PREVENTION OF BROILER CHICK COCCIDIOSIS USING THE INACTIVATED SUBUNIT VACCINE COXABIC®

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Abstract

The efficacy of the vaccination of breeding hens in the prevention of coccidiosis of broiler chicks hatched from eggs laid by hens immunized with CoxAbic® vaccine in comparison to broiler chicks coming from non-vaccinated hens was examined. Breeders were vaccinated 8 and 4 weeks before the beginning of egg laying. The level of Eimeria antibodies was determined using ELISA: in hen sera before and after the vaccination and in chick sera at the first day of age. One hundred and fifty six one-day-old chicks were divided into 4 groups: group 1- chicks from vaccinated hens and groups 2, 3, 4 - chicks from non-vaccinated hens. Chicks of groups 1, 2 and 3 were maintained in pens on concrete floor and chicks of group 4 in cages with wire net floor. One seeder chick in each pen was inoculated by oral gavage at 1 d of age with 50 oocysts each of 3 coccidia species: E. maxima, E. acervulina, E. tenella. Broilers of group 3 were fed diet containing diclazuril At age of 34 d the broilers were challenged with sporulated oocysts (300 000 E. acervulina, 150 000 E. maxima, 60 000 E. tenella per bird). Six d after challenge intestinal lesion scores were recorded. It was demonstrated that CoxAbic® vaccine used to vaccinate breeders protected their progeny against coccidiosis caused by 3 coccidian species. The level of Eimeria antibodies estimated in sera of chicks coming from vaccinated breeders was significantly higher than that in chicks coming from non-vaccinated ones. Broilers from vaccinated breeders were characterized by the lowest lesion score after challenge.

Key words: broiler chickens, coccidiosis, Eimeria, vaccine.

Poultry coccidiosis caused by protozoan parasites of the genus Eimeria has been known for over 130 years. This disease still causes great losses in poultry meat production worldwide. The major means to control coccidiosis is using coccidiostats in feeds. Because of development of drug resistance in many coccidia strains and increasing consumers demands for chemical-free poultry meat different ways of control of this disease have been searched. The vaccination against coccidia seems to be very attractive alternative. Ideally, broiler chicks ought to have the immunity against coccidiosis from the day of hatching. But vaccination in this age may build the immunity only after some weeks. Therefore, some studies (8, 9) on antigens isolated from Eimeria maxima gametocytes, found to be highly conserved for different Eimeria species and pointed out the new way in the prevention of broiler coccidiosis. This way is based on passive maternal transmission of antibodies from breeding hens to their progeny, via the egg yolk. Yolk antibodies can penetrate from blood vessels into tissues. They appear on the surface of mucous membrane of the digestive tract and respiratory system, playing crucial role in the first days of chicks’ life (4). The cross-immunity between E. maxima, E. acervulina and E. tenella was documented (10). It seems that maternal immunity can be very useful in vaccination for the sake of economical reasons. Namely, vaccination of the single breeding hen would lead to the protection of over 100 chicks (7). Maternal immunity is maintained during the first weeks of chicks life (about 3 weeks) and protects them against the invasion of coccidia from the environment (11). In this way the immunity prevents the subclinical coccidiosis and economical losses connected with it. One of the results of the maternal immunity against Eimeria is that the number of oocysts shed by chicks coming from vaccinated breeders was significantly higher than that in chicks coming from non-vaccinated ones. Broilers from vaccinated breeders were characterized by the lowest lesion score after challenge.

The aim of this experiment was the evaluation of the efficacy of the vaccination of breeding hens with CoxAbic® vaccine in the prevention of coccidiosis in the progeny chicks.
**Material and Methods**

One hundred and fifty Cobb-500 pullets were removed to the study from a flock of 7500 hens. In this flock the immunoprophylaxis with attenuated vaccine was used in the first week of chicks life. The separated hens were immunized with the subunit vaccine CoxAbic® (ABIC, Israel). The vaccine was a water-in-oil emulsion containing affinity purified proteins of *E. maxima* gametocytes (230 kDa, 82 kDa, 56 kDa). The breeders were vaccinated 8 and 4 weeks before beginning of egg laying (18 and 22 weeks of age) by injection of 0.5 ml of the vaccine per bird into the breast muscle. The birds were reared and maintained according to usual husbandry conditions.

