International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (4), pp. 001-005, April, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effects of the electromagnetic radiation on oocysts of *Eimeria papillata* infecting mice

Saleh Al-Quraishy[1], Mohamed A. Dkhil[1,2*] and Abdulsalam A. Alkhudhayri[1]

[1]Department of Zoology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.
[2]Department of Zoology and Entomology, Faculty of Science, Helwan University, Helwan, Egypt.

Accepted 23 February, 2019

Electromagnetic radiation (EMR) produced by many telecommunication systems, has short and long term biological effects on living cells. The aim of this study was to investigate the influence of EMR on the outcome of coccidiosis induced by *Eimeria papillata*. Oocysts from *E. papillata* infected mice were exposed to the EMR in the form of gamma rays, ultraviolet rays and radiations emitted from the mobile phone. Sporulation rate, oocysts shedding as well as the histological alterations in jejunum of mice irradiated with oocysts exposed to EMR were determined. Oocyst output was reduced in mice exposed to EMR. The jejunum histopathology was improved after inoculation of mice with irradiated oocysts. We suggest that EMR has anticoccidial activities and its application could serve as an alternative to the anticoccidial drugs currently used in poultry production.

Key words: Electromagnetic radiation, *Eimeria papillata*, mice.

INTRODUCTION

Coccidiosis is a common infectious disease in poultry, causing major economic losses. The protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract of poultry and produces tissue damage, resulting in reduced growth and increased susceptibility to pathogens (McDougald, 2003). The Eimerian parasites are characterized by fast reproduction and by infecting especially young animals (Gres et al., 2003; Pakandl, 2005). Infection begins with oral uptake of Eimerian oocysts, which release infectious sporozoites in the intestine. These, in turn, invade mainly epithelial cells of the intestine, in which they asexually multiply before oocysts are finally discharged with the feces.

It is known that electromagnetic radiation (EMR) produced by many telecommunication systems, has short and long term biological effects on living cells (Galeev, 2000; Gos et al., 2000). Related to this subject, numerous *in vivo* and *in vitro* researches are carried out either on humans and animals or microorganisms (Juutilinen and Seze, 1998; Gos et al., 2000). When researches on the effects of EMF are investigated, it is seen that the number of studies on the influence of electromagnetic radiation on single cell protozoa is very limited (Berk, 1997). EMR have demonstrated the production of cytokines, increased immune parameters and stress effects and concluded that EMR causes stress at the cellular level and that this leads to production of cytokines and consequently biological response, including immune response (Markov et al., 2006).

Exposure of *E. tenella* oocysts to EMR decreased infectivity (Bajwa and Gill, 1977). The current study aimed to investigate the possible anticoccidial activity of the electromagnetic field.

MATERIALS AND METHODS

Animals

Male Swiss albino mice were bred under specified pathogen-free conditions and fed a standard diet and water *ad libitum*. The experiments were performed only with male mice at an age of 8–9 weeks and were approved by state authorities and followed Saudi Arabian rules for animal protection.

*Corresponding author. E-mail: mohameddkhil@yahoo.com. Tel: 00966-14675754. Fax: 00966-14678514.

