Influence of Vaccination of Hens with Attenuated Oocysts of *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima* on the Protection of Offspring against Eimeria Infection

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Abstract

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The feasibility of protection of offspring against Eimeria infection via maternal or passive derived immunity from hens (layers) vaccinated with attenuated oocysts of *Eimeria acervulina*, *E. maxima*, *E. tenella* and *E. necatrix* was investigated. Gross lesion scores on the basis of pathological changes and oocyst count methods were used in the experiment. Seven hens (layers) (Group A) and eighty hens (layers) (Group B) were orally vaccinated with 30,000 to 50,000 oocysts of attenuated *Eimeria tenella*, *E. acervulina*, *E. maxima* and 10,000 oocysts of attenuated *E. necatrix*. A second vaccination with the same dose was given to group B hens, and seven control hens (Group C) were not vaccinated. Offspring of three groups of hens (layers) were challenged with 10,000 oocysts of virulent lines of *Eimeria tenella*, *E. acervulina*, *E. necatrix* and *E. maxima*. Offspring of revaccinated hens showed significantly reduced lesion scores ($p < 0.01$) after challenge with *E. tenella*, *E. necatrix, E. acervulina* and *E. maxima* compared to offspring of non vaccinated hens. Offspring of vaccinated hens showed significant reduction in lesion scores ($p < 0.01$) with *E. maxima* and ($p < 0.05$) with *E. necatrix* compared to offspring of non vaccinated hens (layers). Progeny of revaccinated hens also showed reduced oocyst excretion with $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. tennella*, $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. maxima*, $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. necatrix* and $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. acervulina* compared to offspring of control hens that excreted $(2.4 \pm 0.1) \times 10^6$ oocysts of *E. tenella*, $(2.5 \pm 0.1) \times 10^6$ oocysts of *E. maxima*, $(2.1 \pm 0.1) \times 106$ oocysts of *E. necatrix* and $(2.7 \pm 0.1) \times 10^6$ oocysts of *E. acervulina.* The results demonstrate that revaccination of hens with attenuated oocysts of Eimeria may give better results than single vaccination in protection of offspring against Eimeria challenge infection. The results obtained in the experiment need further investigation.

Chicken, coccidiosis, maternal immunity, lesion scores, oocyst count

Poultry coccidiosis is an intestinal disease caused by protozoan parasites of the genus Eimeria. The disease causes serious economic loses to the poultry meat industry worldwide. As the world's poultry production continues to grow, so do concerns about the control of Eimeria infections that cause coccidiosis, which remains one of the most commonly reported diseases of chickens (Biggs 1982; Xie et al. 2001). Commercial chickens free from coccidia are extremely rare (Williams 1999). Furthermore, there is increasing concern about rising levels of drug resitance (Chapman 1997), consumer pressure to phase out the inclusion of anticoccidial drug additives in the diets of food animals (Young and Craig 2001), concerns about drug residues in poultry products (Young and Craig 2001) and a falling success rate in the discovery of chemical entities as well as high costs of development of new anticoccidial drugs. As a result, there has been in recent years a refocusing of research on biological rather than chemical control of coccidiosis.

One approach to immune-based protection of chickens which should be considered is

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that obtained via maternally derived antibodies. It is known that infection of hens with Eimeria species causes the production of Eimeria-specific immunoglobulin G (IgG) antibodies which are transferred to offsprings via the egg yolk and results in protection of hatchlings against challenge infection for the first 2 to 3 weeks of their early life (Rose 1972; Smith et al. 1994a; 1994b). It has been proved that Eimeria-specific IgG levels in egg yolks and sera of newly hatched chicks correlate very strongly with resistance to infection in the hatchlings (Smith et al. 1994c). The egg yolk of a normal chicken contains milligram quantities of immunoglobulin G (IgG) whereas there are only very small quantities of IgM or IgA in egg white (Lösch et al. 1986). Parasite-specific IgG levels in egg yolk or the sera of young chickens from hens infected with *E. maxima* correlate very well with immunity to Eimeria in chicks, but parasite-specific IgA or IgM antibodies are not detectable (Smith et al. 1994a). Wallach (2002) reported that immunization of breeding hens with affinity purified sexual stage antigens isolated from *E. maxima* prior to the start of lay results in the protection of their offspring. It is likely that the use of maternally derived IgG antibodies to Eimeria species is the right approach in protecting hatchlings against the disease. The aim of this study was to investigate the feasibility of protecting hatchlings via maternal tranfer of protective antibodies from hens (layers) to their offspring by vaccination hens with attenuated oocysts of *Eimeria tenella, E. necatrix*, *E. acervulina* and *E. maxima.*