Eggs laid by the vaccinated breeders and the non-vaccinated breeders were collected for incubation on day 6 of laying. The breeders were observed for egg production, fertility and egg hatchability. Blood from 30 vaccinated and 30 non-vaccinated breeders was sampled before vaccination (sampling No. I), 4 weeks after the first vaccination (II) and 4 weeks after the second vaccination (1 day before starting the incubation) (III). Twenty chicks from vaccinated breeders and 20 from non-vaccinated breeders were bled on day 1.

One hundred and fifty six one-day-old tagged chicks were used in the trial. The chicken house was divided into 12 pens of 1 m² size. The pens were separated from each other by 1.3 m high wire net within groups, however, pens between groups were separated by wood boards. The broiler chicks were divided into 4 groups, each further separated into 3 subgroups, 13 chicks per pen.

Group 1: 39 chicks from vaccinated breeders – fed no coccidiostats.
Group 2: 39 chicks from non-vaccinated breeders – fed no coccidiostats.
Group 3: 39 chicks from non-vaccinated breeders – fed Clinacox® (diclazuril 1 mg per 1 kg of feed) for 25 d. On day 26 the feed was changed to *E. maxima* coccidiostat-free.
Group 4: Chicks from non-vaccinated breeders – fed no coccidiostats, were maintained in pens with wire net floor raised 20 cm above the concrete floor, served as negative controls, intended to prevent them from exposure to coccidia.

Chicks from groups 1, 2, 3 were maintained on litterspread on concrete floor. One chick in each pen (subgroup) was inoculated by oral gavage at 1-day of age with 50 oocysts each of 3 coccidia species: *E. maxima*, *E. acervulina*, *E. tenella*. Seeders were to initiate cycling of oocysts in the litter and to expose the other chicks in the pen. Litter samples were collected at 7, 14 and 28 d of age, and oocysts were counted (OPG) by the modified McMaster method.

At day 34 all broilers were challenged with pathogenic sporulated oocysts of 3 *Eimeria* species by oral gavage. Each challenge dose contained: 300 000 *E. acervulina* oocysts + 150 000 *E. maxima* oocysts + 60 000 *E. tenella* oocysts per bird (13). Six days after challenge all broilers were slaughtered and lesion scoring was determined according to the Johnson and Reid scale (3).

Sera from hens and 1-day-old broilers were assayed to determine the level of circulating *Eimeria* antibodies using ELISA kit (supplied by ABIC, Israel) in relation to specific *E. maxima* gametocyte antigen. Results (optical density) were read in micro ELISA reader at 405 nm and calculated according to formula supplied by the producer. The mean S/P value was calculated: S/P= (Group sample mean – Negative control) / (Positive control – Negative control).

The broilers on trial were vaccinated according to routine prophylactic program. The birds were observed daily for any clinical or behavioural changes. Standard hygiene and husbandry conditions were maintained. Health, feed consumption and body weight were recorded. All chicks were weighed at days 1, 9, 21, 29, 33, 39 of age. Feed consumption was evaluated at days 9, 25, 33 and 40 of age. EU production index (EPI) was calculated (mean body weight x survival rate x 100) / (number of days x feed conversion per 1kg body weight).

