

## SUSPECTED CLINICAL TOXOPLASMOSIS IN PET RABBITS (*ORYCTOLAGUS CUNICULUS*) IN LIMA, PERU

(*Suspeita de toxoplasmose clínica em coelhos de estimação (Oryctolagus cuniculus) em Lima, Peru*)

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**RESUMO:** A toxoplasmose é uma doença prevalente entre coelhos em muitas áreas do mundo. No entanto, a sua apresentação clínica é muito incomum. Na América do Sul, a prevalência de toxoplasmose em coelhos é desconhecida e os relatórios clínicos desta doença são escassos ou inexistentes. Anticorpos contra *Toxoplasma gondii* foram encontrados em quatro coelhos de estimação que foram recebidos na Clínica de Animais Selvagens e Exóticos, Faculdade de Medicina Veterinária, Universidad Nacional Mayor de San Marcos (Lima, Peru). Estes animais foram recebidos em cerca de um ano e pertenciam a diferentes proprietários. Os motivos mais comuns para a apresentação foram anorexia/hiporexia e dificuldade em andar. O sinal clínico mais frequentemente observado foi a mialgia. Todos os coelhos apresentaram anemia, linfopenia e aumento da creatinina. Os 75% (3/4) dos pacientes morreram ou foram humanamente eutanaziados. Este artigo descreve a presença de anticorpos contra *T. gondii* em coelhos clinicamente doentes pela primeira vez no Peru. Com base nestas observações, recomenda-se a pesquisa sobre prevalência de toxoplasmose em coelhos e seus fatores de risco associados.

**Palavras-chave:** Animais exóticos; creatinina; Lagomorpha; protozoários; *Toxoplasma gondii*

**ABSTRACT:** Toxoplasmosis is a prevalent disease among rabbits in many areas of the world. However, its clinical presentation is very uncommon. In South America, prevalence of toxoplasmosis in rabbits is unknown and clinical reports on this disease are scarce or inexistent. Antibodies against *Toxoplasma gondii* were found in four pet rabbits, which were received at the Wild and Exotic Animals' Clinic, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos (Lima, Peru). These animals were received over about one year and belonged to different owners. The most common reasons for presentation were anorexia/hyporexia and difficulty to walk. The most frequently observed clinical sign was myalgia. All rabbits presented anemia, lymphopenia, and increased creatinine. The 75% (3/4) of patients died or were humanely euthanized. This article describes the presence of antibodies against *T. gondii* in clinically ill rabbits for the first time in Peru. Based on these observations, research on prevalence of toxoplasmosis in rabbits and its associated risk factors are recommended.

**Keywords:** Exotic animals; creatinine; Lagomorpha; protozoa; *Toxoplasma gondii*

## INTRODUCTION

Toxoplasmosis is among the most common zoonotic diseases worldwide (it is present in about 30 to 50% of world population); and it is also considered an important opportunistic infection in immunocompromised patients (Torrey and Yolken, 2003; Barakat, 2007; Dubey and Jones, 2008; Muñoz-Zanzi et al., 2010; Flegr et al., 2014). The etiologic agent is *Toxoplasma gondii*, an obligate intracellular protozoan first described in 1908 by Nicolle and Manceaux in USA and by Splendore in Brazil (Black and Boothroyd, 2000; Barakat, 2007; Cenci-Goga et al., 2011). This parasite infects the intestine of domestic and wild felids, which are its definitive host (Méndez et al., 2011). Most birds (recorded in more than 30 species) and mammals (recorded in more than 300 species) are potential intermediate hosts. Australian marsupials, arboreal primates and lemurs are highly susceptible (Bowman et al., 2002; Almería et al., 2004; Flegr et al., 2014; Carlson-Bremer et al., 2015). Moreover, *T. gondii* has recently been detected in snakes, being the first record of natural infection by this parasite in reptiles (Nasiri et al., 2016).

Toxoplasmosis is prevalent in domestic rabbits in USA, Mexico, Egypt, Czech Republic, Germany, and China (Aghwan et al., 2010; Bergmann et al., 1980; Dubey et al., 1992; Percy and Barthold, 2007; Ashmawy et al., 2011; Fisher and Carpenter, 2012; Alvarado-Esquivel et al., 2013; Varga, 2014; Meng et al., 2015). Infection is usually asymptomatic and clinical disease is associated with immunosuppression (Dubey et al., 1992; Dubey and Jones, 2008; Lennox and Kelleher, 2009; Fisher et al., 2012; Varga, 2014); hence it is considered to be an uncommon cause of neurological disease in rabbits (*Oryctolagus cuniculus*) (Percy and Barthold, 2007; Fisher and Carpenter, 2012). Rabbits can get *T. gondii* when

eating grass contaminated with sporulated oocysts originated in cat feces, and congenital transmission of *T. gondii* has been described in rabbits (Harkness et al., 2010a; Fisher and Carpenter, 2012; van Praag, 2014; Varga, 2014). While toxoplasmosis is potentially zoonotic, the only known way of transmission from rabbit to humans is by handling or ingesting contaminated undercooked or raw meat. Toxoplasmosis is not spread by rabbits' feces (Lennox and Kelleher, 2009; Varga, 2014).

