

RESSALVA

Atendendo solicitação da autora, o texto completo desta tese será disponibilizado somente a partir de 02/02/2018.

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL**

**BORRELIOSIS IN HORSES: EPIDEMIOLOGY,
EXPERIMENTAL INFECTION AND THERAPEUTIC**

Roberta Carvalho Basile
Bachelor in Veterinary Medicine

2016

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL**

**BORRELIOSIS IN HORSES: EPIDEMIOLOGY,
EXPERIMENTAL INFECTION AND THERAPEUTIC**

Roberta Carvalho Basile

Advisor: Prof. Antonio de Queiroz Neto, BVM, PhD

Co-Advisor: Prof. Delphim da Graça Macoris, BVM, PhD

Thesis presented to the Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus of Jaboticabal, as partial fulfillment of requirements for the obtention of PhD Degree in Veterinary Medicine (Clinical Medicine).

2016

B311c Basile, Roberta Carvalho
Borreliosis in horses: epidemiology, experimental infection and
therapeutic, Jaboticabal, SP / Roberta Carvalho Basile. --
Jaboticabal, 2016
xvii, 131 p. : il. ; 28 cm

Tese (doutorado) – Universidade Estadual Paulista, Faculdade de
Ciências Agrárias e Veterinárias, 2016

Orientador: Antonio de Queiroz Neto

Coorientador: Delphim da Graça Macoris

Banca examinadora: Jairo Jaramillo Cardenas, Adolorata
Aparecida Bianco Carvalho, Estevam Guilherme Lux Hoppe, Natalino
Hajime Yoshinari

Bibliografia

1. Borrelia burgdorferi . 2. Carrapatos . 3. Infecção. 4. ELISA. 5.
PCR. I. Título. II. Jaboticabal - Faculdade de Ciências Agrárias e
Veterinárias.

CDU 619:616.98:636.1

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação –
Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

CURRICULUM OF THE AUTHOR

Roberta Carvalho Basile - Born in São Paulo, Brazil, on February 20, 1978, daughter of Lucio Godinho de Carvalho and Dolores de Carvalho, married to Marcelo Toledo Basile since January 2005 and mother of Matheus Carvalho Basile, born in March 2012. In December of 2012, she graduated in Veterinary Medicine at the Univ. Estadual Paulista, Jaboticabal, SP. She attended specialization in Clinical and Equine Surgery at the Jaguariúna University, SP, between 2013 and 2014, and Veterinary Acupuncture in this same University between 2011 and 2012. She graduated also in civil engineering at the University of São Paulo, School of Engineering of São Carlos, SP, in 2001 and completed his Master of Aeronautics and Mechanical Engineering at the Technological Institute of Aeronautics in Sao Jose dos Campos, Brazil, in 2003. She has held the Engineer profession as flight test engineer at Embraer between 2003 and 2008. She joined at the Graduate Program in Veterinary Medicine at the Univ. Estadual Paulista, Campus of Jaboticabal, SP, in March 2013 as a PhD student. She has been working as Assistant Professor at Camilo Castelo Branco University since 2015, being responsible for the Large Animals courses and attendances.

“Sometimes, the most interesting and unexpected results come from unlikely jobs.”

M.L.

DEDICATE

To my family, my fortress and greater wealth.

ACKNOWLEDGEMENTS

I am very grateful for my parents' love and its everlasting support, for my husband complicity, love and partnership, and for my son's comprehension and patience for the non played moments.

I am thankful to Prof. Queiroz, which was much more than an Advisor, a real partner whichever I needed during this journey. You are not from here!

To Prof. Delphim Macoris, my tutor which welcomed and sheltered me since the first day and I would say till actual days.

To Dear Virginia Bonoldi and Elenice Mantovani, which masterfully carried out all serological and molecular trials. Without that, nothing of this would be possible.

To my cherished Mariana Vieira and Luiz Felipe, loyal warriors always by my side.

To my Unesp colleagues: Lara, Talissa, Gabriel, Luciana, Aline, Renatha, Mateus, Raquel, Flora, Julia, Tamires, Buca, Walter, Mayara, Jessica, Bel, Gabriela, Vanessa, Yuri, Márcia Ajusi, each one of you had an essential contribution for this project.

To Dear Goido e Deco, my eternal acknowledgment for each drop of sweat and effort spent in these pages.

To the Professors Marcos R. André, Canola, Paulo Aléscio, Guilherme Ferraz, Rosângela Zacarias, each one of you helped guiding myself to overcome very hard times.

