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# EPIDEMIOLOGY OF COCCIDIOSIS IN COMMERCIAL RABBITS (1982-1987) AND RESISTANCE AGAINST ROBENIDINE

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## Introduction

Coccidiosis is an omnipresent parasitic disease in commercial rabbitries. Besides depression of weight gain and low efficiency of feed conversion, the parasite favours the proliferation of *Escherichia coli* in the gut (Licois & Guillot, 1980) and may be associated with the development of the enteritis complex (Whitney, 1970, Prescott, 1978, Sincovics 1984).

Nine different species of eimeria have been described in rabbits (Catchpole & Norton, 1979, Coudert, 1979) All nine species have been detected in Belgium (Peeters et al., 1981). The intestinal species may be separated in species with high pathogenicity (*E. intestinalis, E. piriformis* and *E. flavescens*), causing high mortality and in species with low to moderate pathogenicity, mainly depressing efficency of feed conversion. This depression may cause a rise of the production-price by 5 % (Peeters & Halen, 1980).

Therefore anticoccidial drugs are systematically being incorporated in rabbit feeds. At this moment only two anticoccidials are allowed in the CEE : clopidol and robenidine. Different authors showed most species of rabbit eimeria to be poorly controlled by clopidol (Whitney, 1970, Catchpole & Norton, 1975, Bedrnik & Martinez, 1976, Coudert et al., 1976, Gallazzi, 1976, Peeters & Halen, 1980) and so only robenidine remains as adequate anticoccidial (Coudert, 1978, Peeters et al., 1979). Robenidine has been used first in Belgium in 1982 and since then the evolution of the percentage occurrence of the different eimeria-species has been monitored systematically in commercial rabbitries. Some strains have been isolated in pure culture and were examined on possible resistance against robenidine. This paper deals with the results of this research

## Materials and methods

### *Faecal samples*

Between August 1982 and December 1987 a total of 1489 caecal samples were collected in 61 commercial rabbitries from diarrhoeic rabbits. The rabbits were housed in wire cages which had droppings pits underneath and were fed ad libitum with a commercial pelleted ration containing 50 to 66 ppm robenidine. Since the end of 1985 an increasing number of rabbit breeders have added sulfonamides to the drinking water the first week or weeks after weaning.

### *Parasitology*

Caecal samples were examined by the McMaster egg counting technique. Samples which were found to be negative were re-examined using the salt-flotation-concentration technique. A proportion of the screened faecal suspension was made up in 2.5 per cent potassium bichromate (w/v) and agitated in a water bath at 27°C in order to sporulate for subsequent differentiation in species as described before (Peeters et al., 1981). The frequency of the species present was determined on at least 100 sporulated oocysts.

### *Experimental infection studies*

Four field strains of *E.magna* were obtained from caecal contents from diarrhoeic rabbits. These rabbits originated from four different rabbitries, in which outbreaks of coccidiosis occurred between 1984 and 1987, despite the presence of 66 ppm robenidine in the pelleted feed. Oocysts with the typical morphology of *E.magna* were picked out after sporulation and propagated in coccidia-free rabbits. Cultures were prepared from the faeces of rabbits inoculated with 1,000 oocysts by sieving and flotation in saturated sodium chloride solution. The oocysts were then washed and suspended in a shallow layer of 2.5 % (w/v) potassium dichromate at 27°C. Cultures of sporulated oocysts were counted and stored at 4°C.

A liquid nitrogen stabilate of *E.magna* strain W16 was used as an unexposed control. This strain was isolated from a field case in June 1960, well before the use of robenidine. The strain was passaged seven times between then and 1968, when a stabilate in liquid nitrogen was prepared. This was passaged through another rabbit in 1982 to provide material for a second stabilate. This stabilate was used to infect a coccidia-free rabbit to produce the oocysts necessary for this trial.

A total of 192 coccidia-free New-Zealand White rabbits were used in four experiments. After weaning at 4 weeks, the rabbits were housed individually in heat sterilized wire-floored metal cages and received a commercial pelleted ration ad libitum. At weaning faecal samples were screened on coccidia by the salt-flotation-concentration method, on viruses by transmission electron microscopy and on enteropathogenic *Escherichia coli* and *Clostridium spiroforme* by conventional bacteriological methods. No coccidia nor other disease agents were detected.