Materials and Methods

Chickens and housing

Striped leghorn hens (layers), 51 weeks of age were purchased from Czech Academy of Sciences, Prague, Czech Republic. They were free from diseases and fecal sample examination revealed no coccidia oocysts. The hens were reared in a battery with wire floored cages in a manner which as far as possible fulfilled commercial conditions, in a coccidia-free environment. The premises were equipped with heaters maintaining temperature at 18- 20 °C, relative humidity 70- 75%, automatic ventilators with air exchange speed 0.68- 0.43m^3/h per 1 kg live weight of a hen, nipple water drinkers and manual feeders. High levels of hygiene were maintained to minimize the possibility of infection. The premises and equipment (eg. feed equipment) were disinfected with 25% aqueous ammonia solution after thorough mechanical cleaning of the premises and equipment with 2% chloramin B, before introducing the hens. Hens (layers) were divided into three groups: Seven hens (group A) were vaccinated once, 8 hens (group B) were vaccinated twice and 7 hens (group C) as control were not vaccinated. Their offspring were also grouped according to their parents origin (group A, B and C).They were kept in such a way to avoid contact between the groups. Their premises and equipment were also (eg. feed equipment) disinfected with 25% aqueous ammonia solution after thorough mechanical cleaning of the premises and equipments with 2% chloramin B, before introducing the hatchlings in experimental boxes. Fifteen (15) hatchlings were kept in each group, in wire-floored cages and were raised in isolation in an environment free of coccidia. The premises were equiped with heaters and automatic ventilators with air exchange speed 1.02 m^3/h for 1 kg live weight of a chicken. Heating was regulated according to the actual need of growing chickens with infrared lamps, temperature 22-30 °C, relative humidity 55-65%. They were equiped with manual water drinkers and manual feeders.

Vaccines used

One ml of Quadrivalent vaccine Livacox Q, containing 30,000 to 50,000 oocysts of attenuated lines of *Eimeria tenella*, *Eimeria acervulina*, *Eimeria maxima* and 10,000 oocysts of attenuated line of *Eimeria necatrix* in a 1% solution of chloramine B was used for vaccinating layer chickens.

Challenge inoculum for hatchlings

Progeny were challenged with 10,000 oocysts of virulent lines of *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria maxima*. Quadrivalent Livacox vaccine and challenge inoculum were provided by Biopharm Research Institute of Biopharmacy and Veterinary drugs, Prague, Czech Republic.

Incubation of eggs

Storage and collection of hatching eggs. Egg were collected for seven days and were stored at a temperature of 10 °C to 12 °C, and at a relative humidity of 70 to 80%, before incubation.

The type of incubator used was MIDI-L (HART-BIO a.s.) made in Czech Republic. The incubator was equipped with automatic turning devices that were able to rotate egg trays, a thermometer for measuring temperature and relative humidity and a black box to record temperature and humidity. The incubator fulfilled biological requirements of temperature, humidity and air supply.

Eggs were placed in plastic trays, with broad end higher than the narrow end. Turning of eggs was done automatically hourly through 45° to the vertical. Turning of eggs was done until eggs were transferred to the hatcher on the eighteenth day and turning was discontinued.

Candling of eggs was done on days 7 and 18 of incubation to check for infertility or embryo mortality and infertile eggs and dead embryos were removed. Eggs were incubated at a temperature of 37.8 - 38.0 °C from day 1 to 18 and 37.5 °C for the rest of the incubation period. Temperature was raised during the first 18 days and lowered thereafter in order to give good hatchability.

Relative humidity for hatching eggs was 50 to 65% for the first 18 days of incubation and 65 to 90% for the rest of the incubation period. Proper level of humidity and temperature in the incubator is important for good hatchability.