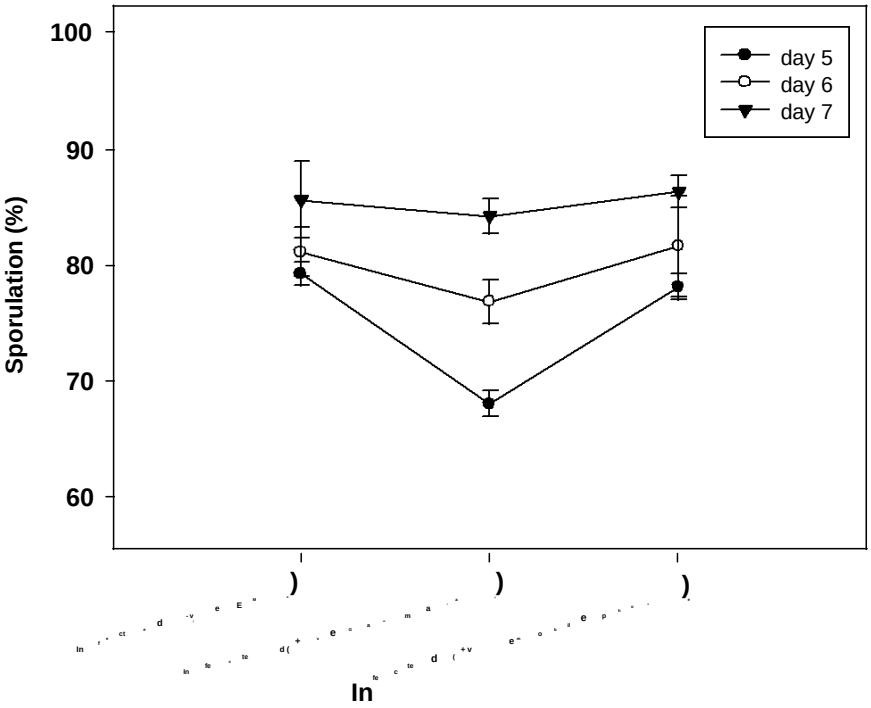

Figure 1. Changes in sporulation of *E. papillata* oocysts due to EMR. Values are means ± SD.

Irradiation of *E. papillata* unsporulated oocysts

E. papillata, kindly provided by Prof. Mehlhorn (University of Duesseldorf, Germany), was previously characterized (Hnida and Duszynski, 1999; Zhao and Duszynski, 2001). *E. papillata* oocysts were obtained from infected mice at day 4 postinfection by both cecal harvest and fecal collection using standard procedures (Schmnatz et al., 1984). Unsporulated oocysts were divided into 4 groups and placed in 4 Petri dishes; each contained at least 5000 oocysts in 10 ml distilled water. The first group was unexposed to the electromagnetic radiation (-ve EMR). The second group was exposed to a dose of 500 Gy gamma-radiation using Gamma Cell 200 Irradiator (Atomic Energy of Canada, Ltd., Ottawa, Canada) utilizing a ^{60}Co source located at the Research Center of College of Science, King Saud University, Saudi Arabia. The third group was exposed to a dose of ultravilot radiation from Molecular Imager Gel Doc XR System (Hercules, California, USA) in a dose of 302 nm for 30 min. The fourth group was exposed to an electromagnetic radiation from a mobile phone (Nokia 6120) receiving a call for 1 h. Directly after exposure of all groups to the EMR, oocysts were allowed to sporulate as described by Schito et al. (1996). Sporulation rate of each group was calculated on day 5, 6 and 7.

Effect of irradiation on oocyst production

Oral gavage of mice from all groups was done with 1000 sporulated oocysts of *E. papillata* suspended in 100 µl sterile saline. Once a day, fresh fecal pellets were collected and weighed for each mouse, and the bedding in the cages was changed to eliminate re-infection. Faecal pellets were diluted in saturated NaCl, causing the oocysts to float. The latter were counted in a McMaster chamber and expressed as number of oocysts per gram of wet feces (Schito et al., 1996). The oocyst output was calculated in day 4 p.i.

Histological analysis

Pieces of jejunum were freshly prepared, fixed in 10% neutral buffered formalin, and then embedded in paraffin. Sections were cut and then stained with hematoxylin and eosin according to Drury and Wallington (1980). According to Dommels et al. (2007), tissue sections were scored for inflammatory lesions (infiltrations by mononuclear cells, neutrophils, eosinophils, and plasmacytes, for fibrin exsudation and lymphangiectasis, for tissue destruction (enterocyte loss, ballooning degeneration, edema, and mucosal atrophy), and for tissue repair (hyperplasia, angiogenesis, granulomas, and fibrosis). A rating score between 0 (no change from normal tissue) and 3 (lesions involved most areas and all the layers of the intestinal section including mucosa, muscle, and omental fat) was given for each aspect of inflammatory lesion, tissue destruction, and tissue repair. The sum of inflammatory lesions, tissue destruction, and tissue repair scores was used to represent the total histological injury score (HIS) for each intestinal section. The sum of the inflammatory lesions was multiplied by 2 to give more weight to this value since the tissue changes were mainly characterized by inflammatory lesions.

Statistical analysis

Statistical analyses were performed using Student's *t*-test at $p < 0.01$.

