SELECTION OF A PRECOCIOUS STRAIN OF EIMERIA ACERVULINA "IN VIVO"

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Key words: Eimeria acervulina, precocious strain, chicken

Abstract: Coccidiosis is a protozoan disease that causes important economic losses in the poultry farms of the world, either because of mortality or due to decreased rate of weight gain. The eimerian species that infect the chicken, especially in the broilers, are E. acervulina, E. tenella and E. maxima. Because of the chimioresistance phenomenon, it appeared the necessity of finding new prevention methods, such as vaccination, existing many available vaccines at the moment. This paper had the purpose of obtaining a precocious strain of Eimeria acervulina, latter used for immunoprophylactic purpose. The preliminary line obtained was characterised concerning the duration of biological cycle, prolificity and degree of pathogenicity and immunoprophylactic efficacy in comparison with the parental strain. The duration of prepatent period of E. acervulina precocious strain decreased from 97 hours after the first passage to 93,49 hours at the fifth passage, registering a decrease of 3,6 hours, and also it had inferior pathogenicity in comparison with parental one, the lesional score being 2 in the precocious line and 4 in the parental line. The highest rate of weight gain was observed in group 3A (positive control), being of 655,71 g/chicken. In the immunized groups the rate was of 611,72 g/chicken in group 1A, respectively 526,36 g/chicken in group 2A. Regarding food conversion, the highest level of consumed feed was registered in group 2A - immunized and infected – 3,20 kg forage/kg rate, followed by group 1A (immunized and uninfected), 2,75 kg foragej/kg rate, the lowest consume being in group 3A – positive control, 2,51 kg feed/kg. Regarding the obtained results it is important to continue the line selection for precocity of E. acervulina and to perform additional studies such as, testing of sensibility and chimioresistance to different coccidiostatics and comparative study of the biological cycle (precocious line – parental line) by histological smears.

INTRODUCTION

Coccidiosis is a protozoan disease that causes important economic losses in the poultry farms of the world, either because of mortality or due to decreased rate of weight gain. The eimerian species that infect the chicken, especially in the broilers, are E. acervulina, E. tenella and E. maxima. Because of the chimioresistance phenomenon, it appeared the necessity of finding new prevention methods, such as vaccination, existing many available vaccines at the moment.

The local species of Eimeria may differ from the vaccine strains, so the applied vaccine will not give sufficient protection against eimerian natural infections. The antigenic variability of the Eimeria strains from different geographic regions may be more frequent as it is thought (Kawazoe et al., 2005).

Jeffers (1974, 1975) was the first who described an obtaining method of a precocious line of Eimeria tenella, showing the possibility of using such lines in the immunoprophylaxy of eimeriosis. Latter, there were obtained precocious lines of other eimerian species that infect
chickens, such as *E. acervulina* (McDonald *et al.*, 1982), *E. mitis* (McDonald and Ballingall, 1983; McDonald and Shirley, 1984), *E. praecox* (Shirley *et al.*, 1984), *E. necatrix* (Shirley and Bellati, 1984), *E. maxima* (McDonald *et al.*, 1986; Shirley and Bellati, 1988), *E. brunetti* (Shirley *et al.*, 1986) and *E. tenella* (McDonald *et al.*, 1986).

Recently, researches for obtaining such *E. acervulina* and *E. necatrix* lines were performed using strains isolated from Australia and Brazilia (Stewart and Jorgensen, 1997; Kawazoe and Manarini, 2001; Kawazoe *et al.*, 2005; Jorgensen *et al.*, 2006, Montes *et al.*, 1998).

The features of a precocious strain are: decrease of prolificity and duration of biological cycle, decrease in pathogenity, keeping their immunogenic capacity and stability (Shirley and Bedrnik, 1997).

**MATERIAL AND METHOD**

**Period:** The studies were performed between September 2005 and May 2006 in the Biobasis and Laboratory of Parasitology and Parasitic Diseases Department of the Faculty of Veterinary Medicine, Cluj-Napoca.

**Aim:** This paper had the purpose of obtaining a precocious strain of *Eimeria acervulina*, latter used for immunoprophylactic purpose. The preliminary line obtained was characterised concerning the duration of biological cycle, prolificity and degree of pathogenicity and immunoprophylactic efficacy in comparison with the parental strain.

**Experimental design**

**Selection of the precocious line of *Eimeria acervulina***. Regarding the morphology and localization/features of the lesions, *E. acervulina* was isolated from a polyspecific suspension of oocysts by experimental infection of 5 chickens of 7 days old. Four days after infection, the chicken were sacrificed and the duodenum isolated. The oocysts isolated from the duodenal content and mucosa were put to sporulate in potassium-bichromat solution at room temperature and constant aeration, followed by computer measurement.

After that, serial passes of the first coproeliminated oocysts were performed, using a dose of 10,000 oocysts/chicken.