Chicken and feed

Hens (layers) in both groups were fed a diet free of any anticoccidial drugs. Feed and water were provided ad libitum.

Offspring were fed a diet containing a synthetic anticoccidial drug (robenidine 33 ppm) from days 1 to 3 of age. Feed and water were provided *ad libitum*. Feed with anticoccidial drug was given to make sure that offspring were free from coccidia parasites as instructed by manufacturer of the Livacox vaccine (Biopharm-Research Institute of Biopharmacy and Veterinary drugs, Prague, Czech Republic). Two days before inoculation of hatchlings with challenge infection, they were fed with diet free of any anticoccidial drug till the end of the experiment. Feed was provided by Biopharm-Research Institute of Biopharmacy and Veterinary drugs, Prague, Czech Republic.

Vaccination schedule for hens (layers) and challenge infection to their offspring

Fifteen hens (seven in group A and eight in group B) were vaccinated orally (using a catheter) with one ml of Quadrivalent Livacox vaccine containing 30,000 to 50,000 oocysts of attenuated lines of *Eimeria tenella*, *Eimeria acervulina*, *Eimeria maxima* and 10,000 oocysts of an attenuated line of *Eimeria necatrix.* Seven hens (group C) were not vaccinated. Four weeks later hens in group B were revaccinated with the same dose. Two weeks after revaccination of hens in group B, eggs were collected for 7 days from all three groups of hens (layers) and incubated. Offspring were kept in three isolated groups (group A, B and C) according to their parents origin. Each goup of 15 hatchlings, were kept in wire floored cages in the experiment boxes. On day 5 of age, offspring from each group were given challenge infection with 10,000 oocysts of virulent lines of *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria maxima*.

Gross lesion determination

Gross lesion scores were examined from 3 groups of offspring: offspring of non-vaccinted (group C), vaccinated (group A) and revaccinated (group B) hens (layers). Ten offspring out of 15 offspring on day 10 of age from each group were sacrificed by cervical dislocation and examined for gross intestinal lesions. The remaining 5 offspring in each group were monitored for oocyst excretion and for lesion score examination at 13 days of age. Gross lesion scores on the basis of pathological/ anatomical changes from four sections of the intestine (upper, middle, lower and caeca) were examined according to 4-point scale (0 = no gross lesions, 4 = severe gross lesions) by Johnson and Reid (1970) for each of Eimeria species (*Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima*).

Oocyst counting

Oocyst count was done according to Eckert et al. (1995), using the remaining 5 offspring in groups A, B and C at day 10, 11, 12, and 13 of age. A microscope with 10 × objective lens was used to count oocysts in a Macmaster chamber.

Statistical evaluation

The statistical assay analysis was performed using Student's *t*-test, Unistat 51 software (University of Veterinary and Phamaceutical Sciences Brno, Department of Veterinary Public Health and Toxicology, Czech Republic). The levels of significance were $p < 0.05$ (*), $p < 0.01$ (**).

Results

The following results were observed following vaccination and revaccination of hens (layers) with attenuated oocysts of *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria maxima*. Gross lesion scores of offspring at 10 days of age: offspring of vaccinated hens showed significantly reduced lesion scores after challenge with *Eimeria maxima* ($p < 0.01$) and *Eimeria necatrix* ($p < 0.05$) (Table 2), compared to offspring of non-vaccinated hens (layers) (Table 1). Offspring of hens receiving two vaccinations showed significantly reduced lesion scores after challenge with *Eimeria acervulina* ($p < 0.01$), *Eimeria maxima* ($p < 0.01$), *Eimeria necatrix* ($p < 0.01$) and *Eimeria tenella* ($p < 0.01$) (Table

Table 1. Gross lesion scores of offspring at 10 days of age of non-vaccinated hens (layers) (Group C)

	Eimeria Species			
Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	3	3	3	3
2	3	3	3	3
3	2	3	3	3
4	3	2	3	2
5	3	2	3	2
6	3	2	2	2
7	2	3	2	3
8	3	3	3	3
9	3	3	3	3
10	3	3	3	3
Average of lesion scores	2.80	2.70	2.80	2.70
Standard Deviation	0.40	0.46	0.40	0.46
Coef. of Variability	0.16	0.21	0.16	0.21