RESULTS

Electromagnetic radiation could induce a change in sporulation rate as indicated in Figure 1. The percentage of sporulation of the irradiated oocysts with 500 Gy

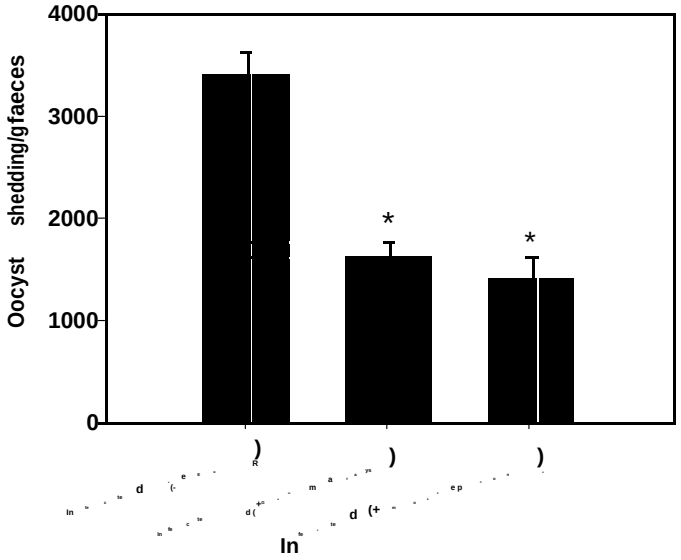

Figure 2. Oocyst output in mice infected with irradiated *E. papillata* oocysts. Values are means ± SD. *, significant difference at (p<0.01) compared to infected –ve EMR group.

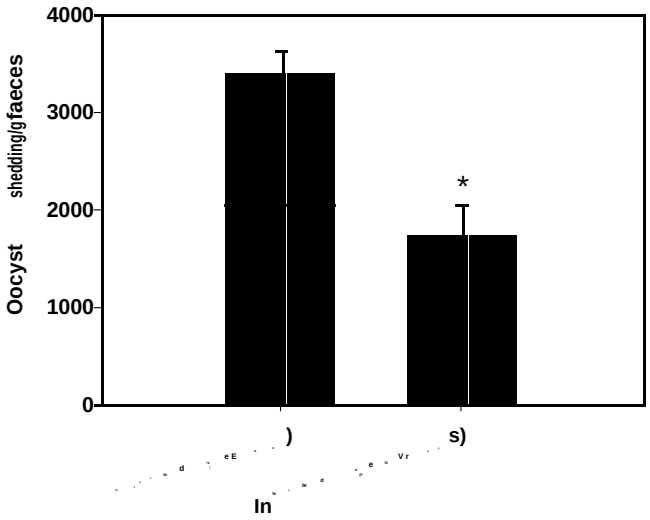

Figure 3. Oocyst output in mice infected with irradiated *E. papillata* sporulated oocysts. Values are means ± SD. *, significant difference at (p<0.01) compared to infected –ve EMR group.

gamma rays was lower in days 5, 6 and 7 than that of the control group. EMR emitted from the mobile phone could also alter the sporulation rate in day 5 post sporulation in dichromate. Surprisingly, unsporulated oocysts exposed to UV for 30 min could inhibit sporulation process.

Oocyst output in faeces of mice at day 4 p.i. were significantly decreased to more than 50% in mice inoculated with oocysts irradiated with gamma rays (Figure 2). EMR emitted from mobile phone could also significantly reduce the shedded oocysts from mice infected with *E. papillata* (Figure 2).

Sporulated oocysts of *E. papillata* post-irradiated with UV for 30 min could significantly ($p < 0.01$) decrease the oocyst out put in faeces of mice (Figure 3).

Histological analysis revealed that mice infected with sporulated oocysts of *E. papillata* suffered a moderate inflammatory injury in jejunum (Figures 4 and 5). This injury was reduced when mice were exposed to EMR (Figure 5). The histological injury score reached approximately 16, 11, 10 and 12 in infected (-ve EMR), infected (+ gamma rays), infected (+ UV rays) and infected (+ mobile phone) groups, respectively (Figure 5).