**Determination of pathogenicity and prolificity of the precocious line of *Eimeria acervulina***. In order to determine the pathogenicity and prolificity of the obtained line we performed comparative studies of precocious and parental strain, regarding the moment of apparition of the first oocyst, number of oocyst per gram of faeces and lesional score.

Thus, two experimental groups were created, each with 6 chicken, as follows: *Group LP* – infected with the precocious line of *Eimeria acervulina*; *Group LI* – infected with the parental line of *Eimeria acervulina*.

**Determination of the immunoprophylactic efficacy of the precocious line of *Eimeria acervulina***. Three groups of 11-12 one day old chickens were created: *Group 1A* – negative control group, immunised and uninfected, 12 chicken, *Group 2A* – immunised and infected, 11 chicken, *Group 3A* – positive control group, unimmunised and infected, 7 chicken. Immunization was performed at the age of 4 days, administering 500 oocysts LP/chicken, via oro-ingluvial tube. The experimental infection was performed at 10 days after immunization with 5000 oocysts LI/chicken.

After infection, we monitored daily: the clinical status of the chicken, the number of oocysts eliminated in faeces using the McMaster method and the lesional score by controlled euthanasia. In the end of the experiment we calculated the conversion food rate, the rate of weight gain and the antieimerian performance percentage of the experimental groups.
The chicken were raised in batteries, with ad libitum feeding and drinking conditions, the used forage being without additional coccidiostatic products or other substances with „static” or „coccidiocide” features. The infection was realised with 5000 oocysts/chicken via the oro-ingluvial tube.

**RESULTS**

**Selection of the precocious line of *Eimeria acervulina***

In order to isolate *E. acervulina*, from experimentally infected chicken, we collected the duodenal segments with specific lesions (lenticular miliar foci, white-grey in colour, easily seen through the serosa) (Fig. 1). The oocysts’ dimensions were of $17,31\pm1,48 \mu m$ in great diameter and $14,41\pm0,84 \mu m$ in small diameter ($17-18/13-15 \mu m$) (Table 1, Fig. 2).

The prepatent period after the passages decrease in duration from 97 hours from the first passage to 93,40 hours at the fifth passage, registering a decreasing of 3,6 hours. At the sixth passage the prepatent period increase to 100,32 hours, value much superior to the initial one. In conclusion, this passage was repeats, the first oocysts appearing at 97 hours after infection (p.i.) (Fig. 3).

Also, we observed the decrease in dimensions of the oocysts with $0,79/0,58 \mu m$ ($16,52\pm1,79/13,83\pm1,49$) comparative to the parental strain (LI) (Table 1).

**Determination of pathogenicity and prolificity of the precoius line of *Eimeria acervulina***

The comparative results of the faecal score, lesional score and oocyst production, in the parental and precocious strain of *E. acervulina* indicated an inferior pathogenity of the precocious strain.

Thus, the faecal score was of 0,07 in the precocious strain and 0,17 in the parental strain. The most important differences were observed in the lesional sore which was 2 in the precocious strain and 4 in the parental strain. The oocyst production was maximum at 124,4 hours in the precocious strain (21600 O.P.G.), meanwhile in the parental strain the maximum was at 136,4 hours (12000) (Table 2).

**Determination of imunoprophylactic efficacy of the precocious line of *Eimeria acervulina***

In the group 1A the level of coproelimination had inferior values, with a maximum on the 8th day p.i., O.P.G. of 86000, the average of coproelimination being 20464 O.P.G. In the group 2A, immunized and infected, appeared a peak on the 14th day p.i. when O.P.G. was of 256800, the average of coproelimination being 44278 O.P.G. In the poitive control 3A group, we registered two more important peaks on the 9th and 11th days (139200 respectively 124800 O.P.G.), the average of coproelimination being 39678 O.P.G. (Fig. 4). The coproelimination level in the immunized and infected group was superior to the poitive control group.

At necropsy, cataral enteritis lesions were present with lenticular, white-greyish foci in the duodenum, with different intensities according to the studied group. The lesional score was inferior in the immunized groups, giving grades of 0,5 in group 1A and 1 in the group 2A. In the poitive control group (3A) the lesional score was 1,5 (Table 3).

The highest rate of weight gain was registered in the case of group 3A (poitive control), being of 655,71 g/chicken. In the immunized groups the rate was of 611,72 g/chicken in group 1A, respectively 526,36 g/chicken in group 2A. The greatest feed conversion rate was in group 2A – immunized and infected – 3,20 kg feed/kg, followed by
group 1A (immunized and uninfected), 2.75 kg feed/kg, the lowest consume being registered in group 3A – positive control, 2.51 kg feed/kg.