Table 2. Gross lesion scores of offspring at 10 days of age of vaccinated hens (layers) (Gorup A)

	Eimeria Species			
Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	3	2	2	3
2	2	2	3	2
3	3	2	2	2
4	3	2	3	2
5	2	3	3	2
6	3	2	2	2
7	2	2	2	2
8	2	2	3	3
9	2	2	2	2
10	2	2	2	2
Average of lesion scores	2.4	2.1	2.4	2.2
Standard Deviation	0.499	0.300	0.489	0.400
Coef. of Variability	0.240	0.090	0.240	0.160
F-test	0.555	0.223	0.555	0.692
t-test	0.074	0.004	0.074	0.024
Significance		**		*

3) compared to offspring of non-vaccinated hens (layers) (Table 1). Gross lesion scores of offspring at 13 days of age: offspring of vaccinated hens showed no significant difference in lesion scores (Table 5) compared to offspring of non-vaccinated hens (Table 4). Offspring of hens (layers) receiving two vaccinations showed significantly reduced lesion scores after challenge with *Eimeria maxima* ($p < 0.01$) and *Eimeria necatrix* ($p < 0.01$) (Table 6) compared to offspring of non vaccinated hens (Table 4).

Offspring of vaccinated hens (layers) had reduced oocyst excretion with $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. tenella*, $(0.4 \pm 0.1) \times 10^6$ oocysts of *E. maxima*, $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. necatrix* and $(0.4 \pm 0.1) \times 10^6$ oocysts of *E. acervulina* compared to offspring of non vaccinated hens with $(2.4 \pm 0.1) \times 10^6$ oocysts of *E. tenella*, $(2.5 \pm 0.1) \times 10^6$ oocysts of *E.*

Table 3. Gross lesion scores of offspring at 10 days of age of revaccinated hens (layers) (Group B)

	Eimeria Species			
Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	2	1	3	2
2	3	2	2	2
3	2	1	2	2
4	2	2	3	2
5	2	2	2	3
6	2	1	1	2
7	2	1	2	2
8	2	2	2	2
9	2	2	2	1
10	2	2	2	2
Average of lesion scores	2.1	1.6	2.1	2
Standard Deviation	0.300	0.489	0.539	0.447
Coef. of Variability	0.090	0.240	0.290	0.200
F-test	0.404	0.846	0.389	0.943
t-test	0.001	0.00011	0.006	0.004
Significance	**	**	**	**

Table 4. Gross lesion score of offspring at 13 days of age of non vaccinated hens (layers) (Group C)

Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	2	3	3	3
2	3	3	3	3
3	3	2	3	3
4	3	3	2	2
5	3	3	3	3
Average of lesion scores	2.8	2.8	2.8	2.8

Table 5. Gross lesion scores of offspring at 13 days of age of vaccinated hens (layers) (group A)

Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	2	2	2	2
2	3	2	3	2
3	3	3	2	3
4	2	2	2	2
5	2	2	3	2
Average of lesion scores	2.4	2.2	2.4	2.2
Standard Deviation	0.489	0.400	0.489	0.400
Coef. of Variability	0.240	0.160	0.240	0.160
F-test	0.704	1.000	0.704	1.000
T-test	0.243	0.067	0.243	0.067
Significance				

maxima, $(2.1 \pm 0.1) \times 10^6$ oocysts of *E. necatrix* and $(2.7 \pm 0.1) \times 10^6$ oocysts of *E. acervulina* (Table 7, 8, 9, 10). Progeny of revaccinated layer chickens had reduced oocyst excretions with $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. tenella*, $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. maxima*, $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. necatrix* and $(0.3 \pm 0.1) \times 10^6$ oocysts of *E. acervulina* compared

Table 6. Gross lesion scores of offspring at 13 days of age of revaccinated hens (layers) (Group B)

Chicken No.	*E. acervulina*	*E. maxima*	*E. tenella*	*E. necatrix*
1	2	1	2	2
2	3	2	3	2
3	2	1	2	2
4	2	2	2	1
5	2	1	1	2
Average of lesion scores	2.2	1.4	2	1.8
Standard Deviation	0.400	0.489	0.632	0.400
Coef. of Variability	0.160	0.240	0.400	0.160
F-test	1.000	0.704	0.396	1.000
t-test	0.067	0.002	0.071	0.008
Significance		**		**

