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EIMERIA PERFORANS AND *E. COECICOLA* MULTIPLICATION RATE AND EFFECT OF THE ACQUIRED PROTECTION ON THE OOCYST OUTPUT

D. LICOIS, P. COUDERT, F. DROUET-VIARD, M. BOIVIN

I.N.R.A. Laboratoire de pathologie du lapin. Station de Pathologie Aviaire et de Parasitologie. 37380 MONNAIE. FRANCE

INTRODUCTION

There is a relatively limited number of papers concerning the studies on *Eimeria* spp. of rabbit. Some studies on the pathogenicity of different species have been done. Life cycle of *E. coecicola* has been recently studied by Pakandl (1989). However, the data on the multiplication capacity or immunogenicity are rare meanwhile the daily excretion curve and the multiplication rate can be considered as a criterion for identification of the coccidial species. The parasitic excretion is also an epidemiological parameter and a datum which let us analyse the acquired immunity of the multiplication of the parasites or assess for example, the efficacy of anticoccidials (Coudert, 1989).

E. perforans and *E.coecicola* are considered as slightly or non pathogenic (Coudert et al., 1979; Cheissin, 1947; Catchpole and Norton, 1979). Nevertheless one of them, namely *E.perforans*, is one of the most frequent species occuring in the rabbit breeding. It is possible that those 2 species play a part as a factor exacerbating pathological reactions in the rabbit intestine, although it has not been studied extensively.

This paper presents the results of the work on multiplication rate and immunogenicity estimated by the oocyst output. The influence of the different strains of *E.perforans* on the acquired immunity is analysed as well.

MATERIALS AND METHODS

- Animals and experimental conditions

All the animals used in this experiment were New Zealand White Rabbits (INRA 1077 strain) originating from the SPF breeding of Station de Pathologie Aviaire et de Parasitologie de l'I.N.R.A. de TOURS. They were free of coccidia, oxyures, *Pasteurella*, *Clostridium* and *E.coli* 0103 (Coudert et al., 1988a). They were inoculated at 6 weeks of age while being transferred to the experimental rooms. They were fed ad libitum with non-supplemented industrial feed. The experimental conditions and desinfection methods were like those previously described by Coudert and al., 1979.

- Experimental procedure

E.coecicola - 30 animals were placed in 5 groups, each was of 3 cages of 2 rabbits. 4 groups were inoculated per os with *E.coecicola* pure strain, freshly multiplied and sporulated (26°C), at the doses of 10, 100, 1.000 and 5.000 oocysts. The 5th group remained non-inoculated as a control. 15 days after the first inoculation all the animals were challenged with 5.000 oocysts the same strain. And finally, as *E.coecicola* infects the appendix vermiform which is a lymphoid organ that can possibly play a part in the immunological phenomena, the animals were infected for the third time in order to assess the specificity of acquired immunity. This inoculation took place 15 days after the second one and was composed of 3 other species : *E.magna*, (10.000 oocysts/animal) *E.flavescens* and *E.intestinalis* (8.000 oocysts/animal).

The faeces of the animals infected with 100 and 5.000 oocysts were collected daily starting from day 7 up to day 14 following the first inoculation. After the second and the third inoculation all the faeces excreted between day 7 and day 14 were collected only once.

The oocysts counts were performed according to the technique described by Licois and Coudert, 1980. The detection threshold of this method is 10^4 oocysts in the faeces excreted during 24h or 50 oocysts per gram of feaces.

E.perforans - 52 animals were allocated into 13 groups of 2 cages of 2 rabbits each one. They were inoculated per os with the pure strain of *E. perforans* at the following doses :
- the BAL strain - 10, 50, 100, 500, 1.000, 5.000 and 10.000 oocysts.
- the PAP and ZUN strains - 100, 500 and 5.000 oocysts.

The faeces of the animals infected with 50, 500, 5.000 oocysts of the BAL strain were harvested daily between day 4 and day 10.