To the Veterinarians Rodolfo Lauricella, Cassiano Ricardo Rios, Paulo H. Machado, Renata Calegari, Reinaldo de Campos, Mariana Invernezzi, Claudia Leschonsky, Roberto Macedo, Alexandre Bloem, Rafael Daia which were somehow available to contribute in this project.

To the properties Sociedade Hípica Araraquara, Fazenda São Sebastião, Haras Vila Colonial, Manege Steel Horse, Haras das Mangueiras, Haras Interagro, Haras Raízes, Haras Las Vegas, Haras Santa Fé, Joquey Clube de SP, Haras TH, Haras Mont Serrat, Haras Vassoural, Universidade do Cavalo, Haras Lagoinha, Haras

Tango, Equinocultura Unesp Jaboticabal, which opened their gates and made their horses available allowing this study possible.

To the horses Pérsico, David Off, Ross, Duque d'Alba, Trufa, Alegria, Mara, Minie, Carriete, Angélica, Tigresa, Seif Alah, Cindy (*In Memoriam*), there is no words to describe, only my feelings.

To the Professors who attended my examining board, dedicating their times for revising and contributing towards the improvement of this work, as my personal and professional development.

For the Faculdade de Ciências Agrárias e Veterinárias (UNESP Jaboticabal), their teachers and staff, for sheltering me throughout my graduation and Doctor degree.

To the agency FAPESP and São Paulo's State Government, which funded this project.

To God, who never abandoned me and has slowly and wisely shown that nothing was in vain. For my Guardian Angel, who often blows in my ear the right paths to follow.

SUMMARY

1. INITIAL CONSIDERATIONS	1
1.1 INTRODUCTION.....	1
1.2 OBJECTIVES.....	3
1.3 RATIONALE.....	4
2. LITERATURE REVIEW	6
2.1 REFERENCES	18
3. ARE THE CLINICAL SIGNS OF LYME BORRELIOSIS REALLY UNDERSTOOD IN HORSES?	29
3.1 EDITORIAL	29
3.2 REFERENCES	30
4. CLINICAL, HEMATOLOGICAL AND RISK FACTORS ASSOCIATED WITH BORRELIOSIS IN HORSES FROM SAO PAULO, BRAZIL	33
4.1 INTRODUCTION	34
4.2 MATERIAL AND METHODS	36
4.2.1 <i>Epidemiologic assessment</i>	36
4.2.2 <i>Case-Control clinical trial</i>	39
4.2.3 <i>Statistical analysis</i>	40
4.3 RESULTS	40
4.3.1 <i>Epidemiologic assessment</i>	40
4.3.2 <i>Case-control clinical trial</i>	45
4.4 DISCUSSION.....	48
4.5 REFERENCES	52
5. EXPERIMENTAL LYME BORRELIOSIS IN TWO ADULT HORSES.....	58
5.1 INTRODUCTION	59
5.2 MATERIAL AND METHODS.....	61
5.3 RESULTS	66
5.4 DISCUSSION.....	74
5.5 REFERENCES	78

6. ANAPHYLACTOID REACTION INDUCED BY SODIUM CEFTRIAZONE IN TWO HORSES INFECTED BY BORRELIA BURGENDORFERI.....	84
6.1 BACKGROUND	85
6.2 CASE PRESENTATION.....	88
6.3 REFERENCES	107
7. FINAL CONSIDERATIONS	112
7.1 GENERAL CONCLUSIONS	112
7.2 FUTURE RESEARCH.....	113

TABLES

Table 1 – Distribution of horses according to their antibody titers against <i>B. burgdorferi</i> sensu lato, obtained through ELISA, in the 11 cities of São Paulo state, Brazil, with the most reported cases of suspected Lyme disease in humans.	42
Table 2 – Scoring points for the risk and clinical history (5 years) parameters of a subgroup of 124 adult horses (59 seronegatives and 65 seropositives for <i>B. burgdorferi</i>), stabled on 22 properties from 11 cities of São Paulo State.	43
Table 3 – Number of cases of seropositivity and seronegativity for <i>B. burgdorferi</i> sensu lato in a subgroup of 291 horses from Sao Paulo, Brazil, according to the type of stabling.	45
Table 4 – Hematological parameters of 20 seronegative and 20 seropositive horses in a case-control clinical trial, from São Paulo, Brazil.	46
Table 5 – Qualitative parameters for the clinical evaluation of seven horses during 90 days of experimental infection. Groups: C-: negative control (n=2), INF: experimentally infected horses (n=2), Immun: experimentally immunized horse (n=1), C+: naturally immunized horses (n=2).....	68
Table 6 – Hematological test results of seven horses during 90 days of experimental infection with <i>Borrelia burgdorferi</i> strain G39/40. Groups: C-: negative control (n=2), INF: experimentally infected horses (n=2), Immun: experimentally immunized horse (n=1), C+: naturally immunized horses (n=2).	70
Table 7 – Biochemical serum analysis of seven horses during 90 days of experimental infection with <i>Borrelia burgdorferi</i> strain G39/40. Groups: C-: negative control (n=2), INF: experimentally infected horses (n=2), Immun: experimentally immunized horse (n=1), C+: naturally immunized horses (n=2).	72
Table 8 – Gelding red blood cell count before and after anaphylactic reaction to sodium ceftriaxone, which occurred on day 1.....	91
Table 9 – Gelding blood gas analysis after anaphylactic reaction to sodium ceftriaxone, which occurred on day 1.	92
Table 10 - Gelding serum biochemical parameters before and after anaphylactic reaction to sodium ceftriaxone, which occurred on day 1.....	96