Table 7. Oocyst excretion of offspring at 10 days of age of non vaccinated, vaccinated and revaccinated hens (layers)

Eimeria species	Oocyst excretion of offspring at 10 days of age of non-vaccinated hens	Oocyst excretion of offspring at 10 days of age of vaccinated hens	Oocyst excretion of offspring at 10 days of age of revaccinated hens
E.tenella	$2417163 = (2.4 \pm 0.1) \times 10^6$	$341512 = (0.3 \pm 0.1) \times 10^6$	$318256 = (0.3 \pm 0.1) \times 10^6$
E.maxima	$2501215 = (2.5 \pm 0.1) \times 10^6$	$398734 = (0.4 \pm 0.1) \times 10^6$	$314127 = (0.3 \pm 0.1) \times 10^6$
E.necatrix	$2116150 = (2.1 \pm 0.1) \times 10^6$	$321516 = (0.3 \pm 0.1) \times 10^6$	$305316 = (0.3 \pm 0.1) \times 10^6$
E.acervulina	$2667342 = (2.7 \pm 0.1) \times 10^6$	$372034 = (0.4 \pm 0.1) \times 10^6$	$313002 = (0.3 \pm 0.1) \times 10^6$

Table 8. Oocyst excretion of offspring at 11 days of age of non vaccinated, vaccinated and revaccinated hens (layers)

Eimeria species	Oocyst excretion of offspring at 11 days of age of non--vaccinated hens	Oocyst excretion of offspring at 11 days of age of vaccinated hens	Oocyst excretion of offspring at 11 days of age of revaccinated hens
E.tenella	$2502365 = (2.5 \pm 0.1) \times 10^6$	$350109 = (0.4 \pm 0.1) \times 10^6$	$341107 = (0.3 \pm 0.1) \times 10^6$
E.maxima	$2578308 = (2.6 \pm 0.1) \times 10^6$	$390136 = (0.4 \pm 0.1) \times 10^6$	$320272 = (0.3 \pm 0.1) \times 10^6$
E.necatrix	$2206315 = (2.2 \pm 0.1) \times 10^6$	$317217 = (0.3 \pm 0.1) \times 10^6$	$314065 = (0.3 \pm 0.1) \times 10^6$
E.acervulina	$2307116 = (2.3 \pm 0.1) \times 10^6$	$367128 = (0.4 \pm 0.1) \times 10^6$	$312117 = (0.3 \pm 0.1) \times 10^6$

Table 9. Oocyst excretion of offspring at 12 days of age of non vaccinated, vaccinated and revaccinated hens (layers)

Eimeria species	Oocyst excretion of offspring at 12 days of age of non--vaccinated hens	Oocyst excretion of offspring at 12 days of age of vaccinated hens	Oocyst excretion of offspring at 12 days of age of revaccinated hens
E.tenella	$2492506 = (2.5 \pm 0.1) \times 10^6$	$342132 = (0.3 \pm 0.1) \times 10^6$	$312132 = (0.3 \pm 0.1) \times 10^6$
E.maxima	$2502170 = (2.5 \pm 0.1) \times 10^6$	$388214 = (0.4 \pm 0.1) \times 10^6$	$315269 = (0.3 \pm 0.1) \times 10^6$
E.necatrix	$2302015 = (2.3 \pm 0.1) \times 10^6$	$316169 = (0.3 \pm 0.1) \times 10^6$	$309122 = (0.3 \pm 0.1) \times 10^6$
E.acervulina	$2218330 = (2.2 \pm 0.1) \times 10^6$	$352149 = (0.4 \pm 0.1) \times 10^6$	$311214 = (0.3 \pm 0.1) \times 10^6$

to offspring of non vaccinated layer chickens with $(2.4 \pm 0.1) \times 10^6$ oocysts of *E. tenella*, $(2.5 \pm 0.1) \times 10^6$ oocysts of *E. maxima*, $(2.1 \pm 0.1) \times 10^6$ oocysts of *E. necatrix* and $(2.7 \pm 0.1) \times 10^6$ oocysts of *E. acervulina* (Table 7, 8, 9, 10).