Toxoplasmosis in rabbits has three presentations: latent infection (i.e. asymptomatic, commonly seen in immunocompetent adult rabbits), chronic infection (i.e. old rabbits presenting cyst in muscles and nervous tissue, with intermittent episodes of clinical disease which are associated to immunosuppression and stress), and acute infection (i.e. commonly seen in young rabbits, which die in about 2 to 8 days post infection) (van Praag, 2014). Clinical signs are frequently observed in acute episodes of disease, among them are described: anorexia, pyrexia, ataxia, hind limbs paresis, tetraplegia, seizures, and death (Fisher and Carpenter, 2012; Varga, 2014). Survival rates are very low and prognosis is poor due to the sudden and quick progression of parasitosis (van Praag, 2014).

Information related to clinical cases of toxoplasmosis in rabbits is scarce in South America and practically non-existent in Peru. This article describes the clinical presentation, clinicopathological findings, and therapeutics in pet rabbits presenting clinical manifestation suggesting toxoplasmosis and showing antibodies against *T. gondii*.

## MATERIAL AND METHODS

### *Review of clinical records*

A systematic review of clinical records of pet rabbits received from

January to December 2015 at the Wild and Exotic Animals' Clinic, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos. Informed consent from each rabbit's owner was obtained in order to use information from clinical records. Data from rabbits with both serologic diagnosis and clinical signs of toxoplasmosis were selected. Sex, age, diet, living with cats, clinical signs, hematological findings, biochemical findings, therapeutics, outcome, and survival time were recorded. This study was approved by the Ethics and Animal Welfare Committee, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos (Authorization No. 017-2016).

### Statistical Analysis

Mean, standard deviation, and range were estimated for age and survival time data. Sex, clinical signs, hematological findings, and biochemical findings were summarized using frequencies.

## RESULTS

A total of 255 clinical records were reviewed, in which four cases of rabbits with clinical diagnosis of suspected toxoplasmosis were found. Hence, frequency of suspected clinical toxoplasmosis in rabbits received at our clinic during 2015 was 1,6% (CI95% 0,4 – 4,0%). Clinical data of these rabbits are presented in Table 1. Mean age of rabbits with toxoplasmosis was  $5,25 \pm 1,5$  (range 4-7) years and 75% (3/4) were female. Survival time (from clinical signs presentation to death or humane euthanasia) was  $6,7 \pm 1,2$  (range 1-7) days and only in one of the four cases the patient recovered from illness. The only recovered rabbit was treated during 6 weeks, until clinical signs disappeared and antibodies against *T. gondii* were

not detected. Such animal was re-checked 6 months later and was found clinically healthy but in poor body condition.

**Table 1.** Clinical, hematological, and biochemical data of rabbits presenting antibodies against *T. gondii*.

	Case 1	Case 2	Case 3	Case 4
Sex	Female	Female	Male	Female
Age	7 years	6 years	4 years	4 years
Diet	Vegetables and pellets	Vegetables and pellets	Vegetables and pellets	Vegetables, pellets, fruits
Living with cats	No	No	No	No
Reason of consultation	Inappetence	Difficulty for walking	Difficulty for walking	Constipation
Body condition	3/5	2.5/5	2.5/5	2/5
Clinical signs	Anorexia, rales, myalgia	Paresis, myalgia, hyperthermia	Paresis, hypothermia, myalgia	Constipation, myalgia, abdominal pain, hyperthermia
Radiographic findings	Undefined radiopaque structures in hepatic parenchyma, mild pleural effusion, apparent cardiomegaly and increased cranial mediastinum	Reduced intervertebral space, sclerosis and spondylosis in C4-C5 and C5-C6	NA*	Reduced intervertebral space in T13-L1, Moderate amount of gas in cecum and reduced visibility of abdominal organs (suggesting ascites)
Hematological findings	Anemia, thrombocytosis, lymphopenia	Anemia, thrombocytopenia, leukopenia, neutropenia, lymphopenia, basophilia	Anemia, lymphopenia, basophilia	Anemia, thrombocytopenia, leukocytosis, lymphopenia
Biochemical findings	Increased creatinine, Increased creatine kinase	Increased creatinine	Increased creatinine	Increased creatinine, Increased creatine kinase
Outcome	Death	Euthanasia	Recovery	Death