The index and percentage of the anticoccidian performance had superior values in group 3A (positive control), the I.C. (coccidiostatic index) being of 725.79 with a P.P. (antieimerian performance percentage) of 103.96%. In the immunized groups the mentioned parameters were: 698.12 I.C. with P.P. of 100% in group 1A, meanwhile in group 2A (immunized and infected) we registered the lowest values, 603.38 I.C. with P.P. of only 86.42% (Table 3).

Table 1: *E. acervulina*: parental and precocious lines oocyst dimensions

<table>
<thead>
<tr>
<th>Diameter (µm)</th>
<th><em>E. acervulina</em> (parental strain)</th>
<th><em>E. acervulina</em> (precocious strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great φ</td>
<td>17.31±1.48</td>
<td>16.52±1.79</td>
</tr>
<tr>
<td>Small φ</td>
<td>14.41±0.84</td>
<td>13.83±1.49</td>
</tr>
</tbody>
</table>

Table 2: Comparative lesion score, faecal score and the coproelimination quantity in LI and LP of *E. acervulina*

<table>
<thead>
<tr>
<th>Duration (hours)</th>
<th>O.P.G</th>
<th>Faecal score</th>
<th>Lesional score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LI</td>
<td>LP</td>
<td>LI</td>
</tr>
<tr>
<td>100.4</td>
<td>0</td>
<td>900</td>
<td>0.17</td>
</tr>
<tr>
<td>112.4</td>
<td>200</td>
<td>21600</td>
<td></td>
</tr>
<tr>
<td>124.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>136.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: LI – parent line; LP – precocious line

Table 3: The lesion score, weight gain, food conversion, index and percentage of anticoccidian performance in the experimental groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>O.P.G.</th>
<th>LESIONAL SCORE</th>
<th>WEIGHT GAIN (G/CHICKEN)</th>
<th>FOOD CONVERSION (KG FOOD/KG SPORE)</th>
<th>I.C.</th>
<th>P.P. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>20464</td>
<td>0.5</td>
<td>611.72</td>
<td>2.753</td>
<td>698.12</td>
<td>100</td>
</tr>
<tr>
<td>2°</td>
<td>44278</td>
<td>1</td>
<td>526.36</td>
<td>3.205</td>
<td>603.38</td>
<td>86.42</td>
</tr>
<tr>
<td>3A</td>
<td>39678</td>
<td>1.5</td>
<td>655.71</td>
<td>2.510</td>
<td>725.79</td>
<td>103.96</td>
</tr>
</tbody>
</table>

Figure 1: Duodenum: miliar lenticular foci, white-greyish induced by *Eimeria acervulina* (original)

Figure 2: *E. acervulina* evoluated oocyst (X40)
The prepatent period of *E. acervulina* after five passages decreased with 3.6 hours, from 97 hours to 93.40 hours. At the 6th passage, the first oocysts were found at 100.31 hours after infection, so this passage was repeated.

McDonald *et al.* (1982) obtain a precocious line of *E. acervulina* Houghton strain, in which the prepatent period decreases from 89 hours to 72-62 hours. Long and Johnson (1988) observed a decrease from 97 to 72 hours in the H” and „C” strain, meanwhile Stewart and Jorgensen (1997) obtain a decrease from 124 hours (parental line) to 92 hours (precocious line). There were obtaine also precocious ionophor tolerant lines of *E. acervulina*, in which it was obtained a decrease of the prepatent period to 22 hours (Li *et al.*, 2004). In the last two years Kawazoe *et al.* (2005) isolate 2 precocious lines of *E. acervulina* („I”„Cu”) in Brazil, after 25 passages, with the abatement of the biological cycle duration from 96 hours to 81, respectively 82 hours.

Generally the precocious line of *Eimeria* have much reduced pathogenity compared with the parental lines, condition observed in our studies too, the lesional score in the parental line being 4 and in the precocious line 2. Other authors showed that the reduction of pathogenicity induced the decrease of reproductive capacity, for example in *E. tenella* (Bednik *et al.*, 1986, Kawaguchi *et al.*, 1988), condition which wasn’t observed in this experiment. The oocyst number of *E. acervulina* per gram of faeces at 124.4 hours was of 21600 in the precocious line, meanwhile in the parental line at 136.4 hours the coproelimination quantity was of 12000 O.P.G.

Also, we observed the decrease of oocyst dimensions. The reduction of pathogenity and prepatency of the precocious lines of *E. acervulina, E. mitis, E. praecox* and *E. maxima* is due generally lack of one or more schizogonic generations. Regarding *E. necatrix* other causes were mentioned, such as the smaller dimensions of the schizonts of the second generation (49.4X35.5 µm parental line, 22.3X17.1 µm precocious line) (Montes *et al.*, 1998).

