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PREFACE

This dissertation has been organized as a series of manuscripts which were or will be submitted for publication to peer-reviewed scientific journals. Thus, some repetition of introductory or methodology material is unavoidable.

ABSTRACT

Lawsonia intracellularis causes proliferative enteropathies in juvenile mammals. The porcine (PPE) and equine (EPE) diseases are worldwide. Rabbits and hamsters are naturally susceptible, the latter being a classic modeling-host for PPE. None is known for EPE, besides foals. An *in vitro* evaluation of antimicrobial efficacy against *L. intracellularis* is difficult. This study aimed to validate a laboratory animal EPE model and to investigate pharmacokinetics (PK) and efficacy of gallium maltolate (GaM) as an alternative antimicrobial therapy. Infected animals were inoculated with cell-cultured *L. intracellularis* and infection was verified with clinically utilized diagnostic tests.

Initially, 2 groups of EPE-infected rabbits were compared to 1 uninfected group. After inoculation (PI), EPE-infected rabbits showed mild clinical signs; detectable seroconversion, fecal shedding, gross lesions in intestinal tissues (IT), and early immuno-histochemistry labeling of *L. intracellularis* antigen. Thus, a humane EPE-rabbit model was achieved. Subsequently, EPE-infected hamsters were compared to uninfected and PPE-infected hamsters; whereas, PPE-infected rabbits were compared to EPE-infected rabbits. EPE-hamsters did not develop infection, unlike PPE-infected controls; and PPE-rabbits did not develop IT lesions or seroconversion comparable to EPE-rabbits.

Therefore rabbits were chosen as the EPE modeling-host for the GaM studies. First, GaM PK and IT concentrations of Ga and Fe were measured. Then, GaM efficacy was compared to a current EPE antimicrobial treatment. During sampling, the intra-arterial catheters in the rabbits' ears were protected with a novel moleskin-cover, allowing repeated sampling while minimally restrained.

The PK study was based on the comparison of EPE-infected and uninfected rabbits, after a single treatment with GaM, collection of serial blood samples and IT samples. The only differing PK parameter, between groups, was a decrease in the terminal phase rate constant of the EPE-rabbits, so a 48h dosing interval was chosen for the efficacy study.

In the efficacy study, 3 groups of EPE-infected rabbits were treated with GaM, doxycycline and a placebo, respectively. No differences were noted between treatments, in terms of lesions and fecal shedding. GaM appears no more efficacious than doxycycline in EPE-rabbits. In conclusion, albeit GaM tolerance appeared adequate in rabbits, results do not support its use in EPE-infected animals.

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The accomplishment of this dissertation includes people that were not directly involved in my advisory committee, but due to their dedication, constant presence and support they felt like they were. Thus, my gratitude goes to Dr. Andrew Allen (University of Saskatchewan, Saskatoon, SK), who guided me through those pathology branches that I've never appreciated so deeply, as a clinician, as I do now; To Drs. Connie Gebhart and Fabio Vannucci (University of Minnesota, St. Paul, MN, USA) who taught me about *Lawsonia intracellularis* and its genetics, the immunohistochemistry, serology techniques, cell cultures and infection model challenges, more than I ever imagined; To Drs. Nicola Pusterla and Samantha Mapes (University of California Davis, Davis, CA, USA) I express my gratitude for their precious contribution of extensive qPCR analysis and neverending support in "our bunny" research.

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Finally I would like to acknowledge the animals (hamsters and rabbits) that were used for these experiments, hoping that our experimental results will encourage other researchers, near and far, to be respectful of what animal models can provide and teach.

DEDICATION

This dissertation is dedicated to several people in my life, as their constant presence made it possible for me to achieve such a prestigious goal with passion and determination.

To my husband, Kevin, as he kindly supported me through every step of this adventure with patience, encouragement and love.

To my parents, Giuseppe and Rita, as their encouragement and unconditional love never faltered through a far distance and… the third thesis;

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Last but not least, this dissertation is dedicated to my yet unborn son, to show him that commitment runs in the family and I'll be committed to him the same way I'm committed to good science. All my life.

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LIST OF ABBREVIATIONS

AFMA	Americans for Medical Advancement
AM(s)	Antimicrobial(s)
AUC	Area under the curve
Cl_O	Oral clearance
C_{max}	Maximum concentration obtained in serum after drug exposure
DMT-1	Divalent metal transporter-1
DPI or PI	Days after inoculation or Post infection
ELISA	Enzyme Linked Immuno-sorbent Assay
EPE	Equine proliferative enteropathy
Fe(II)	Ferrous Iron
Fe(III)	Ferric Iron
[Fe]	Iron concentration
FISH	Fluorescent in-situ hybridization
[Ga]	Gallium concentration
Ga(III)	Elemental Gallium
GaM	Gallium maltolate
GIT	Gastrointestinal tract
H&E	Haematoxylin and Eosin staining
IFAT	Indirect Fluorescent Antibody Test
IHC	Immunohistochemistry
IPMA	Immunoperoxidase monolayer assay
IPX	Immunoperoxidase
IRE	Iron responsive elements
IRP	Iron regulatory protein (1, 2,…)
ISH	In-situ hybridization
IT	Intestinal tissues
K_m	Michaelis-Menten affinity constant
mM	Millimole
MRT	Mean residence time
OGAT	Oral glucose absorption test
PCR	Polymerase chain reaction
PE	Proliferative enteropathy (in general, for every species)
PHE	Porcine hemorrhagic enteritis
PIA	Porcine intestinal adenomatosis
PK	Pharmacokinetics
PPE	Porcine proliferative enteropathy
PT	Post treatment
qPCR	Quantitative PCR

$t_{½}$	Half-life
T_{max}	Time to reach maximum concentration after drug exposure
V_{max}	Maximum initial rate of an enzyme (in this case) catalysed reaction
VESPERS	Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron
vs.	versus
WS	Warthin-Starry staining
λ	Elimination rate constant (non-compartmental analysis)
μM	micromole