\*NA = Non-assessed

All rabbits presented anemia and lymphopenia. Other hematological findings were basophilia (3/4), thrombocytopenia (2/4), leukopenia (1/4), leukocytosis (1/4), neutropenia (1/4), thrombocytosis (1/4), eosinophilia (1/4), and neutrophilia (1/4). Increased creatinine was observed in all animals, followed in frequency by increased creatine kinase (2/4). Hematological and biochemical findings are detailed in Table 2. Serological diagnosis was performed using the Indirect Hemagglutination test and titration with 2-Mercaptoethanol (Toxotest HAI®, Wiener Lab, Argentina) following manufacturer's instructions. IgM was detected only in one animal (i.e. Case 4), thus revealing acute infection. Such diagnosis was based on antibodies titration decreasing in two dilutions when using 2-Mercaptoethanol in comparison

to titer observed without it. These results are shown in Table 3.

**Table 2.** Hematological and biochemical results of seropositive rabbits.

Analyte (unit)	Case 1	Case 2	Case 3	Case 4	Reference range <sup>a, b</sup>
Erythrocytes (10 <sup>6</sup> /ul)	6	4	3	3	4 – 7.2
Hemoglobin (g/dL)	8.7	8.4	9.5	7	10 – 15.5
Packed Cell Volume (%)	38	28	31	26	36-48
Mean Corpuscular Volume (fL)	63.3	70	103.3	86.7	-
Mean Corpuscular Hemoglobin (g/dL)	14.5	21	31.7	23.3	-
Mean Corpuscular Hemoglobin Concentration (g/dL)*	22.9	30	30.6	28	-
Platelets (10 <sup>3</sup> /uL)	916	180	372	50	200 – 1000
Leukocytes (cells/uL)	5380	4080	7400	22300	7000 -13500
Segmented neutrophils (%)	89	52	59	88	20 – 35
Segmented neutrophils (cells/uL)	4788	2122	4366	19624	-
Lymphocytes (%)	8	41	37	8	55 – 80
Lymphocytes (cells/uL)	430	1673	2738	1784	-
Monocytes (%)	0	2	1	1	1 – 4
Monocytes (cells/uL)	0	82	74	223	-
Eosinophils (%)	3	2	1	2	0 – 4
Eosinophils (cells/uL)	161	82	74	446	-
Basophils (%)	0	3	2	1	2 – 10
Basophils (cells/uL)	0	122	148	223	-
Creatinine (mg/dL)	2	2.6	2.4	3.2	0.8 – 1.8
Alanine aminotransferase (U/L)	160	40	16	114	112-350
Total proteins (g/dL)	5.3	NA*	NA*	5.7	2.8 – 10
Albumin (g/dL)	3	NA*	NA*	3.1	2.7 – 4.6
Creatine kinase (U/L)	799	590	667	1990	<700

Source: \*Harkness et al. 2010b; <sup>b</sup>Huerkamp 2003. \*NA = Non-assessed

Therapeutic protocol used in all cases was trimethoprim/sulfamethoxazole (40mg/kg PO q/12h x 28 days) (Bactrim<sup>®</sup>, Roche, Switzerland) and doxycycline (4mg/kg PO q/24h x 28 days) (Doximicina<sup>®</sup>, Labodec, Peru) (van Praag 2014; Varga 2014). Also, fluid therapy using Lactated Ringer's (100ml/kg SC) (Hartmann's solution<sup>®</sup>, Baxter, Colombia) was given and a nutritional supplement was prescribed (Hemolitan Pet<sup>®</sup>, Vetnil, Brazil) during 30 days. Pain management was administered using flunixin meglumine (1mg/kg IM q/24h x 3 days) (Flunixin<sup>®</sup>, Genfar, Colombia) and then tramadol (10mg/kg PO q/12h x 7 days) (Tramadol<sup>®</sup>, John Martin, Argentina) (Morrisey and Carpenter, 2012). Moreover, if a patient showed any other condition (e.g. constipation), it received additional medication targeting such problem (e.g. gastroprokinetic agents).

