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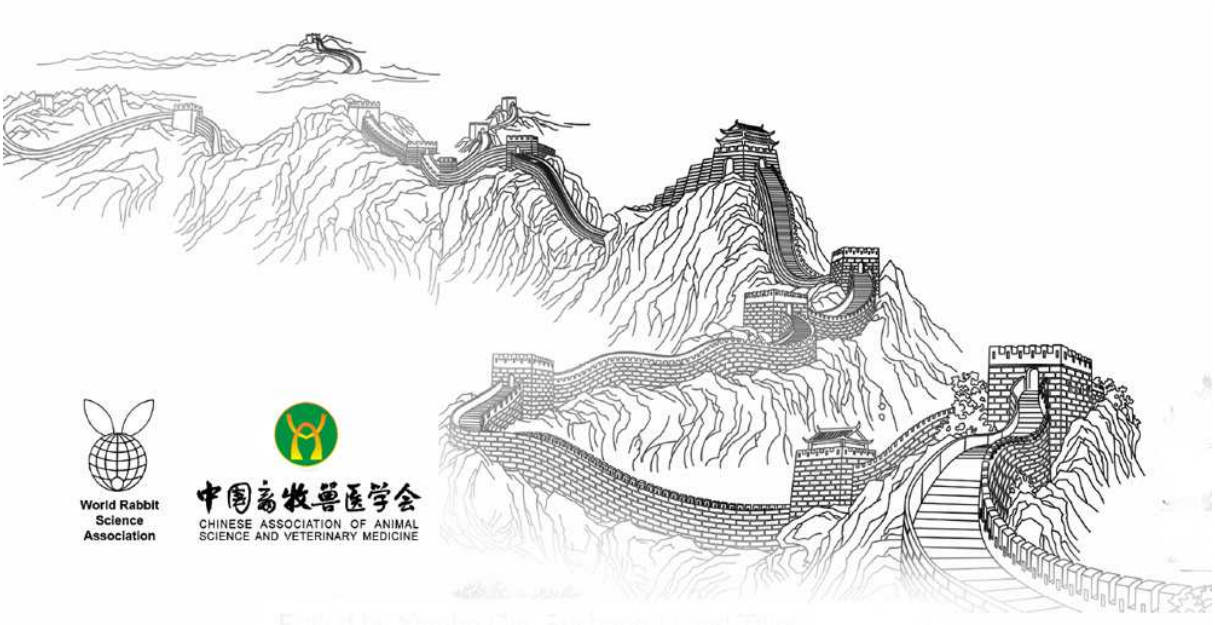

METHODOLOGY OF REPRODUCTION OF COLIBACILLOSIS ON RABBITS: EFFECT OF THE AGE AT INOCULATION AND OF THE STRAIN

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ABSTRACT

Digestive disorders are responsible for significant mortality and morbidity that have a negative impact on rabbit's growth. Some antibiotics are used against these diseases, but other products must be developed to help animals to deal with these disorders. For this purpose, it is necessary to have models to test these products. The aim of this trial is to develop a model to reproduce colibacillosis by studying the effects of the date of inoculation and inoculated *Escherichia coli* O103 strain. This trial does not use specific pathogen free rabbits in order to have representative farm rabbits. Inoculated rabbits near to weaning generated too high mortality. In this trial, a later inoculation of rabbits with the strain A gives interesting results in terms of survival and growth performance. This combination seemed to be a good compromise to experimentally reproduce colibacillosis to test new products as solution against the disease.

Key words: Rabbit, Colibacillosis, *Escherichia coli* O103, Model.

INTRODUCTION

Digestive diseases are among the most important problems encountered in rabbit production and occur mainly in young rabbits after weaning (Marlier et al., 2003). Digestive disorders are responsible for significant mortality and morbidity that have a negative impact on rabbit's growth. Among these diseases, Epizootic Rabbit Enteropathy (ERE), coccidiosis and colibacillosis are very common. Some antibiotics are used against these diseases, but others alternatives should be developed to prevent their emergence. To this aim, it is necessary to have models to test these new products. Regarding ERE and coccidiosis, models are developed (Boisot et al, 2005). The model for colibacillosis was missing. The aim of this trial is to develop a model to reproduce colibacillosis in order to test new products to help animals to deal with this disease. The effects of age at inoculation and of the inoculated *Escherichia coli (E. coli)* strain are studied.

MATERIALS AND METHODS

This protocol was validated by the French Ethical committee n°52, under the project n° 03835.03.