**Results**

There were no lesions detected at site of injection, and no undesirable reactions were observed in breeders vaccinated with CoxAbic®. No negative influence was observed in egg laying, fertility nor in egg hatchability. The level of *Eimeria* antibodies in vaccinated breeders, expressed by mean S/P value, amounted 0.81 and in non-vaccinated ones 0.73. (Fig.1). It must be stressed that the producer of ELISA kit declared that values 0.4 and higher were positive. Bigger differences were noted in the level of *Eimeria* antibodies in 1-day-old broilers from vaccinated breeders (mean S/P=0.57) and in broilers from non-vaccinated breeders (mean S/P=0.19). The mean S/P value in chicks from non-vaccinated breeders was lower than 0.2 (S/P<0.2 obtained in broiler serum is considered by kit producer as positive level of *Eimeria* antibodies).

Small number of oocysts was found on days 7 and 14 only in one of 3 pens in group 1 and oocysts were not found in other groups. However, on day 28 oocysts were found in all groups: the lowest oocyst number was found in group 4 (on wire net floor) and amounted 385 oocysts /1g of faeces. The highest number of oocysts was found in group 2 – broilers from non-vaccinated breeders 263 478 oocysts per 1g of litter (OPG). Oocyst excretion in broilers from vaccinated breeders (group 1) amounted to 154 268 OPG and was lower than in broilers fed coccidiostats (group 3) – 220 430 OPG. Reduction of oocyst excretion in comparison to control group 2 was: 41% in group 1 and only 16% in group 3.

Broilers coming from vaccinated breeders (group 1) had the lowest mean lesion score – 1.39 (Fig. 2). A score higher than 2.0 was observed only in one broiler in this group. The highest value of mean lesion scores (2.46) was noted in group 4 (negative controls on wire floor). In this group 2 broilers died after challenge and 7 birds had lesion scores higher than 3.0.
The EU production index (EPI) in group 1 was the most favourable during whole experiment ranging from 180 to 292 (Table 1). After 25 d in group 1 EPI was significantly higher (P≤0.05) than in other groups, after 33 d than in groups 3 and 4 and after 40 d than in group 4.

**Discussion**

The interest in the passive transmission of *Eimeria* antibodies increased when Rose (5) had published her study in which she described the important role of passive immunization in preventing broiler coccidiosis. This author had infected chicks with *E. maxima* oocysts and after 2-3 weeks she sampled blood from them. Serum obtained from these birds she injected into other chicks infected with *E. maxima*. Fifty percent reduction of oocyst excretion was obtained in comparison to control birds. Other studies (6, 7, 9, 10) documented that anti-*Eimeria* antibodies transferred to chicks from hens immunized with *E. maxima* gametocyte antigen very effectively limited coccidiosis.
caused by 3 species of *Eimeria* (*E. acervulina, E. maxima, E. tenella*). In our experiment the high level *Eimeria* antibodies was noted in chicks coming from vaccinated breeders. This level expressed by mean S/P value was about 3 times higher than that in chicks from non-vaccinated breeders. It confirmed the good efficacy of vaccination with CoxAbic®. Wallach et al. (10) detected the high level of *Eimeria*-specific IgG antibodies in egg yolk and hatching sera coming from immunized hens. In contrast the authors did not detect *Eimeria*-specific IgA and IgM. According to other authors (1, 2) 3 the main protein fractions of CoxAbic® vaccine antigen (230 kDa, 82 kDa, 56 kDa), isolated from *E. maxima* gametocytes, are localized in or around the wall-forming bodies (WFBs) of the macrogametocytes and in material that is associated with the oocyst wall itself. Moreover, there is documented evidence that these 3 protein fractions were highly conserved and identical in different strains of *Eimeria* species (10). In contrast the authors did not detect antibodies in egg yolk and hatchling sera coming from vaccinated hens obtained the good performance traits.

Our experiment demonstrated that there were no lesion at site of CoxAbic® vaccine injection, and no undesirable reactions were observed in breeders vaccinated with the dose recommended by the manufacturer. Moreover, there were no negative influences for egg laying and hatching. *Eimeria* antibodies were transferred to chicks. It did not decrease the development of acquired immunity against coccidiosis, which was confirmed by the reduction of oocysts excretion after challenge.

### References