**Table 3.** Indirect Hemagglutination (IHA) test results.

	Case 1	Case 2	Case 3	Case 4
Ig titer (IHA without 2ME)	1/64	1/128	1/32	1/128
Ig titer (IHA with 2ME)	1/64	NA*	1/16	1/32

NA\* = Non-assessed

## DISCUSSION

To the authors' knowledge, this article reports the presence of antibodies against *Toxoplasma gondii* in clinically ill pet rabbits (*Oryctolagus cuniculus*) in Peru for the first time. Previous research in Peru has reported the presence of antibodies against *T. gondii* in other domestic animal species such as in dog (*Canis lupus familiaris*), sheep (*Ovis aries*), pig (*Sus scrofa*), llama (*Lama glama*), alpaca (*Vicugna pacos*), and cat (*Felis catus*) (Suárez-Aranda et al., 2000; Ruíz et al., 2012; Angulo et al., 2014; Cerro et al., 2014; Chávez-Velásquez et al., 2014; Pinedo et al., 2014; Bernal et al., 2015). Moreover, toxoplasmosis is a prevalent disease in Peruvian human population (Maguiña et al., 2004). From a public health perspective, it should be highlighted that people handling or eating raw or undercooked meat from infected rabbits might get toxoplasmosis (Lennox and Kelleher, 2009; Varga, 2014). Hence, based on our findings it is strongly recommended to assess the prevalence of *T. gondii* in rabbits bred for human consumption. Furthermore, as other small mammals (e.g. hedgehogs, ferrets, guinea pigs, pot-bellied pigs, sugar gliders) kept as exotic pets are susceptible to toxoplasmosis, this disease should be considered among differential diagnosis for such species.

The observed prevalence of clinical toxoplasmosis reported in this study agrees with Dubey et al. (1992) who signaled such kind of presentation in rabbits is considered rare. Despite the diagnostic test kit used in the described cases has not specifically been validated for rabbits, the same technique (i.e. Indirect Hemagglutination) has previously been employed in

lagomorphs (e.g. rabbits and hares) (Ahmed et al., 2011; Luo et al., 2017). The seropositive rabbits analyzed did not live with cats and all of them were fed diets including fresh vegetables. Based on this, the most likely source of infection is postulated to be the ingestion of vegetables contaminated with cat feces containing *T. gondii* oocysts, as suggested by Dubey et al. (1992). All seropositive rabbits were adult or aged animals. According to what is described by van Praag (2014), such animals may have been chronically infected with toxoplasmosis and presented with an acute episode, potentially due to stress or immunosuppression associated with disease or aging. Active infection (expressed by the detection of IgM) was diagnosed only in one patient. Most patients were female, but association between disease and gender was not statistically assessed due to the limited sample size. In most presented cases, clinical disease progressed to death or humane euthanasia of animals, due to poor prognosis and lack of therapeutic success. Mean survival time was less than one week. These findings agree with the high mortality rate and poor prognosis described by van Praag (2014) for acute toxoplasmosis. Unfortunately, *post mortem* examination was not authorized in any case. Consequently, a definitive diagnosis of toxoplasmosis could not be fully established by means of highly sensitive and specific techniques such as histopathology and PCR.

With regard to increased creatinine, van Praag (2014) has described it as commonly recorded in rabbits with toxoplasmosis. However, it should not be considered a main characteristic of this disease. The most frequent reason for patient consultation was difficulty for walking (2/4), whereas the most commonly found clinical sign was myalgia (diagnosed by palpation of forelimb and hindlimb muscles and

observation of response suggesting pain) (4/4). Hyperthermia and hypothermia were observed in 50% and 25% of toxoplasmosis cases, respectively; and poor body condition was recorded in 75% of patients. Based on clinical presentation of paresis and poor body condition, encephalitozoonosis (a disease previously reported in rabbits in Lima, Peru) should be considered as a differential diagnosis (Chilón, 2014; van Praag, 2014). However, diagnostic test for encephalitozoonosis is not currently available in Peru. Additionally, in two cases vertebral alterations were detected which may explain the development of paresis but not hyperthermia and other observed clinical signs. Finally, constipation observed in one of the patients (i.e. Case 4) might be associated to pain as suggested by Meredith (2008), and this would have worsened the health status of the animal leading to its death.

## CONCLUSIONS

For the first time in Peru, antibodies against *T. gondii* were detected in rabbits (*Oryctolagus cuniculus*) presenting clinical signs suggesting toxoplasmosis. Further studies regarding the seroprevalence of toxoplasmosis in rabbits in Peru are required for a better understanding of its epidemiology and potential risk to human health.

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## CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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