Table 10. Oocyst excretion of offspring at 13 days of age of non-vaccinated, vaccinated and revaccinated hens (layers)

Eimeria species	Oocyst excretion of offspring at 13 days of age of non--vaccinated hens	Oocyst excretion of offspring at 13 days of age of vaccinated hens	Oocyst excretion of offspring at 13 days of age of revaccinated hens
E.tenella	$2398167 = (2.4 \pm 0.1) \times 10^6$	$339228 = (0.3 \pm 0.1) \times 10^6$	$331167 = (0.3 \pm 0.1) \times 10^6$
E.maxima	$2481263 = (2.5 \pm 0.1) \times 10^6$	$367212 = (0.4 \pm 0.1) \times 10^6$	$365150 = (0.4 \pm 0.1) \times 10^6$
E.necatrix	$2311275 = (2.3 \pm 0.1) \times 10^6$	$313409 = (0.3 \pm 0.1) \times 10^6$	$231017 = (0.2 \pm 0.1) \times 10^6$
E.acervulina	$2209246 = (2.2 \pm 0.1) \times 10^6$	$310679 = (0.3 \pm 0.1) \times 10^6$	$221511 = (0.2 \pm 0.1) \times 10^6$

Discussion

Investigation of the feasibility of protection of offspring against Eimeria infection via maternal or passive derived immunity from hens (layers). Revaccination of hens (layers) with oocysts of attenuated lines of *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix* and *Eimeria tenella* protected offspring against challenge infection with *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix* and *Eimeria tenella* as they showed reduced lesion scores (Table 3) compared to offspring of non-vaccinated hens (Table1) and reduced oocyst shedding (Table 7, 8, 9, 10). Offspring of vaccinated hens showed increased resistance against *Eimeria maxima* and *Eimeria necatrix* infections (Table 2) and reduced ooycst excretion (Table 7, 8, 9, 10). At thirteen days of age offspring of revaccinated hens showed resistance against *Eimeria maxima* and *Eimeria necatrix* infections (Table 6) but offspring of vaccinated hens did not show any resistance against Eimeria infection (Table 5). This seems to suggest that passive or maternal immunity wanes or declines with increasing time after infection of the offspring. Also some of the lesion scores were high in the offspring of vaccinated and revaccinated hens (layers) while the fecal oocyst counts were low, perhaps due to aggravation by microflora (like *Escherichia coli* or *Clostridium perfringens*) interacting with intestinal mucosal damage as well as the developmental stages of Eimeria parasites. From our experimental results, revaccination of hens (layers) gives better protection against Eimeria infections to their offspring than a single vaccination.

There are few reports concerning matenally derived immunity from hens (layers) and protection of the offspring against Eimeria infections. Smith et al. (1994abc) demonstrated that infecting hens (layers) with *Eimeria maxima* induces production of Eimeria-specific immunoglobulin G (IgG) antibodies which are transferred to hatchlings via the egg yolk and confer a high degree of maternal immunity against homologous challenge and partial immunity to infection with other species, for example *Eimeria tennella.* Wallach et al. (1992, 1995), and Wallach (1997) also demonstrated that immunization of hens (layers) with *Eimeria maxima* gametocyte antigens results in a protective immunoglobulin G (IgG) response, which is transferred to the embryo providing protection to their offspring. Wallach (2002) showed that a subunit vaccine (CoxAbic vaccine) consisting of affinity purified sexual stage (gametocyte) antigens isolated from *Eimeria maxima,* based on the concept of transmission-blocking immunity protects offspring against Eimeria infection. Wallach (2002) reported that immunization of breeding hens prior to the start of lay gives rise to a protective immunoglobulin G (IgG) response which is transferred to the developing embryo providing protection to their offspring against *Eimeria acervulina*, *Eimeria tenella* and *Eimeria maxima*. In a field trial it was observed that hens vaccinated with subunit vaccine (CoxAbic vaccine) showed reduced oocyst shedding of the three major species of Eimeria (*Eimeria tenella*, *Eimeria acervulina* and *Eimeria maxima*) and they performed at least as well as the broiler control fed a coccidiostat (Michael 2002).