13 days after the first inoculation all the rabbits were challenged with 10.000 oocysts of the PAP strain. All the faeces excreted between day 5 and day 9 after the second inoculation were collected only once.

RESULTS

E. coecicola

- Excretion curve (Fig. 1) - At the dose of 100 oocystes the excretion started on the day 9 after inoculation. Some oocysts may be observed on day 8 after the infection with the dose of 5.000. The peak of excretion occured on day 10. The majority of oocysts (95 %) were passed between day 9 and day 11 after inoculation.

- Multiplication Rate - Fig. 2 Shows that the number of oocysts discharged increased with the dose of inoculum up to 1.000 oocysts. Beyond that dose the total oocyst output did not differ any more whatever the dose and its peak was about 4.10^8 oocysts.

- Acquired protection against the production of the oocysts of a homologous strain. In the animals initially inoculated with 10 oocysts, the output recorded after the challenge with 5.000 oocysts decreased meanly by 36,4 %. Nevertheless it stayed still at a relatively high level (fig. 3). The fall (>99,5%) was also observed in rabbits given 100 oocysts at the first

inoculation. The protection was nearly absolute after only one inoculation with 1.000 or 5.000 oocysts, since for each of those doses no oocysts were detected after the challenge in any of 3 cages.

- Acquired protection against other Eimeria spp. The mean total output of the oocysts of *E.intestinalis*, *E.flavescens* and *E.magna* was $2,38.10^9$, $5,72.10^8$ and $2,63.10^8$ respectively. That are the numbers usually obtained after the first inoculation with those species (Coudert et al., 1988b).

E.perforans

- Excretion curve (Fig. 4) - Whatever the dose the oocyst production started on day 5 following the inoculation and decreased on day 10. The peak of excretion took place on day 6. According to the doses 83-87 % of the total oocyst output was obtained between day 6 and day 8.

- Multiplication Rate of the strains : BAL, PAP, ZUN (Fig. 5) The total oocyst output was proportional to the dose up to the inoculum of 100 oocysts. Beyond this dose the increase of the ouput was not observed any longer. The total oocyst production reached its maximum at the level of 3 - 5 $.10^8$ oocycsts. It is difficult to interpret the differences between the strains since there were not enough cages for each treatment.

- Acquired protection against the oocyst production (Fig. 6) Let's remind that all the animals were challenged with 10.000 oocysts of the PAP strain of *E.perforans*.

In the animals initially inoculated with the ZUN or the BAL strains (heterologous strains) one could observe a significant reduction (of 67-87%) of the oocyst output after the challenge. Nevetherless the excretion as an absolute number stayed still at the high level of $4-9.10^7$ oocysts.

Much stronger protection has been noticed in the animals infected with the PAP strain (homologous strain). The reduction of excretion depends on the initial dose : the higher the initial dose, the lower oocyst output following the challenge.

DISCUSSION

The multiplication rate, the evolution of oocyst production and the acquired protection against multiplication of the parasite were estimated in 2 species of rabbit coccidia: *E.perforans* and *E.coecicola*. While some data on the pathogenicity of these species have been published, there are no informations concerning the oocyst production, at least as far as we know. Yet, in our opinion, this detail is an important epidemiological factor which should not be neglected. In order to control it during the anticoccidial treatment or vaccination it is necessary to know its evolution.

The daily oocyst output confirms our previous results concerning the prepatent periods which are 5 days for *E.perforans* and 8 days for *E.ceocicola* (Coudert et al., 1988b).

The analysis of the multiplication capacity shows that the oocyst output of both species increases according to the infection dose but only to a certain level. Beyond that level the total oocyst output does not vary any more. This level is reached by the relatively low doses. A simple theoretical calculation shows that the plateau of excretion is reached with about 200

oocysts of *E.coecicola* and less shan 100 oocysts of *E.perforans*. The total number of oocysts passed oscillated between 2 and 5.10^8 for *E.coecicola*, but also for the most of the other species (Coudert et al., 1988b).

