# FREQUENCY OF THE CARRIAGE AND ENUMERATION OF ESCHERICHIA COLI IN CAECAL CONTENT OF 15 TO 49-DAY-OLD RABBITS

## PADILHA M.T.S.<sup>1,2</sup>, LICOIS D.<sup>1</sup>, COUDERT P.<sup>1</sup>

<sup>1</sup> I.N.R.A., Laboratoire de Pathologie du Lapin, Station de Pathologie Aviaire et de Parasitologie, 37380 Nouzilly, France. <sup>2</sup> Université Fédérale Santa Catarina, Brazil.

**Abstract** - The frequency of the carriage and the enumeration of *Escherichia coli* was determined in caecal content of 15- to 49-day-old, SPF rabbits. Results, expressed as the percentage of rabbits in the different class intervals of the log of *E. coli* populations, showed a high proportion of rabbits with no detectable *E. coli* (<10<sup>2</sup> *E. coli*/g, which is the treshold of the method used): 25% on day 15, 12% on day 22 and 30% later. Consequently the distribution of animals in the different classes is statically not normal. The mean level of *E. coli* population in rabbits harbouring colibacilli were at least of 10<sup>7</sup> bact/g, on days 15 and 22 and then decreased to  $10^4$ - $10^5$  bact/g, on days 42-49. As a result, this makes the interpretation of a general mean including the first class interval (< $10^2 E. coli/g$ ), very difficult. Moreover, the distribution of animals does not allow variance analyses if this class interval is included. This means that, in the rabbit, it would be necessary to dissociate the two parameters which are the frequency of animals without detectable *E. coli* and the enumeration of colibacilli in animals harbouring this bacteria.

#### INTRODUCTION

It is well known that among mammals, the healthy weaned rabbit harbour a particular caecal microflora. This flora is dominated by strict anaerobes, mainly *Bacteroides* sp. The facultative anaerobic flora is mainly represented by streptococci while lactobacilli are usually never found. The colibacilli flora is present at a low level (SMITH, 1965a, b; GOUET and FONTY, 1973, 1979). Moreover, *Escherichia coli* are often not recovered from the caecal content of the weaned rabbit. Few studies have taken this characteristic into consideration (MORISSE *et al.*, 1990) and the mean numeration of colibacilli flora in healthy rabbit was reported at  $10^2$ - $10^3$  bacteria/g of caecal content (SMITH and CRABB, 1965; MORISSE *et al.*, 1985) or  $10^3$ - $10^4$  (LICOIS *et al.*, 1992). The aim of this paper was to determine more precisely the frequency of animals in which no *E. coli* is detectable and the level of *E. coli* in rabbits harbouring this enterobacteria, between 15 and 49 days of age.

#### MATERIAL AND METHODS

The study presented herein is a compilation of data obtained from different experiments fully described by PADILHA (1995). Only results dealing with *E. coli* will be reported. The experiments retained were those in which only Specific Pathogen Free (SPF) rabbits were used and for which no experimental factor was studied. Animals, housing and feeding were described in details by PADILHA (1995). Briefly, animals (INRA strain A1077) were free of known pathogens including enteropathogenic strains of *E. coli* (COUDERT *et al.*, 1988). They were fed with a complete peletted feed without any antibiotic. Experimental rooms and materials were disinfected by steam at 120°C followed by formol vapor and this procedure was carried out twice before each experiment. In all the experiments, the rabbits were euthanatized at 15, 22, 29, 35, 42 and 49 days of age for the study of the microflora from the caecal content. The numbers of animals used (depending on the age), are indicated on figure 1. *E. coli* counts were performed after ten fold dilutions (wt/vol) of caecal content plated onto Drigalski agar. Results were expressed as the percentage of rabbits in the different class intervals of the log of *E. coli* populations. Intervall class <2 corresponds to a number of *E. coli* inferior to  $10^2$  *E. coli*/g of caecal content and is referred as « not detected » in the text, which is the treshold of the method used. Data were treated by variance analysis and Tukey's test for comparison of the means.





### **RESULTS AND DISCUSSION**

No clinical sign of disease was observed in studied animals. For each day, no significant difference was found between the different experiments analysed in this study. Thus all the results showing the percentage of rabbits in the different class intervals, for each day, are mentioned on Figure 1. On days 15 and 22, the distribution of *E. coli* populations were similar with a few number of animals in classes 2 to 5. From a statistical point of view, this distribution is not normal. The percentage of rabbits with a non detectable *E. coli* flora, at 25% on day 15, significantly decreased to 12% on day 22 (P<0.001). On the other hand, between 15 and 22 days of age, *E. coli* counts in rabbits harbouring colibacilli were at least of  $10^7$  bact/g of caecal content (fig. 2). Later, the mean level of *E. coli* population decreased to  $10^4$ - $10^5$  bact/g on days 42-49.

From day 29 onwards to day 49, the profiles for the different class intervals were similar and the distribution, if we exclude the first class (<2), seemed normal. The percentage of rabbits without detectable *E. coli* did not differ according to the age (30% on average).

Our results show that the level of the colibacilli flora is noticeably higher than that usually described in literature in healthy rabbit. But it could be advisable to know the treshold of the method used by the authors and the frequency of animals not bearing *E.coli*, in their experimental conditions, that is rarely mentioned.