Lösch et al. (1986) reported that exposure of hens to immunogens causes production of

antibodies which are secreted into the egg yolk during its formation in the ovary. These antibodies are of the immunoglobulin G (IgG) (also known as IgY in birds) class and are able to protect hatchlings against a variety of disease-causing organism. The mechanism of maternally derived immunity to *Eimeria maxima* has been well defined. Cellular immune effectors are very unlikely to persist in eggs during their incubation and IgA and IgM although present in egg white in small amounts (micrograms) are not Eimeria-specific (Lösch et al. 1986). Rose (1972) demonstrated that gamma-livetin globulin fraction of egg yolks from hens are able to be injected subcutaneously into naive chickens and thereby protects them against infection. Furthermore Rose (1967) reported that there is evidence of cross resistance between *Eimeria tenella* and *Eimeria necatrix,* however, as pointed out by Rose (1987), these reports have not gained wide acceptance as providing evidence for the existance of cross-species resistance because the results of the *E. maxima* and *E. brunetti* study were not reproducible (Hein 1971) and the cross-protection observed in other studies was relatively low, limited to certain developmental stages and apparently infective-site dependent.

Thus protective maternally derived immunity can presumably be used to protect chickens from coccidiosis, since immunization of chicken layers prior to the start of lay, gives protective immunoglobulin G (IgG) to hatchlings and protects them against challenge infection.

The results of this experiment demonstrate that two vaccinations of layer chickens with attenuated oocysts of Eimeria might give protection to offspring against challenge infection. The results need further investigation.

Vliv vakcinace rodičů kura domácího atenuovanými oocystami *E. acervulina*, *E. maxima*, *E. tenella* a *E. necatrix* na chráněnost potomstva proti infekcím eimeriemi

Tato práce sleduje pasivní chráněnost kuřat pocházejících od rodičů vakcinovaných atenuovanými oocystami *E. acervulina*, *E. maxima*, *E. tenella* a *E. necatrix* proti infekcím eimeriemi. Vnímavost kuřat byla testována na základě hodnocení patologických změn (Gross lesion scores) a metodou vylučování oocyst (oocyst count scores). Sedm nosnic (skupina A) bylo vakcinováno, 8 nosnic (skupina B) bylo revakcinováno atenuovanými oocystami *E. acervulina*, *E. maxima* a *E. tenella* v dávce 30 000 až 50 000 oocyst a *E. necatrix* v dávce 10 000 oocyst; 7 nosnic kontrolní skupiny nebylo vakcinováno. Potomstvo nosnic všech 3 skupin bylo čelenžováno dávkou 10 000 virulentních oocyst *E. acervulina*, *E. maxima*, *E. tenella* a *E. necatrix*. Potomstvo revakcinovaných nosnic vykazovalo statisticky významnou redukci patologických změn ($p < 0{,}01$) u *E. acervulina*, *E. maxima*, *E. tenella* a *E. necatrix* v porovnání s potomstvem nevakcinované skupiny nosnic. Kuřata od vakcinované skupiny nosnic vykazovala statisticky významnou redukci patologických změn ($p< 0{,}01$) pouze u *E. maxima* a u *E. necatrix* ($p < 0{,}05$) v porovnání s potomstvem kontrolní skupiny. Také celkový počet vylučovaných oocyst v trusu potomstva revakcinovaných nosnic byl snížený na $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. tenella*, $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. maxima*, $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. necatrix* a $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. acervulina* v porovnání s potomstvem nevakcinované skupiny nosnic, kde byl celkový počet vylučovaných oocyst v trusu $(2{,}4 \pm 0{,}1) \times 10^6$ oocyst *E. tenella*, $(2{,}5 \pm 0{,}1) \times 10^6$ oocyst *E. maxima*, $(2{,}1 \pm 0{,}1) \times 10^6$ oocyst *E. necatrix* a $(2{,}7 \pm 0{,}1) \times 10^6$ oocyst *E. acervulina*. Potomstvo vakcinované skupiny nosnic vykazovalo snížené vylučování oocyst na $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. tenella*, $(0{,}4 \pm 0{,}1) \times 10^6$ oocyst *E. maxima*, $(0{,}3 \pm 0{,}1) \times 10^6$ oocyst *E. necatrix* a $(0{,}4 \pm 0{,}1) \times 10^6$ oocyst *E. acervulina* v porovnání s kontrolní skupinou. Zjištěné výsledky dokazují, že revakcinace nosnic atenuovanými oocystami rodu Eimeria může poskytout účinnější ochranu potomstva proti čelenžní infekci.