In four consecutive infection experiments forty-eight rabbits were separated in five groups with the same average weight (coefficient of variation = 9 %). One group comprising eight rabbits was neither medicated nor infected, whereas two groups of twenty rabbits were infected respectively with the unexposed sensitive W16 strain and one of the four exposed field strains. Each rabbit was inoculated orally with 50,000 sporulated oocysts. Within each infected group of 20, half the rabbits received a pelleted feed containing 66 ppm robenidine from 5 days before inoculation, and the other two groups of 10 rabbits received the same ration without robenidine. Animals were checked daily for clinical signs or mortality until three weeks post-infection (p.i.). Diarrhoea was assessed as follows : 0 = no diarrhoea; 1 = increased water content of faecal pellets; 2 = pulpy diarrhoea; 3 = liquid diarrhoea. A mean score was calculated by dividing the sum of scores between 5 and 10 days p.i. by the number of scored stools. The individual weight gain, feed consumption and feed conversion ratio were determined between day 0 and 7 p.i. and individual total oocyst output was recorded between day 7 and 12 p.i.

## Results

The first year of the introduction of the anticoccidial robenidine in commercial rabbit production, only 6 % of caecal samples from commercial rabbits contained more than 100 oocysts per gramme (o.p.g.) and only three different species were detected : *E.magna, E.media* and *E.perforans* (Table 1). Afterwards this figure increased to 18 % in 1983 and to 48 % in 1984. More and more species re-appeared and in 1985 all nine species of *Eimeria* were detected again. However, while most other species remained relatively rare, the percentage occurrence of *E.magna, E.media* and *E.perforans* rose progressively between 1982 and 1984. Between 1985 and 1987 the occurrence of these species remained unchanged.

The experimental infection experiments with an unexposed field strain of *E. magna*, isolated before the robenidine period (W16) and with 4 exposed field strains showed that they all caused significant growth depression, reduced feed intake and diarrhoea in unmedicated rabbits. Mortality induced by the *E. magna* -strains was low (3/80). No difference in pathogenicity has been established between the unexposed W16 isolate and the four exposed

field strains (Table 2). As the absence of other disease agents was confirmed by coprological examination of the rabbits from experiments. 1, 2 and 3, *E. magna* accounts for the observed pathology. Only in experiment 4 rotaviruses were detected in faecal samples 7 days p.i. They depressed weight gain and reduced efficiency of feed conversion.

Table 1. Percentage occurrence of nine species of eimeria in commercial diarrhoeic rabbits fed with pelleted rations containing an anticoccidial drug

| Anticoccidial | Sulfa-quinoxa-line (1) | Robenidine | | | Robenidine + sulfa mezathine | | |
|---|---|---|---|---|---|---|---|
| Year | 1979 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 |
| Number of samples | 191 | 96 | 159 | 245 | 314 | 415 | 260 |
| $< 10^2$ | 15 | 94 | 82 | 52 | 59 | 69 | 53 |
| $10^2$ | 16 | 2 | 3 | 13 | 7 | 5 | 7 |
| $10^3$ | 28 | 2 | 6 | 12 | 12 | 13 | 13 |
| $10^4$ | 34 | 0 | 7 | 13 | 12 | 7 | 17 |
| $\geq 10^5$ | 7 | 2 | 2 | 10 | 10 | 6 | 10 |
| *E.coecicola* | 4 | 0 | 0 | 2 | 1 | 0 | 0 |
| *E.flavescens* | 26 | 0 | 1 | 2 | 4 | 1 | 4 |
| *E.intestinalis* | 22 | 0 | 0 | 3 | 2 | 1 | 2 |
| *E.irresidua* | 8 | 0 | 0 | 1 | 1 | 2 | 3 |
| *E.magna* | 62 | 6 | 3 | 18 | 26 | 25 | 23 |
| *E.media* | 75 | 5 | 5 | 28 | 26 | 26 | 36 |
| *E.perforans* | 87 | 9 | 20 | 46 | 32 | 34 | 33 |
| *E.piriformis* | 6 | 0 | 1 | 0 | 1 | 0 | 0 |
| *E.stiedai* | 0 | 0 | 0 | 4 | 1 | 0 | 1 |

(1) Results from a previous study in healthy rabbits (Peeters et al., 1981).

Incorporation of 66 ppm robenidine in the feed of rabbits inoculated with the unexposed W16 strain prevented the clinical manifestations of the parasite almost completely. Only feed conversion was somewhat higher than in non-inoculated control rabbits. Total oocyst output was reduced by 92 to 98 %. In experiment 4, the influence of the drug on reduction of clinical signs was less clear, as simultaneous rotavirus infection affected the animals. Medication of rabbits infected with each of the four exposed field strains of *E. magna* on the contrary was not able to prevent clinical signs or to reduce total oocyst output (Table 2).