DISCUSSION

Oocysts of *E. papillata* spend a part of their life cycle outside the host when reproductive stages are released from the host into the external environment, where they wait to be transmitted to a new host. Hence, the fitness of the parasite depends, at least in part, on its ability to resist adverse external conditions before transmission. Our results clearly show that exposure of unsporulated oocysts to the EMR significantly reduced the oocysts output in faeces of mice. This reduction occurrence may be due to the reduction in the viability of the oocysts (Martinaud et al., 2009).

Some of the researchers investigating the biological effects of EMR, used mobile phones (Irmak et al., 2002) for their experiments. We preferred to produce EMR by means of a GSM mobile phone turned to speech position for 1 h. Also, we used UV rays and gamma which produce electromagnetic waves. EMR from these sources affected the sporulation process of *E. papillata* as well as the oocysts output after inoculation into mice.

EMR mediated a reduction of the severity of lesion in the jejunum of the infected mice. These results generally support the observations of Gilbert et al. (1998) who found that irradiation of sporulated oocysts of *Eimeria* reduced the pathogenic effects when oocysts were inoculated into animals and reduced the ability of sporozoites to develop in cultured cells.

Remarkably, the jejunum of mice infected with *E. papillata* is characterized by low inflammation (Al-Quraishy et al., 2011). Exposure to EMR may have anti-inflammatory effect (Vallbona and Richard, 1999; Jasti et al., 2001), and electromagnetic signals have been reported to stimulate the production of cytokines, mediating an enhanced immune response (Blank et al., 1992; Mevissen et al., 1998; Simko and Mattsson, 2004).

The novel observation was that exposure of the unsporulated oocysts to EMR antagonized the effect of infection with *E. papillata*. Further work on the effect of EMR exposure on coccidiosis infection is needed. Perhaps EMR could serve as an alternative to the anticoccidial drugs currently in use.

