suddenly. These same authors also claim that because clinical toxoplasmosis is more severe in cats infected by the transplacental route, the affected kittens may die even before weaning, which may have occurred with the other kittens that came to death.

The PCR was selected as method for diagnosis, a resource provided by the hospital itself, which is a diagnostic option described by Vidotto et al. (2015). For Lappin (2010), the ideal would be to correlate the PCR with the serological test, analyzing specific antibodies for *T. gondii*, such as IgM and IgG, because IgM has a better correlation with clinical feline toxoplasmosis, since it is rarely present in serum of healthy cats.

However, for Dubey; Lindsay; Lappin (2009), newborn cats from chronically infected mothers receive a concentration of IgG through colostrum that can persist for up to 12 weeks after birth, which might not be reliable in this case.

Complementary tests performed were hemogram (table 1) and coproparasitology, suggested by Vidotto et al. (2015) and Dubey; Lindsay; Lappin (2009).

Table 1 – Demonstration table with values of the patient's hemogram exam, Exam realized at the Laboratory of Clinical Pathology of the Veterinary Hospital – UEM.

| Erythrogram | Results | Units | Reference values |
|------------------------|---------|-----------------------------|----------------------------------|
| RBC | 5,1 | millions/ μ l | 5,0 - 10,0 millions/ μ l |
| Hemoglobin | 8,2 | g/dL | 8 - 15 g/dL |
| HCT | 26,6 | % | 24 - 45 % |
| MCV | 52,2 | fL | 39 - 55 fL |
| MCHC | 30,8 | g/dL | 30 - 36 g/dL |
| Metaribricytes | | % | 0% |
| PPT | 6,3 | g/dL | 6,0 - 8,0 g/dL |
| Morphology and notes: | | RBC normal morphology | |
| Leukogram | Results | | Reference values |
| | % | μ L | Percentage Absolute |
| WBC | 14.900 | | 100 5.500 - 19.500 / μ L |
| Segmented neutrophils | 86 | 12.814 | 35 - 75% 2.500 - 12.500/ μ L |
| Neutrófilos bastonetes | 0 | 0 | 0 - 3% 0 - 300/ μ L |
| Metamyelocyte | 0 | 0 | 0% 0 / μ L |
| Lymphocytes | 11 | 1.639 | 20 - 55% 1.500 - 7.000/ μ L |
| Monocytes | 1 | 149 | 1 - 4% 0 - 850/ μ L |
| Eosinophils | 2 | 298 | 2 - 12% 0 - 1.500/ μ L |
| Basophils | | 0 | Raro Raro |
| Morphology and notes: | | WBC normal morphology | |
| Thrombogram | Results | | Reference value |
| Platelets | 492.000 | | 200.000 - 500.000/uL |
| Morphology and notes: | | Platelets normal morphology | |

*RBC (red blood cells); MCV (mean corpuscular volume); MCHC (mean corpuscular hemoglobin concentration); PPT (total plasma protein); WBC (white blood cells).

Coproparasitological exam is the way to detect the presence of oocysts in the faeces of animals infected by both *T. gondii* and *Cystoisospora spp.* Oocysts of *Cystoisospora spp.* are easily observed through centrifugal-flotation techniques using zinc sulphate solution (Faust technique) or saturated sugar solution (Sheater technique) or even flotation in saturated

sodium chloride solution (Willis technique), as described by Bresciani; Bunny; Paiva (2015). They report that the main species of *Cystoisospora* that cause infection in felines are: *Cystoisospora felis* and *Cystoisospora rivolta*, and the size of oocysts are between 39 and 48 μ m in length by 26 to 37 μ m in width and 20 to 24 μ m in length by 15 to 20 μ m wide, respectively.