The acquired immunity is efficient with the dose of 100 oocysts of *E.coecicola* and almost complete with the dose of 1.000 and more oocysts. The low doses of other species are also sufficient to prevent the multiplication of the coccidia, particularly those of *E.flavescens* and *E.irresidua* (Norton et al., 1979) or *E.intestinalis* (Coudert et al., 1990).

Considering the acquired protection against the multiplication of the parasite one can observe a clear difference between these 2 species. The inoculation of 1.000 oocysts of *E.perforans* does not protect the animals totally againts the challenge with a homologous strain. The protection is still worse in the animals reinfected with the heterologous strains.

The immunity acquired with *E.perforans* is relatively weaker than that obtained with the other species. This might partially explain the most frequent prevalence of *E.perforans* in rabbit husbandry.

Despite its localization in an immunocompetent organ, no cross immunity was observed in the animals infected with *E.coecicola* and challenged with *E.magna*, *E.flavescens* or *E.intestinalis*. Those results show once again that the acquired immunity is specific not only for each *Eimeria* species but also for each strain of a given species, as there are several antigenic variations among the strains of the same species.

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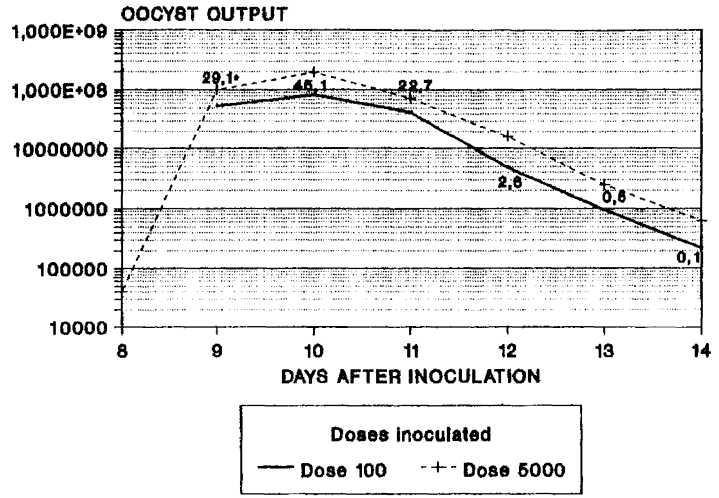
ABSTRACT

The multiplication rate and acquired protection estimated by measuring output has been studied in 2 species of rabbit coccidia : *Eimeria perforans* and *E.coecicola*. The maximal total oocyst production is similar in both species and it is about 4.10^8 oocysts. The peak of excretion occurs on day 6 and day 10 for *E.perforans* and *E.coecicola* respectively. The immunity towards the excretion of oocysts of *E.coecicola* is absolute after only one inoculation with 1000 oocysts. No cross protection was observed in the animals immunized with *E.coecicola* and subsequently inoculated with *E.magna*, *E.flavescens* or *E.intestinalis*. Inoculation with 1000 oocysts of *E.perforans* does not protect the animals totally against a challenge with the homologous strain (the oocyst production is still maintained at the level of 4.10^5). This protection is still worse in the animals reinfected with the heterologous strains (the oocyst output is over 5.10^7).

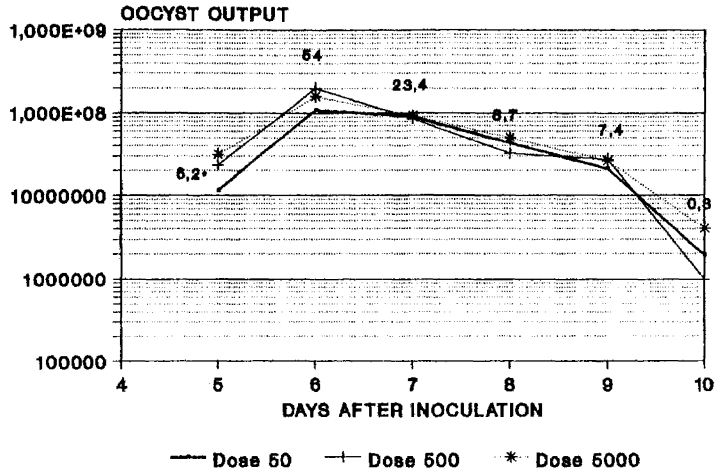
Those results show that apart from the specificity of a species there is also another specificity : that one which characterizes the strains within the same species and this specificity is important for the acquired protection.