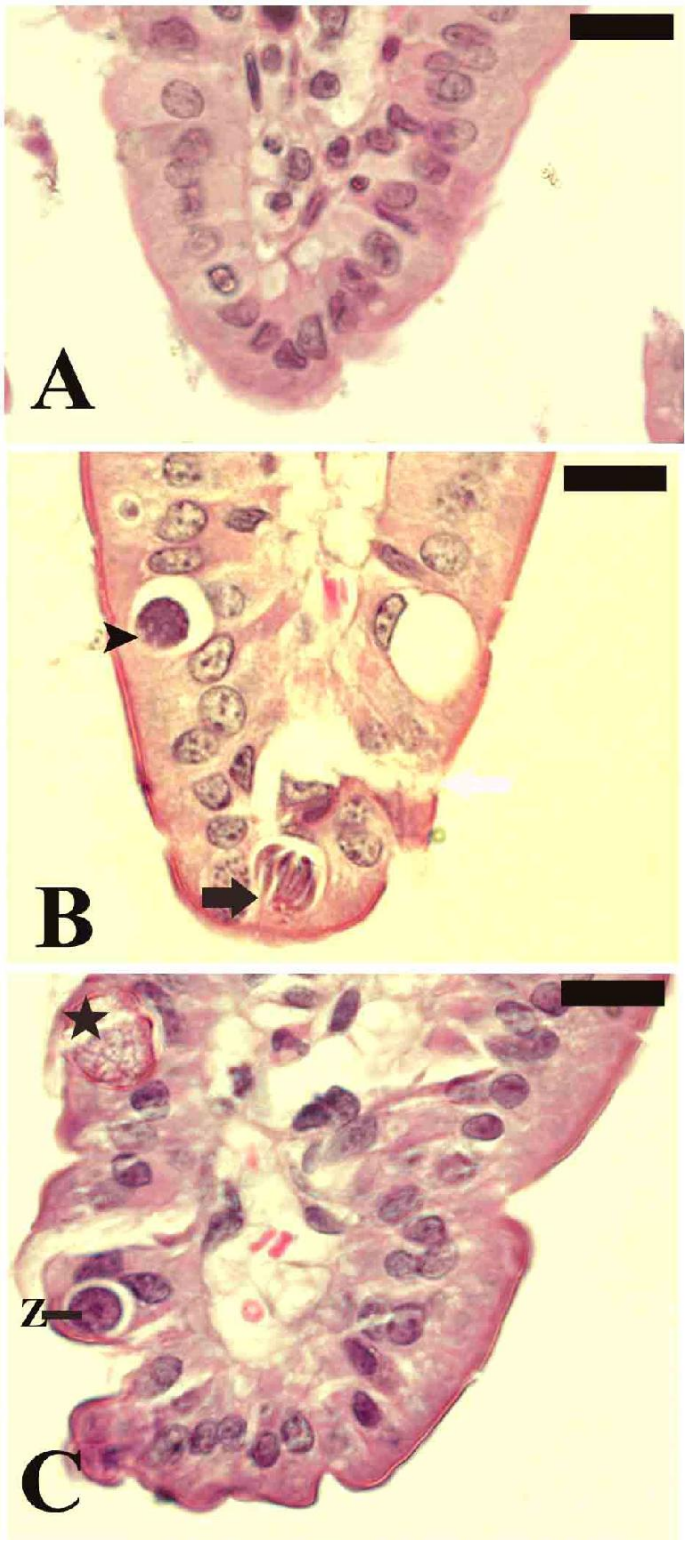

Figure 4. Sections of mouse jejunum infected with *E. papillata* on day 4 p.i. A, Non-infected jejunum with normal architecture. B and C, Infected jejunum with some pathological changes and developmental stages appearing in the inner epithelium. Destructive wall (white arrow). Arrow heads indicate Macrogamete, black arrow indicates Merozoites. Zygote (Z) and oocyst (star) appeared in C section. Sections are stained with hematoxylin and eosin. Bar=25 µm.

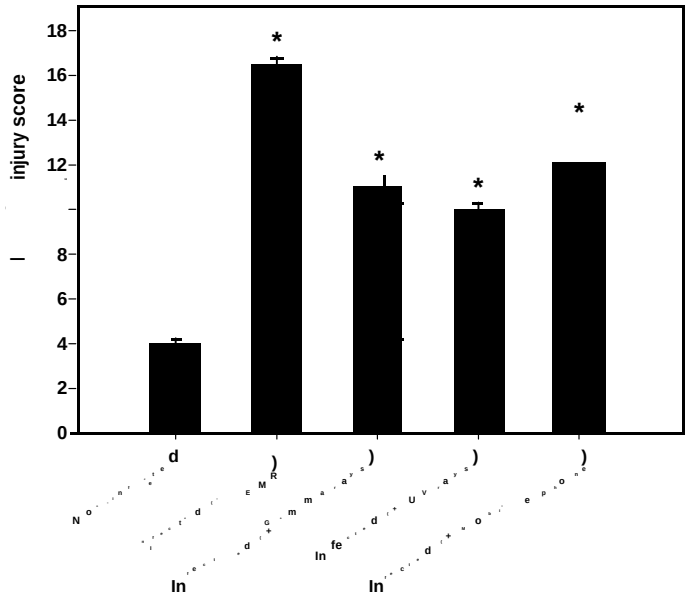

Figure 5. Total HISs in jejunum of mice infected with *E. papillata* on day 4 p.i.. Values are means±SD. Asterisk significant difference vs. the control ($p<0.01$) and section sign significant difference between infected and infected + EMR exposed mice ($p<0.01$).

ACKNOWLEDGMENT

The author extends his appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGPVPP-002.

REFERENCES

Al-Quraishy S, Delic D, Sies H, Wunderlich F, Abdel-Baki AA, Dkhil MA (2011). Differential miRNA expression in the mouse jejunum during garlic treatment of *Eimeria papillata* infections. Parasitol. Res., DOI: 10.1007/s00436-011-2266-yI.

Bajwa RS, Gill BS (1977). Effect of irradiation (gamma rays) on the biology of *Eimeria tenella* oocysts. Ann. Rech. Vet., 8: 181-186.

Berk SG, Srikanth S, Majahan SM, Ventrice CA (1997). Static uniforms magnetic fields and amoeba. Bioelectromagnetics, 18: 81-84.

Blank M, Khorkova O, Goodman R (1992). Changes in polypeptide distribution stimulated by different levels of electromagnetic and thermal stress. Bioelectrochem. Bioenerg., 3: 109–114.

Dommels YE, Butts CA, Zhu S, Davy M, Martell S, Hedderley D, Barnett MP, McNabb WC, Roy NC (2007). Characterization of intestinal inflammation and identification of related gene expression changes in mdr1a$^{(-/-)}$ mice. Genes Nutr., 2: 209–223.