References

BIGGS PM 1982: The world of poultry disease. Avian Pathol **11**: 281-300

CHAPMAN HD 1997: Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl. Avian Pathol **26**: 221-244

ECKERT J, BRAUN R, SHIRLEY MW, CONDERT P 1995: COST 89/820. (Ed.), Biotechnology: Guidelines on techniques in coccidiosis reaserch. European Commission, Luxembourg. pp. 31-35

HEIN H 1971: Eimeria brunetti: cross infections in chickens immunized to *E. maxima*. Exp. Parasitol. **29**: 367-374

JOHNSON J, REID MW 1970: Anticoccidial Drugs: Lesion scoring techniques in battery and floor- pen experiments with chickens. Exp. Parasitol **28**: 30-36

LÖSCH U, SCHRANNER I, WANKE R, JÜRGENS L 1986: The chicken egg, an antibody source. J. Vet. Med. SER B **33**: 609-619

MICHAEL A 2002: The practical use of maternal vaccine against coccidiosis. World Poult. **18**: 2-4

ROSE ME 1972: Immunity to coccidiosis: Maternal transfer in *Eimeria maxima* infection. Parasitology **65**: 273-282

ROSE ME 1967: Immunity to *Eimeria brunetti* and *Eimeria maxima* infections in the fowl. Parasitology **57**: 365-370

ROSE ME 1987: Eimeria, Isospora and cryptosporidium. In Immunology, Immunopathology and Immunoprophylaxis of Parasitic Infections, vol. 3, Protozoa and Arthropods (ed. Soulsby, EJL), Boca Raton, Florida, USA: CRC Press Inc. pp. 275-312

SMITH NC, WALLACH M, PETRACCA M, BRAUN R, ECKERT J 1994a: Maternal transfer of antibodies induced by infection with *Eimeria maxima* partially protects chickens against challenge with *Eimeria tenella*. Parasitology **109**: 551-557

SMITH NC, WALLACH M, MILLER CATHERINE MD, MORGENSTERN R, BRAUN R, ECKERT J 1994b: Maternal transmission of immunity to *Eimeria maxima*: Enzyme-linked Immunosorbent assay of protective antibodies induced by infection. Infect Immun **62**: 1348-1357

SMITH NC, WALLACH M, MILLER CMD, BRAUN R, ECKERT J 1994c: Maternal transmission of immunity to Eimeria maxima: Western blot analysis of protective antibodies induced by infection. Infect Immun **62**: 4811-4817

WALLACH M, HALABI A, PILLEMER G, Sar-SHALOM O, MENCHER D, GILAD M, BENDHEIM U, DANFORTH HD, AUGUSTINE PC 1992: Maternal immunization with gametocyte antigens as a means of providing protective immunity against Eimeria maxima in chickens. Infect Immun **60**: 2036-2039

WALLACH M, SMITH NC, PETRACCA M, MILLER CM, ECKERT J, BRAUN R 1995: Eimeria gametocyte antigens: potential use in a subunit maternal vaccine against coccidiosis in chickens. Vaccine **13**: 347-354

WALLACH M 1997: The importance of transmission-blocking immunity in the control of infections by apicomplexan parasites. Int J Parasitol **27**: 1159-1167

WALLACH M 2002: The development of CoxAbic a novel vaccine against coccidiosis. World Poult **18**: 2-4

XIE M, CAI J, LI A, PENG X 2001: Coccidiosis of domestic fowl in China. Proceedings of the VIIIth international coccidiosis conference, Palm ove, Sydney, Australia, pp. 153-154

WILLIAMS RB 1999: A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int J Parasitol **29**: 1209-1229

YOUNG R, CRAIG A 2001: The use and misuse of antibotics in UK Agriculture – Part 3. Residues of dangerous drugs in intensively produced chicken meat and eggs. Bristol, UK: The soil association