## Discussion

In 1979 we examined healthy looking commercial rabbits on coccidiosis (Peeters et al., 1981). As was common practice before 1982, they received a pelleted ration with 82.5 ppm sulphaquinoxaline/pyrimethamine as anticoccidial drug. The study revealed the omnipresence of eimeria despite the presence of the drug : 85 % of samples contained more than $10^2$ o.p.g., of which 41 % with more than $10^4$ o.p.g. All 9 species were found. *E.magna, E.media* and *E.perforans* were present in 62 % to 87 % of samples, whereas the dangerous species *E.flavescens* and *E.intestinalis*, which cause high mortality, were established in 26 and 22 % of samples respectively. Similar results were obtained in Britain (Catchpole and Norton, 1979) and in France (Zundel et al., 1980).

The incorporation of robenidine as anticoccidial drug in rabbit feeds since 1982 caused a spectacular modification of the coccidiosis pattern in commercial rabbits. Most of the species disappeared completely and only very low levels of coccidia were detected. Although the coccidial infection ratio has risen again since 1983, still low numbers of coccidia are being detected as compared to 1979 (Table 1). The benificial effect of robenidine in the field confirms previous laboratory studies (Coudert, 1978, Peeters et al., 1979).

Although most species, especially the killer species *E.flavescens* and *E.intestinalis* are still relatively rare in commercial rabbits after 4 years of continuous use of robenidine, the increased occurrence of *E.magna, E.media* and *E.perforans* was striking. Especially the rise of *E.magna* is of particular concern as this species, although not a killer type, may cause considerable economic losses by growth retardation and bad feed conversion. In 1987 the species was present in 23 % of faecal samples of diarrhoeic rabbits. The increasing coccidiosis problems by *E.magna* in the field are reflected by the increasing use of sulphonamides in the drinking water at weaning since 1985.

The results with the unexposed control strain W16 indicate that 66 ppm robenidine was effective against *E.magna* when the drug was first introduced in rabbits in 1982. The results with the four exposed field strains of *E.magna* on the contrary show that medication was not able to prevent clinical signs or to reduce total oocyst output. It is therefore concluded that resistance to robenidine has been acquired by some *E.magna* strains, since the introduction of robenidine.

Table 2. Influence of 66 ppm robenidine on weight gain, feed intake, feed conversion, clinical signs and oocyst output of rabbits infected with 50,000 sporulated oocysts of field strains of *E. magna*

| Exp. | Strain | Medi-ca-tion | Daily weight gain (d 0 - 7) | Daily feed intake (d 0 - 7) | FCR | Mean diarrhoea score x 10 (d 5 - 10) | Morta-lity (d 0 - 21) | Mean total oocyst output x 10 (d 7 - 12) |
|---|---|---|---|---|---|---|---|---|
| 1 | - | No | 39 ± 6a(1) | 91 ± 19a | 2.33 | 0 | 0/8 | < 1a |
| | W16 (2) | No | 16 ± 6 b | 71 ± 12b | 4.44 | 12.1 | 0/10 | 95 ± 64b |
| | W16 | Yes | 35 ± 11a | 92 ± 25a | 2.63 | 2.2 | 0/10 | 2 ± 3c |
| | U84/314 (3) | No | 18 ± 5b | 64 ± 9b | 3.56 | 14.2 | 1/10 | 109 ± 59b |
| | U84/314 | Yes | 6 ± 15c | 53 ± 29b | 8.83 | 15.4 | 1/10 | 107 ± 61b |
| 2 | - | No | 37 ± 11a | 90 ± 20a | 2.43 | 0 | 0/8 | < 0.05a |
| | W16 | No | 14 ± 10b | 62 ± 14b | 4.43 | 9.5 | 1/10 | 52 ± 39b |
| | W16 | Yes | 35 ± 10a | 98 ± 15a | 2.80 | 1.0 | 0/10 | 4 ± 6c |
| | U84/284 (3) | No | 19 ± 6 b | 73 ± 18b | 3.84 | 10.3 | 1/10 | 77 ± 51b |
| | U84/284 | Yes | 10 ± 10b | 54 ± 15b | 5.40 | 9.4 | 3/10 | 92 ± 56b |
| 3 | - | No | 39 ± 10a | 89 ± 17a | 2.28 | 0 | 0/8 | < 0.05a |
| | W16 | No | 14 ± 10b | 72 ± 11b | 5.14 | 11.1 | 0/10 | 123 ± 85b |
| | W16 | Yes | 39 ± 11a | 93 ± 16a | 2.38 | 1.2 | 0/10 | 3 ± 4 c |
| | U85/118 (3) | No | 20 ± 9 b | 68 ± 11b | 3.40 | 10.5 | 0/10 | 118 ± 60b |
| | U85/118 | Yes | 18 ± 12b | 71 ± 18b | 3.94 | 7.9 | 1/10 | 106 ± 62b |
| 4 | - | No | 31 ± 11 (4) | 87 ± 17 (4) | 2.82(4) | 2.0 (4) | 0/8 | < 0.05a |
| | W16 | No | 19 ± 10 | 79 ± 10 | 4.14 | 5.5 | 0/10 | 66 ± 60b |
| | W16 | Yes | 24 ± 11 | 76 ± 13 | 3.20 | 6.4 | 0/10 | 4 ± 4c |
| | U86/445 (3) | No | 17 ± 10 | - (5) | - (5) | 6.8 | 0/10 | 100 ± 56b |
| | U86/445 | Yes | 20 ± 13 | 72 ± 10 | 3.60 | 8.8 | 0/10 | 92 ± 57b |