According to Javinsky (2017), the oocysts of *Toxoplasma gondii* are about a quarter of the size of the oocysts from *Cystoisospora felis*. The major difficulty in identifying *T. gondii* oocysts is the differentiation between *Hammondia* or *Besnoitia* spp. oocysts, since they are morphologically indistinguishable. In this case, the author proposes the use of PCR of stool sample to detect the presence or not of *T. gondii* oocysts. Vidotto et al. (2015) and Dubey; Lindsay; Lappin (2009) point out the difficulty of finding *T. gondii* oocysts, since felines eliminate these oocysts through faeces only once in their lives, with rare exceptions, and in a short period of time.

The coproparasitological exam performed at the veterinary hospital was a flotation in saturated sodium chloride solution (Willis technique), sufficient to identify *Cystoisospora* spp. The sample was analyzed by the professor responsible for the parasitology sector of the hospital, who was able to confirm the presence of *Cystoisospora* spp. oocysts considering especially morphological characteristics described in literature and knowing that *Cystoisospora* spp. oocysts are bigger when compared to *Toxoplasma gondii*, through experience in the evaluations of this specific exam. PCR was also performed on the faeces of the patient in order to identify the presence of *T. gondii* oocysts, which resulted in a negative result. Thus, it was concluded, analysing stool sample, that only *Cystoisospora* spp. oocysts were identified, through coproparasitological exam.

Pasty feces to lumpy liquids with traces of blood presented by the animal in this report may have been secondary to both *Cystoisospora* spp. and *T. gondii*, since diarrhea and hematochisis are symptoms of both diseases. However, none of the consulted authors reported clinical manifestations of locomotor difficulty, cervical ventroflexion and dyspnea, similar to the case described, as originating from Isosporosis.

Costa et al. (2015) report that the development of clinical toxoplasmosis is associated with factors such as age, simultaneous infections, pathogenicity of the protozoal and immunosuppression. The first two factors were present in this case, since the animal had a coinfection with *Cystoisospora* spp. To Bresciani; Bunny; Paiva (2015), *Cystoisospora* spp. may contribute to complications of the clinical situation in several diseases, which probably occurred in this case. We may also consider that the kitten was probably immunosuppressed, a common characteristic in orphan. These aspects imply in an acute clinical condition and neuromuscular disorders observed.

The anti-toxoplasmosis treatment based on sulfamethoxazole and trimethoprim is able to control the infection and decrease the symptomatology (BRESCIANI et al., 2016; ELMORE et al., 2010; VIDOTTO et al., 2015). Therefore, this protocol was used, selecting a drug that can reach significant concentrations in the central

nervous system, moreover the same therapeutic protocol is effective against cystoisosporosis (BRESCIANI; PAELHO, 2015; CRYSTAL, 2009).

As the patient was considered a newborn animal, Mata's guidelines were followed; Papich (2011) recommend decreasing the antibiotics dose up to 50% in these patients, thus the initial dose was lower (15mg / kg) administered orally and daily for 7 days; starting from the second week, the interval between medications was decreased at a dose of 15mg / kg administered every 16 hours for 21 days. The treatment period (28 days) was consistent with that reported by Vidotto et al. (2015) and Lappin (2010).

Glutamina® (NutriSana) and Lactobac Cat® (containing probiotic and prebiotic) were instituted as supportive therapy, two multivitamins composed of a combination of nutrients that helped with a fast response and improvement of the health status of the animal, besides acting as adjuvant in the microbiota recomposition of the digestive tract, since the animal had shown symptoms of enteritis.

Finally, the therapeutic response was considered satisfactory and the animal exhibited significant clinical improvement within three days after treatment onset, healing after the end of the therapeutic protocol established, totaling thirty days of treatment.

CONCLUSION

The present paper reported a case of toxoplasmosis in a neonate feline with nonspecific and acute symptoms infected with *T. gondii*, detected by molecular test (PCR). The clinical status was probably aggravated due to coinfection with *Cystoisospora* spp., confirmed by coproparasitological examination. However, a effective and fast therapeutic response was obtained with the administration of sulfa-trimethoprim, administered for approximately 30 days, in addition to the nutritional supportive therapy.