Drury RAB, Wallington EA (1980). Carleton's histological technique, 5th edn. Oxford University Press, Oxford, New York, Toronto, pp. 188–189, 237–240, 290–291.

Galeev AL (2000). The effects of the microwave radiation from mobile telephones on humans and animals. Neurosci. Behav. Physiol., 30: 187-194.

Gilbert JM, Fuller AL, Scott TC, McDougald LR (1998). Biological effects of gamma-irradiation on laboratory and field isolates of *Eimeria tenella* (Protozoa; Coccidia). Parasitol. Res., 84: 437-441.

Gos P, Eicher B, Kohli J, Heyer WD (2000). No mutagenic or recombiogenic effects of mobile phones fields at 900 MHz detected in the yeast *Saccharomyces cerevisiae*. Bioelectromagnetics, 21: 515-523.

Gres V, Voza T, Chabaud A, Landau I (2003). Coccidiosis of the wild rabbit (*Oryctolagus cuniculus*) in France. Parasite, 10: 51–57.

Hnida JA, Duszynski DW (1999). Taxonomy and phylogeny of some *Eimeria* (Apicomplexa:Eimeriidae) species of rodents as determined by polymerase chain reaction/restriction-fragment-length polymorphism analysis of 18S rDNA. Parasitol. Res., 85: 887–894.

Irmak MK, Fadillioğlu E, Güleç M, Erdoğan H, Yağmurca M, Akyol O (2002). Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell Biochem. Funct., 20: 279-283.

Jasti AC, Wetzel BJ, Aviles H, Vesper DN, Nindl G, Johnson MT (2001). Effect of a wound healing electromagnetic field on inflammatory cytokine gene expression in rats. Biomed. Sci. Instrum., 37: 209–214.

Juutilinen J, Seze R (1998). Biological effects of amplitude-modulated radiofrequency radiation. Scand. J. Work Environ. Health, 24: 245-254.

Markov MS, Nindl G, Hazlewood CF, Cuppen JJM (2006). Interactions between electromagnetic fields and immune system: Possible mechanism for pain control. In: S.N. Ayrapetryan and M.S. Markov (eds.), Bioelectromagnetics, Springer, pp. 213-225.

Martinaud G, Billaudelle M, Moreau J (2009). Circadian variation in shedding of the oocysts of *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptative trait against desiccation and ultraviolet radiation. Int. J. Parasitol., 39: 735–739.

McDougald LR (2003). Coccidiosis, In: Saif, Y.M., Barnes, H.J., Fadly, A.M., Glisson, J.R., McDougald, L.R. Swayne, D.E. (Eds) Poultry Diseases, 11th edn, Iowa, Iowa State Press, pp. 974-991.

Mevissen M, Häussler M, Szamel M, Emmendörffer A, Thun-Battersby S, Löscher W (1998). Complex effects of long term 50 Hz magnetic field exposure *in vivo* on immune functions in female Sprague-Dawley rats depend on duration of exposure. Bioelectromagnetics, 19: 259–270.

Pakandl M (2005) Selection of a precocious line of the rabbit coccidium *Eimeria flavescens* Marotel and Guilhon (1941) and characterisation of its endogenous cycle. Parasitol. Res., 97: 150–155.

Schito ML, Barta JR, Chobotar B (1996). Comparison of four murine *Eimeria* species in immune competent and immunodeficient mice. J. Parasitol., 82: 255–262.

Schmnatz DM, Crane MSJ, Murray PK (1984). Purification of *Eimeria sporozoites* by DE-52 anion exchange chromatography. J. Protozool., 31: 181-183.

Simko´ M, Mattsson M (2004). Extremely low frequency electromagnetic fields as effectors of cellular responses *in vitro*: Possible immune cell activation. J. Cell. Biochem., 93: 83–92.

Vallbona C, Richard T (1999). Evolution of magnetic therapy from alternative to traditional medicine. Physiol. Med. Rehab. Clin. N. Am., 10: 729–754.

Zhao X, Duszynski DW (2001). Molecular phylogenies suggests the oocyst residuum can be used to distinguish two independent lineages of *Eimeria* spp in rodents. Parasitol. Res., 87: 638–643.