Table 11 – Mare red blood cell count before and after anaphylactic reaction to sodium ceftriaxone, which occurred on day 1.....	98
Table 12 - Mare serum biochemical parameters before and after anaphylactic reaction to sodium ceftriaxone, which occurred on day 1.....	98
Table 13 – Timetable of the events described for the anaphylactic reaction to sodium ceftriaxone of a horse and a mare.....	101

FIGURES

- Figure 1 – Cluster analysis of the risk factors and clinical parameters of 124 adult horses (59 seronegatives and 65 seropositives for *B. burgdorferi*) stabled in 22 properties from 11 cities of São Paulo state.44
- Figure 2 - Principal component analysis of the clinical and hematological parameters of 20 seropositive and 20 seronegative horses (immunoglobulins anti-*Borrelia burgdorferi*). Creat: creatinine, Bil unc: bilirubin unconjugated, Glob: globulins, AST: aspartate aminotransferase, PT: total protein, Alb: albumin, GGT: gamma glutamyl-transferase, Es: eosinophils, RBC: red blood cells, Hb: hemoglobin, PCV: packed cell volume, Mo: monocytes, Ly: lymphocytes, Plat: platelets, WBC: white blood cells, (cont.) (continued) NB: neutrophils (band), NT: neutrophils (mature), ELISA: enzyme-linked immunosorbent assay, CPK: creatine phosphokinase.47
- Figure 3– A – Subcutaneous application of *Borrelia burgdorferi* diluted in BSK medium near the cervical chain of lymph nodes in a mare. B – Increased volume below the injection site one day after application of the inoculum.67
- Figure 4 – Antibody titers evaluated by indirect ELISA for seven horses, two of which received experimental infection. The large arrow indicates the day of infection with *Borrelia burgdorferi* strain G39/40 in two horses (Infected horses) and the first immunization of another horse (immunized horse), (cont.)73
- Figure 5 - Abdominal ultrasound of the gelding with tympanic colic as a result of anaphylactic reaction to sodium ceftriaxone. Image obtained by ventral-abdominal positioning of the probe, caudal to xiphoid. Note the thickening of intestinal wall, distention, atony and *ileus* in segments of the small intestine. Diagnostic Imaging Sector - FCAV - UNESP - Jaboticabal.93
- Figure 6 - Exposure of the gelding cecum and jejunum during exploratory celiotomy. Full line arrow: cecum. Dashed line arrow: jejunal serosa showing edema due to anaphylaxis caused by sodium ceftriaxone, in lighter color compared to other intestinal loops.....94
- Figure 7 - Infrared images to monitor the evolution of the laminitis treatment in the mare that had colic secondary to anaphylactic reactions to ceftriaxone sodium. Day 6 was the second day of temperature rise in the hoof. The crosses indicate the

temperature of the hoof crown for each member. Temperature profile of the hooves was back to normal on Day 20. Spot 1: right limb, Spot 2: left limb.....99

Figure 8 – Infrared image of the four limbs of the mare two days after treatment started (Day 8). The crosses indicate the temperature of the hoof crown of each member. Spot 1: right forelimb, Spot 2: left forelimb, Spot 3: right hindlimb, Spot 4: left hindlimb.100



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal



CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 001968/13 do trabalho de pesquisa intitulado "**Caracterização clínica, laboratorial e terapêutica de equinos infectados pela Borreliose de Lyme Símile (brasileira)**", sob a responsabilidade do Prof. Dr. Antonio de Queiroz Neto está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 05 de fevereiro de 2013.

Jaboticabal, 05 de fevereiro de 2013.