(1) means with different superscripts are significantly different (P<0.05); (2) unexposed strain; (3) exposed strains; (4) results were influenced negatively by simultaneous rotavirus infection; (5) high wastage of feeds

Similar observations have been carried out in chickens infected with eimeria. According to Chapman (1978) resistance against robenidine may develop as early as after 6 passages in chickens. In rabbits, resistance of *E.magna* against robenidine was already observed after 18 months of continuous use. It is remarkable that after five years of medication of commercial rabbits, the very pathogenic species *E.intestinalis* and *E.flavescens* are still almost absent from commercial units, despite the constant presence of the species in domestic rabbitries. Previous results showed that *E.magna* was the most difficult intestinal species to control , as far as reduction of oocyst output is concerned (Peeters and others, 1983). It is not surprising,

therefore, to see that resistance developed first in this species. As complete resistance against robenidine may develop quickly, further monitoring of rabbit coccidiosis in the field is necessary in order to detect resistance in other species.

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**Summary**

Caecal samples were collected from 1,489 diarrhoeic rabbits issued from 61 commercial rabbitries. They were screened for coccidiosis. In 1982, the year of introduction of the anticoccidial robenidine in commercial rabbit feeds, a dramatic decrease of coccidial infection ratio was detected in commercial rabbitries, as compared to the 1979 figures. Only *Eimeria magna, E.media* and *E.perforans* were detected, whereas the highly pathogenic species *E.flavescens* and *E.intestinalis* had disappeared from commercial units. After 5 years of continuous use of robenidine, infection ratio rose progressively, though still far below the 1979 levels. Most of the species reappeared, but only in very low proportions (1 - 4 % of samples). The percentage occurrence of *E.magna, E.media* and *E.perforans* on the contrary rose progressively to 23, 36 and 33 % resp.in 1987, suggesting drug resistance. Four strains of *E. magna* have been cultured from four different rabbitries in which losses by coccidiosis occurred, despite the presence of robenidine in the feed. Experimental infection experiments confirmed that the four strains were completely resistant to robenidine, explaining the field outbreaks of *E. magna* induced coccidiosis in the presence of robenidine.

**Résumé**

Un total de 1489 matières caecales a été recolté chez des lapereaux présentant de la diarrhée et provenant de 61 élevages intensifs. L'examen parasitaire des échantillons de 1982, l'année de l'introduction de la robénidine en élevage intensif, a démontré une chute considérable des taux d'infection coccidienne en comparaison avec les résultats de 1979. Seulement *Eimeria magna, E. media* et *E. perforans* ont été détectés, alors que les espèces hautement pathogènes comme *E. flavescens* et *E. intestinalis* étaient complètement absentes. Après 5 ans d'utilisation de la robénidine en continu, les taux d'infection ont augmenté progressivement, mais restaient nettement inférieurs aux taux de 1979. La plupart des espèces sont réapparues, mais en proportions très faibles (1 à 4 % des échantillons). La fréquence de *E. magna, E. media* et *E. perforans* au contraire a progressé en continu et a atteint un taux respectivement de 23, 36 et 33 % en 1987, ce qui suggère l'apparition d'une résistance. Quatre souches d'*E. magna* ont été isolées en culture pure. Ces souches provenaient de quatre élevages différents, dans lesquels des pertes par coccidiose étaient présents, malgré la présence de robénidine dans l'aliment. Des infections expérimentales ont confirmé que ces 4 souches étaient complètement résistantes à la robénidine, ce qui explique certains problèmes de coccidiose à *E. magna* sur le terrain en présence de la robénidine.

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## EPIDEMIOLOGIE DE LA COCCIDIOSE EN ELEVAGE INTENSIF (1982-1987) ET RESISTANCE A LA ROBENIDINE

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