REFERENCES

- BARBOSA, B. F. et al. Enrofloxacin is able to control *Toxoplasma gondii* infection in both in vitro and in vivo experimental models. **Veterinary Parasitology**. v. 187, p. 44– 52, 2012.
- BRESCIANI, K. D. S. et al. Epidemiological Aspects of Feline Toxoplasmosis. **Archives of Veterinary Science**. v.21, n.2, p.01-08, 2016.
- BRESCIANI, K. D. S.; COELHO, W. M. D.; PAIVA, F. Isosporose. In JERICÓ, M. M.; NETO, J. P. A.; KOGIKA, M. M. **Tratado de Medicina Interna de Cães e Gatos**. 1 ed. Rio de Janeiro: Roca, 2015. v. 1, cap. 76, p. 695-698.
- COSTA, R. C. B. et al. Infecção pelo toxoplasma gondii e pelo vírus da imunodeficiência felina em gatos domésticos (*felis catus*). **Enciclopédia Biosfera**, Centro Científico Conhecer - Goiânia, v.11, n.22; p. 1994, 2015.

CRYSTAL, M. A. Coccidiose. In NORSWORTHY, G. D. et al. **O Paciente Felino**. 3 ed. São Paulo: Roca, 2009. cap. 23, p. 50-51.

DUBEY, J. P.; LINDSAY, D. S.; LAPPIN, M. R. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. **Vet. Clin. Small Anim.** v 39, p. 1009-1034, 2009.

ELMORE, S. A. et al. Toxoplasma gondii: epidemiology, feline clinical aspects, and prevention. **Trends in Parasitology**, v. 26, n. 4, p. 190-196, 2010.

JAVINSKY, E. Sistema Digestivo, Fígado e Cavidade Abdominal: Parasitos Gastrintestinais. LITTLE, S. E. **O GATO: Medicina Interna**. 1 ed. Rio de Janeiro: Roca, 2016. cap. 23, p. 479-495.

LAPPIN, M. R. Doenças infecciosas: Infecções Polissistêmicas por Protozoários. In NELSON, R. W.; COUTO C. G. **Medicina Interna de Pequenos Animais**. 4 ed. Rio de Janeiro: Elsevier, 2010. cap. 99, p. 1367-1370.

MATA, J.; PAPICH, M. G. Considerações Farmacológicas em Pacientes Jovens. In PETERSON, M. E.; KUTZLER, M. A. **Pediatria em Pequenos Animais**. 1 ed. Rio de Janeiro: Elsevier, 2011. cap. 27, p. 233-243.

MUNHOZ A. D. et al. Toxoplasmosis in cats in northeastern Brazil: Frequency, associated factors and coinfection with Neospora caninum, feline immunodeficiency virus and feline leukemia vírus. **Veterinary Parasitology: Regional Studies and Reports**, v. 8, p. 35-38, 2017.

NORSWORTHY, G. D.; GRACE, S. F. Toxoplasmose. In NORSWORTHY, G. D. et al. **O Paciente Felino**. 3 ed. São Paulo: Roca, 2009. cap. 138, p. 335-336.

STRITAL, A. D. et al. Estudo epidemiológico e avaliação de fatores de risco da infecção por Toxoplasma gondii e achados clinico-patológicos da infecção aguda em cães admitidos em um Hospital Escola Veterinário. **Pesq. Vet. Bras.** v. 36, n. 10, p. 993-998, 2016.

VIDOTTO, O. et al. Toxoplasmose. In JERICÓ, M. M.; NETO, J. P. A.; KOGIKA, M. M. **Tratado de Medicina Interna de Cães e Gatos**. 1 ed. Rio de Janeiro: Roca, 2015. v. 1, cap.78, p. 703-712.

ZULPO, D. L. et al. Toxoplasma gondii: A study of oocyst re-shedding in domestic cats. **Veterinary Parasitology**, v. 249, p.17-20, 2018.