Prof. Dr. Andriago Barboza De Nardi
Coordenador - CEUA

BORRELIOSIS IN HORSES: EPIDEMIOLOGY, EXPERIMENTAL INFECTION AND THERAPEUTIC

Abstract – Lyme borreliosis is a disease caused by the spirochete *Borrelia burgdorferi* sensu lato, cosmopolitan and transmitted by the bite of ticks which remain adhered to the host for more than 24 hours. In humans, it can cause articular, cardiac and neurological diseases. In horses, so far the disease had been described by means of case reports and extrapolations of its pathogenesis in humans. This study aimed to investigate the clinical signs and hematological changes of Lyme disease in horses. Furthermore, it is also assessed the feasibility of treating infected horses with sodium ceftriaxone. To this end, the experiment consisted of three main phases. The first phase consisted of an epidemiological survey of the disease in São Paulo State, specifically in cities with suspected cases of Lyme borreliosis in humans. It was collected blood samples and clinical history of 760 horses that resulted in an average of 21% seropositivity in the state. In this stage, it was concluded that there was a high relationship between seropositivity, *Amblyomma sculptum* tick presence, the presence of capybaras in the property, lymphopenia, abortion and retained placenta. The second phase consisted of an experimental infection of two adult horses with *B. burgdorferi* strain G39 / 40. The horses were evaluated for 90 days of infection and we found that the animals showed nonspecific clinical signs and hematologic changes only in the first 11 days of infection. It was noted the presence of mild hypochromic normocytic anemia, muscle pain, pale mucous membranes, lethargy and swollen lymph nodes, signs that can easily be confused with chronic piroplasmosis. During phase 3 of the experiment, the two horses experimentally infected underwent treatment with intravenous sodium ceftriaxone. Already during the first application, both developed an anaphylactoid reaction moderate to severe with colic syndrome as consequence for one horse and laminitis to the other. Both recovered and were finally treated with oxytetracycline.

Keywords: *Borrelia burgdorferi*, tick, ELISA, PCR, sodium ceftriaxone.

BORRELIOSE EM CAVALOS: EPIDEMIOLOGIA, INFECÇÃO EXPERIMENTAL E TERAPÊUTICA

Resumo – A Borreliose de Lyme é uma doença causada pela espiroqueta *Borrelia burgdorferi* sensu lato, cosmopolita, transmitida por meio da picada de carrapatos que permanecem aderidos ao hospedeiro por mais de 24 horas. Em humanos, pode provocar doenças articulares, cardíacas e neurológicas. Nos equinos, até o presente momento a doença havia sido descrita por meio de relatos de caso e extrapolações de sua patogenia nos humanos. Por meio do presente estudo, pretende-se pesquisar os sinais clínicos e alterações hematológicas da borreliose de Lyme nos equinos. Além disso, avaliou-se também a viabilidade de se tratar os equinos infectados com ceftriaxona sódica. Para tanto, o experimento foi composto por três principais fases. A primeira fase foi composta por um levantamento epidemiológico da doença no Estado de São Paulo, especificamente nas cidades com casos suspeitos de borreliose de Lyme em humanos. Coletou-se amostras de sangue e histórico clínico de 760 equinos e obteve-se média de 21% de soropositividade no estado. Desta fase, concluiu-se que existe grande relação entre a soropositividade, presença de carrapatos *Amblyomma sculptum*, presença de capivaras na propriedade, linfopenia, abortamento e retenção de placenta. A segunda fase foi composta por uma infecção experimental de dois equinos adultos com *B. burgdorferi* cepa G39/40. Os equinos foram avaliados durante 90 dias de infecção e foi possível verificar que os animais apresentaram sinais clínicos e alterações hematológicas inespecíficas somente nos primeiros 11 dias de infecção. Notou-se a presença de anemia normocítica hipocrômica discreta, dores musculares, palidez de mucosas, letargia e aumento de linfonodos, sinais que podem facilmente ser confundidos com a piroplasmose crônica. Durante a fase 3 do experimento, os dois equinos infectados experimentalmente foram submetidos ao tratamento com ceftriaxona sódica por via intravenosa. Já durante a primeira aplicação, ambos desenvolveram uma reação anafilactóide de moderada à severa, com consequência de síndrome cólica para um deles e laminite para o outro. Ambos se recuperaram e foram finalmente tratados com oxitetraciclina.

Palavras-chave: *Borrelia burgdorferi*, carrapato, ELISA, PCR, ceftriaxona sódica.

