Title

The *Eimeria*-host cell interaction in broiler chickens

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Dedication

To, Ali, my dear son

My kind son, my best friend who always gives me strength and force to tolerate everything....

I am so sorry; I may fulfill your wish for going back to your country, Iran, where you told me many people like you and you want to live there. I will never forget that you told me; "Dad please finish your study and backing to Iran". I tried, but failed to grant your wish,

It is not your fault but mine. I know a day will come when you can make the decision for yourself and you can choose your country for the rest of your life.

I shall never forget you and the way that you put your arm around my neck.

I do not know if there will come a day when we can meet each other or not, but Ali, you know that I love you forever. Wherever I will not forget you until my heart stops beating.

Your father

Table of contents

List of Tables

List of figures

List of plates

Summary

Coccidiosis is an enteric infection of chickens caused by protozoan parasites of the genus *Eimeria*. Coccidiosis is a worldwide disease with an economic impact on broiler chicken production. An outbreak of disease can reduce weight gain and feed digestion in the entire flock, reducing the production of processed meat for market. The major characteristics of *Eimeria* species are the invasion of specific sites in the intestine of chickens and specificity of the immune response.

To date, prophylaxis and vaccination are used to control coccidiosis. However, the continuous use of chemotherapeutics has led to increased drug resistance by *Eimeria*. In the case of vaccination, immunity against *Eimeria* is species-specific, hence, there is a need to vaccinate chickens against all species of *Eimeria* for complete protection. The *Eimeria*-host cell interaction is the first stage in the reproductive cycle in chickens that produces the damage in the chicken intestine. A more complete understanding of the environmental factors within the intestinal tract that influence this interaction will be useful to control the disease. The lack of a suitable method to study the interaction between *Eimeria* and host cells derived from different areas of intestine has hampered our understanding of the disease. The cell type of interest for this study was the chicken enterocyte. A layer of mucus is secreted by goblet cells in the intestinal epithelial to protect the enterocytes. *Eimeria* sporozoites have to cross the mucus layer in order to invade the epithelial cells. It is reasonable to assume that this mucus may have some involvement in *Eimeria*-enterocyte attachment. The objectives of this study were to investigate the roles of the enterocyte and intestinal mucus in the attachment process and the subsequent penetration of host cells by *Eimeria* sporozoites.

Newly hatched, and 3-week-old chickens, were killed and intestinal segments were collected for developing an *in vitro* method *ex vivo* (organ culture system, isolated enterocytes and a frozen section method) to study the *Eimeria* interaction with intestinal epithelial cells. *Eimeria* sporozoites were extracted from oocysts and then labelled with a fluorescent dye (PKH-67). The frozen section model was found to be superior to the use of isolated enterocytes and organ culture systems, and was used for subsequent experiments in this project. This method was used to

investigate the *Eimeria*-enterocyte attachment at preferred and non-preferred sites on the surface of enterocyte membranes. Indeed, the use of this method demonstrated that D-galactose on the surface of sporozoites had an important role in the attachment of *E. tenella* sporozoites to caecal enterocytes, with caecal and duodenal mucus both functioning as a physical barrier to *E. tenella.* In addition, two other major developments resulted from this project, these being; the development of a PCR protocol that can specifically identify different *Eimeria* species in a mixed sample containing at least 0.05 ng/µl of *Eimeria* DNA and a propidium iodide method that is a suitable indicator tool to assess the viability of oocysts and sporocysts. Finally, the inclusion of $MgCl_2$ in the extraction buffer increases the hatchability of sporozoites from sporocysts.

In conclusion, this study led to development of a frozen section method which can be used *ex vivo* to investigate further the role of mucus from vaccinated and non-vaccinated chickens, diets with different compositions, anticoccidial drugs, and the identification of the specific receptors in different areas of the chicken intestine. Finally, the propidium iodide method in combination with the PCR protocols can be used as a quality assurance tool in the production of *Eimeria* vaccines.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Abbreviations used in thesis

µg	Microgram
µl	Microlitre
µm	Micrometer
°C	Degrees centigrade
C.	*Cryptosporidium*
cells/ml	Cells per millilitre
cm	Centimetre
cm^2	Square Centimetre
DE-52	Dimethylaminoethyl cellulose
D-gal	D-galactose
DNA	Deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'-triphosphate
E.	*Eimeria*
E.a	*Eimeria acervulina*
E.t	*Eimeria tenella*
FBS	fetal bovine serum
g	Gram
g/L	Gram per litre
HBSS	Hanks' Balanced Salt solution
IU	International unit
IU/ml	International units per milliliter
MAbs	Monoclonal antibodies
MDBK	Madin Darby Bovine Kidney
MEM	Minimum Essential Medium Eagle
mg/ml	Milligram per millilitre
ml	Millilitre

mM	Millimole
mm	Millimetre
NBF	Neutral buffered formalin
ng	Nanogram
nm	Nanometre
OD	Optical density
PCR	Polymerase chain reaction
PBS	Phosphate buffered saline
pM	Picomole
PI	Propidium iodide
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic acid
rpm	Revolutions-per-minute
SD	Standard deviation
SCAR	Sequence-characterized amplified regions
TAE	Tris acetate-EDTA
Taq	Thermostable DNA polymerase
Tris	Tris[hydroxymethyl]aminomethane
x 40	A magnification of x 40
xg	Times gravity
UV	Ultraviolet
v/v	Volume by volume
W/v	Weight by volume