As for the oocyst coproelimination, superior coproeliminations were noticed in the lines immunized and infected with the parental line. Williams (1994) observed that in broilers, breeders and layer chicken, vaccinated with Paracox (attenuated vaccine) in drinking
water, at one week of age, the first peak of coproelimination being at 2-4 weeks. This is the result of the vital cycle development of the vaccinal strains. Between weeks 4 and 7, there is a new peak, a little higher than the initial one, which probably stimulates once more the already installed immunity. It’s the result of the infection with the natural strains from the shelter and controlled by the immunity induced after vaccination (Williams și col., 1999). Furthermore, a low number of oocysts were observed in the litter, due to the installed immunity. Some of the oocysts are destroyed by the adverse conditions from the litter (Williams, 1995).

The chicken immunized on the first day of life with Paracox-5, by feed, had oocyst coproelimination 7 days after vaccination and reached the maximum level at 28 days after vaccination. The chimioprevented chicken from the same place presented late oocyst coproeliminations but reaching the maximum level in a shorter time. The maximum level was registered at the same time, but it was superior to the values of the vaccinated chicken. *Eimeria* spp. local strains were present but no clinical signs of disease occurred in the studied groups.

In the present study of the immunized groups, the results were according to data given by Williams *et al.* (1999), registering a single peak, but inferior to the other groups on the 6th-8th days p.i., probably because of the spontaneous contamination. The conditions observed in the immunized and infected groups compared to the positive control group, could be explained by the crowding effect. According to this, there is a direct relation between the infectious dose and oocyst production, the parasites fecundity decreasing with the increase of the infectious dose (Brackett and Bliznik, 1952, cit. by Johnston *et al.*, 2001). After Brackett and Bliznik (1952, cit. by Johnston *et al.*, 2001) the primary immune response has an important role in the crowding effect because of the fact that an infectious dose may induce a rapid and efficient primary immune response.

Although the oocyst number per gram of faeces was high in group 2, the lesional score was inferior to that of positive control group, which was of 0,5-1 in the immunized groups and 1,5 in the positive control group (3A).

In the past, the lesional score at the sacrificed chicken in different moments of time gave important clues about the induced protection, but only this criterium or in correlation with the performances may induce us in error, because in the vaccinated chicken may appear lesions induced by infections with *Eimeria* spp., but without affecting the performances. Thus, the rate of weight gain and the rate of feed conversion are considered important criteria for evaluation an antieimerian vaccine (Williams and Catchpole, 2000).

The rate of weight gain and the food conversion in the immunized groups were inferior to the positive control group. Other authors, after 25 passages obtain superior values of these parameters in the precoious lines of *E. acervulina* (Kawazoe *et al.*, 2005; McDonald and Ballingall, 1983).

Regarding the obtained results it is important to continue the line selection for precocity of *E. acervulina* and to perform additional studies such as, testing of sensibility and chimioresistance to different coccidiostatics and comparative study of the biological cycle (precoious line – parental line) by hystological smears.

**CONCLUSIONS**

The researches for obtaining a precocious line of *Eimeria acervulina* from local eimerian strains and their application in eimerian immunoprophylaxy in the chicken, performed between Semptember 2005 – May 2006 in the Biobasis and Laboratory of
Parasitology and Parasitic Diseases Department of the Faculty of Veterinary Medicine Cluj-Napoca, showed the following results:

- the duration of prepatent period of *E. acervulina* decreased from 97 hours after the first passage to 93.49 hours at the fifth passage, registering a decrease of 3.6 hours;
- the precocious line of *E. acervulina* had inferior pathogenicity to the parental one, the lesional score being 2 in the precocious line and 4 in the parental line;
- the average of coproelimination was superior in group 2A (immunized and infected) with a value of 44278 O.P.G., meanwhile in group 3A (unimmunized and infected) the value was of 39678 O.P.G.; in group 1A (immunized and uninfected) the value was inferior during the whole experimental period, the average being of 20464 O.P.G.
- at necropsy, cataral enteritis lesions were present, with white-grey lenticular foci, the lesional score being of 0.5 in group 1A and 1 in group 2A, and in the positive control group (3A) the lesional score was 1.5;
- the highest rate of weight gain was observed in group 3A (positive control), being of 655.71 g/chicken. In the immunized groups the rate was of 611.72 g/chicken in group 1A, respectively 526.36 g/chicken in group 2A;
- the highest level of consumed feed was registered in group 2A - immunized and infected – 3.20 kg forage/kg rate, followed by group 1A (immunized and uninfected), 2.75 kg foragej/kg rate, the lowest consume being in group 3A –positive control, 2.51 kg feed/kg;
- the antieimerian index and the percentage of antieimerian performance in the immunized groups were inferior to that of the positive control group.

**Acknowledgements:** this study was financed by CNCSIS, within the framework of Td project, number 307, project manager Adriana TITILINCUC. Veterinarian epizootologist from AVIS Deva, is thanked for support.

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