1. INITIAL CONSIDERATIONS

1.1 INTRODUCTION

Lyme borreliosis is a multisystemic disease discovered in the mid-1970s in the United States, in children of Connecticut with polyarthritis without determined causes. It is a long term studied and diagnosed condition in the Northern Hemisphere, caused by the spirochete *Borrelia burgdorferi* present in Ixodidae family of ticks. This pathogen is able to infect mammals including humans and each ones have different clinical responses upon the infection. The most important reservoirs in Brazil should be the capybaras, which also could serve as carriers and disseminators of ticks in pastures and near local lakes and rivers.

My history with borreliosis began in 2004, while still working as an engineer I fell in love with the horses. I began to enjoy horseback riding in a training center in Sao Jose dos Campos in the end of my working days, which came up to contribute immensely to this PhD project. Everything was doing well until the day I started to feel pain in the carpal joints. The pain only regressed with anti-inflammatory drugs and started get around other joints such as elbows and knees. In a few months, I could not sleep soundly because the pain progressively increased. At this time I did not remember having found any tick, actually I did not even know what it was.

My joints were swollen, purplish and painful. In the morning, I did have no strength to turn the door handle, it became a common routine pushing the toothpaste tube with my elbow, and to step down the stairs of my house seated. I have visited many physicians as clinical, orthopedic, homeopaths, rheumatologists, and numerous tests were carried out without any clear diagnosis. In their view, I had rheumatoid arthritis, lupus, fibromyalgia, stress, I was eating wrong, etc.... Everything

seemed to be a cause, unless an infectious disease. I have achieved a slight improvement with a physician who told me, despite my inconclusive tests, I should treat me to lupus with steroids and chemotherapy.

Since then, four years have passed and I started a degree in veterinary medicine. In the third year, I remember having attended a zoonosis class where the teacher introduced me to this illness for the first time. I was thoughtful, remembering what I had passed a few years ago. The information was stored, waiting to be used.

When I was in fourth year of my under-graduation, we worked with some horses in the university. This site was known to be full of ticks and, one day, I found one stuck in my body, I did not know exactly how long. I removed it immediately, and in the following days it was formed a generous inflammatory circle around the bite.

A few days later, I started feeling extremely tired; it looked like building a house every day. I generally arrived at home early evening always feverish, around 38°C. I felt weak, discouraged, with headaches, depression and pain in the throat that does not regress with painkillers. I went to an endocrinologist, because we really thought I could have some thyroid problem. It was when, during the attendance, my husband asked me to comment with him that I had recently been bitten by tick. When he said this, two seconds became six years in my head. I looked at his face and thought: "Eureka!". And the doctor looked at my face thinking "Is your husband crazy?"

Without explaining the reasons, immediately I asked the doctor if he could request a serology for Lyme. And he spoke with doubt "Laaaaiiiiiiiiiiiiiiiiiiiiiiiii is the spotted fever?". I replied: "No doctor, also transmitted by ticks, but the spotted fever

is transmitted by a rickettsia and Lyme, borrelia." And then the doctor added: "OK, how it is spelled?" And finally asked for IgG and IgM serology for Lyme.

My disease seemed to be finally diagnosed and I start seeking for a medical advice in this field. In my brief research I found Dr. Natalino Yoshinari, the pioneer physician in the study of the disease in Brazil and member of the research group of this project. I was treated with antibiotics and anti-inflammatory drugs for a period of 60 days, when we later found out that I was pregnant. But the treatment was a successful and the pregnancy. Since 2012, I have had mild annual recurrences, which also was controlled with chemotherapeutic agents.

7. FINAL CONSIDERATIONS

7.1 GENERAL CONCLUSIONS

This experiment provided more information about possible clinical signs of Lyme borreliosis in horses, besides the disease remains obscure. In its early phase, horses can present non-specific clinical signs such as lethargy, pale mucous, muscular back pain and enlarged lymphonodes. The hemolytic activity of *Borrelia burgdorferi* front of the red blood cells of horses can promote a mild anemia in the first week of infection. This mild alterations can induce veterinarians to misdiagnose piroplasmosis in horses infected by *Borrelia burgdorferi*. It is also possible to verify the occurrence of abortion and retained placenta associated with laminitis in mares. Even without the horses are treated, after 90 days of infection, it is no longer possible to detect antibodies in blood. The seropositivity of horses can be related to the presence of *Amblyomma sculptum* and capybaras in the property. The treatment of the infection with ceftriaxone can cause an anaphylactoid reaction in horses that may be associated with massive bacterial death, with consequences like colic syndrome. The horses treated with oxytetracycline for 21 days tolerated the therapy with no side effects presentation.