FIG. 1- DAILY OOCYST OUTPUT OF *E. COECICOLA*



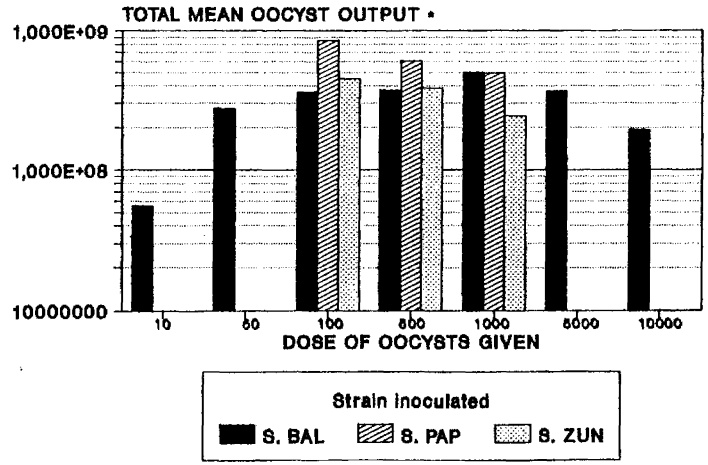
* Value in ù of the total excretion at the dose of 100 oocycts

FIG. 2- DAILY OOCYST OUTPUT OF *E. PERFORANS*



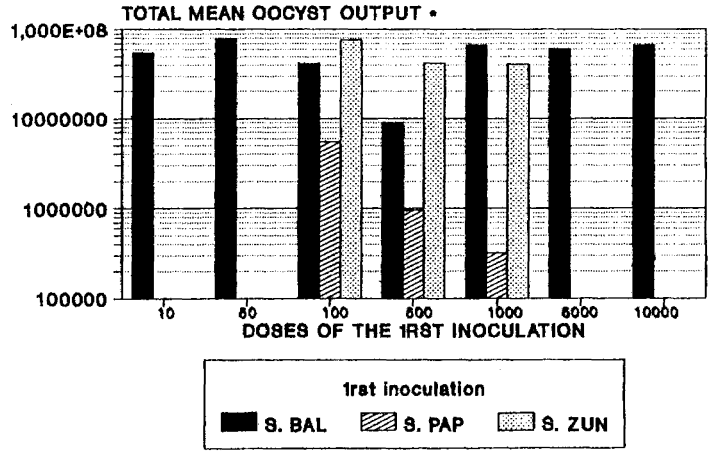
* Value in % of the total excretion at the dose of 500 oocysts

FIG. 3- MULTIPLICATION RATE OF *E. PERFORANS*



* Total oocyst output from D5 to D10
For the pap & zun strains only the doses of 100, 500 et 1000 oocysts were tested

FIG. 4- IMMUNOGENICITY OF *E. PERFORANS*



* Total oocyst output from D5 to D10 following the challenge with 10000 oocysts of the PAP strain of *E. perforans*

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TABLE 1 -MULTIPLICATION RATE OF E. COECICOLA
Oocyst output depending on the dose inoculated

Dose of oocysts given	Total mean oocyst output ($x10^{-6}$)
10	26
100	183
1000	412
5000	383

TABLE 2 -IMMUNOGENICITY OF E. COECICOLA
Oocyst output after a challenge with 5000 oocysts

Dose of oocysts given at the 1rst inoculation	Total mean oocyst output after the challenge ($x10^{-6}$)
10	253
100	1,2
1000	0,003
5000	0,003