It is not frequent to find such a low level of colibacilli in the caecum as well as a high number of animals not bearing this bacteria among the animal species which have been studied. Some similarities have been noticed in some strains of mice (SCHAEDLER and DUBOS, 1962) or in guinea pig (SEELIGER and WERNER, 1963). DHO and LAFONT (1982) also have observed such a distribution of *E. coli* populations, in the trachea of experimentally infected chickens.

As a result, the high proportion of weaned rabbits with non detectable colibacilli, makes the interpretation of a general mean including the first class interval (<2), very difficult. Moreover, the distribution of animals according to the level of the colibacilli flora, does not allow variance analyses if this class interval is included. This means that, in the rabbit, it would be necessary to dissociate the two parameters which are the frequency of animals without detectable *E coli* and the enumeration of colibacilli in animals harbouring this bacteria.





Acknowledgement - We wish to thank M. BOIVIN, Y. BREUZIN, A. FRANCINEAU, P. BOUVIER and M. DUPUY for their skilful technical assistance.

#### REFERENCES

- COUDERT P., LICOIS D., BESNARD J., 1988. Establishment of a specified pathogen free breeding colony (SPF) without hysterectomy and hand-rearing procedures. In : HOLDAS (ed). Proceedings of the 4th World Rabbit Congress, Budapest, 137-148
- DHO M., LAFONT J.P., 1982. *Escherichia coli* colonization of the trachea in poultry : comparison of virulent and avirulent strains in gnotoxenic chickens. *Avian Diseases*, 26, 787-797.
- GOUET PH., FONTY G., 1973. Evolution of the intestinal microflora of conventional rabbits from birth to weaning. Ann. Biol. anim. Biochim. Biophys. 13, 733-755.
- GOUET PH., FONTY G., 1979. Changes in the digestive microflora of holoxenic rabbits from birth until adulthood. Ann. Biol. anim. Biochim. Biophys. 19, 553-566.
- LICOIS D., GUILLOT J.F., MOULINE C., REYNAUD A., 1992. Susceptibility of the rabbit to an enteropathogenic strain of *Escherichia coli* O103 : effect of animal's age. Ann. Rech. Vét. 23,: 225-232.
- MORISSE J.P., LE GALL G., MAURICE R., COTTE J.P., BOILLETOT E., 1990. Action chez le lapereau d'un mélange de fructo ologo saccharides sur certains paramètres intestinaux et plasmatiques. Sèmes Journées de la Recherche Cunicole, 12-13 Dec, Paris, Comm. Nº 53.

- PADILHA M.T.S., 1995. Etude des relations entre la microflore et l'activité fermentaire caecale chez le lapereau, pendant la période péri-sevrage. Thesis. François Rabelais University. Tours, France.
- SCHAEDLER R.W., DUBOS R.J., 1962. The fecal flora of various strains of mice, its bearing on their susceptibility to endotoxin. J. Exp. Med. 115, 1149-1161.
- SEELIGER H.P.R., WERNER H., 1963. Recherches qualitatives et quantitatives sur la flore intestinale de l'homme. Ann. Inst. Pasteur. 105, 911-936.
- SMITH H.W., 1965a. Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Pathol. Bact. 89, 95-122.
- SMITH H.W., 1965b. The development of the flora of the alimentary tract in young animals. J. Pathol. Bact. 90, 495-513.
- SMITH H.W., CRABB W.E., 1961. The faecal bacterial flora of animals and man. Its development in the young. J. Pathol. Bact. 82, 53-66.

#### La fréquence du portage et le nombre d'Escherichia coli du contenu caecal chez les lapins âgés

de 15 à 49 jours - La fréquence du portage et le nombre d'*Escherichia coli* du contenu caeacal, ont été déterminés, chez des lapins SPF âgés de 15 à 49 jours. Les résultats sont exprimés en poucentage d'animaux présents dans les différentes classes du log du nombre d'*E. coli* mesurés chez ces animaux. Ils montrent une forte fréquence de lapins pour lesquels la flore colibacillaire n'est pas détectable ( $<10^2 E. coli/g$ , qui est le seuil de détection de la méthode utilisée) : 25% et 12% respectivement chez les lapereaux de 15 et 22j et 30% chez les animaux plus âgés. En conséquence, d'un point de vue statistique, la distribution des lapins. dans les différentes classes n'est pas normale. Par ailleurs, le niveau moyen de la flore colibacillaire chez les lapins qui hébergnet des *E. coli* est au moins de 10<sup>7</sup> bact./g à 15 et 22j et régresse à 10<sup>4</sup>-10<sup>5</sup> bact./g chez ceux de 42-49j. L'interprétation d'une moyenne générale qui incluerait les animaux de la 1re classe (<10<sup>2</sup> *E. coli/g*) paraît donc difficile. De plus la distribution des animaux n'autorise pas d'analyses de variance si cette classe est incluse. Par conséquent, il semble nécessaire chez le lapin de dissocier les deux paramètres que sont la fréquence des animaux sans flore colibacillaire détectable et d'autre part la numération des *E. coli* chez les lapins porteurs de cette bactérie.