Animals and inoculation

Six hundred and forty non specific pathogen free rabbits were allotted at weaning (36d), according to their live body weight the day before weaning, their sex and their litter origin in six groups. Animals were inoculated with 500µL of one of the two strains of *Escherichia coli O103* tested (strain A or B, respectively 10^6 and 10^7 CFU/mL) and 3 dates of inoculation were studied; "early" (few days after weaning), "middle" and "late" (respectively 2 and 5 days after early inoculation) to investigate the effect of age at inoculation. Six groups were created by the combination of these 2 factors. Feed was distributed ad libitum and did not contain any coccidiostatic or antibiotic.

Model validation
Feces were taken from 2 cages per group, between 6 and 7 days after inoculation, bacterial identification and serotype determination were performed on feces. Necropsies were done on 15 rabbits and a caecal bacterial count was done on these animals.

Health status and growth performances
Mortality was controlled every day and results were given as cumulative mortality at each weighing. Live weight, feed intake and morbidity (presence of health disorders) were controlled at 43, 49, 56, 63 and 70 days of age. Average Daily Gain (ADG) was calculated from live weight data.
End point was defined as followed: animal having a weight loss greater than 45 g/d; animal prostrated, hardly moving, without strength; glassy look, which does not eat anymore; having or having had diarrhea. Animals concerned were euthanized. These rabbits were counted as dead rabbits.

Statistical Analysis
Mortality and morbidity were analyzed by a Chi-square test. Results of live weight, live weight gain and feed intake were analyzed according to a general linear model using ANOVA and testing group effect. Results were significantly different when p value was under 0.05, and means with different letters on the same rows differed significantly.

RESULTS AND DISCUSSION

Model validation
Necropsies confirmed the death is due to colibacillosis. Bacterial counts of *E. coli* in caecum content are between $2.8x10^8$ and $1x10^{10}$ CFU/g, the serotype O103 is also confirmed. In feces, the bacterial count of *E. coli* between 6 and 7 days after inoculation are between $6x10^5$ and $4x10^8$ in inoculated groups, serotype O 103 is also found. It is well known that in rabbits, *E. coli* content is relatively low; less than 10^4 -10^5 CFU/g of caecal contents (Marlier *et al*., 2003; Licois *et* Marlier, 2008). In this study, bacterial counts in caecal contents are higher than values usually observed in rabbits, and excretion of *E. coli* in feces is high. These high levels of *E.coli* O103 observed in this trial allow us to confirm that rabbits died of colibacillosis, validating thus the experimental reproduction of the disease.

Health status

Table 1: cumulative mortality in % according to the strain inoculated and inoculation date

Strain Inoculation date	A "early"	A "middle"	A "old"	B "early"	B "middle"	B "old"	p value
D43	1.1	0.0	0.0	0.0	1.2	0.0	NS
D49	20.0 ^{bc}	15.6 ^{bc}	1.1 ^{a}	21.5 ^{c}	8.6 ^{b}	0.0 ^{a}	<0.001
D56	60.0 ^{c}	37.1 ^{bc}	10.3 ^{a}	43.3 ^{bc}	31.3 ^{b}	10.0 ^{a}	<0.001
D63	62.7 ^{c}	45.5 ^{bc}	12.9 ^{a}	52.4 ^{c}	37.5 ^{bc}	18.9 ^{a}	<0.001
D70	68.4 ^{c}	47.7 ^{bc}	20.0 ^{a}	60.0 ^{c}	37.5 ^{abc}	29.4 ^{ab}	0.0021

Means with different letters on the same rows differ significantly.

At 43 days of age, mortality is low in all groups, including the 2 groups "early" (Table 1). From D49 to D63, some differences appear among groups; mortality is significantly higher in "early" and "middle" inoculated rabbits compared to "late" inoculated rabbits, whatever the strain. This observation is supported by the study of Licois *et al*., 1992, in which they showed a strong effect of the animal's age at inoculation on mortality; mortality for young rabbits is very high compared to rabbits inoculated later. This effect of age on mortality could be explained by the fact that young rabbits have a not totally mature immune system between 4 and 8 weeks of age, as proposed by Combes *et al*., 2013.
At the end of the present trial, mortality varies from 20.0 to 68.4% depending of the group. In this model of experimental reproduction of colibacillosis, it is important not to have a too high mortality, because effects of products are measured on alive rabbits at the end of the experiment. Using non SPF

rabbits allows the use of rabbits similar to those found on farms. In this trial, mortality in the 2 groups "early" appears to be too high (68.4% and 60.0%).
Depending on the groups, at 43 days of age morbidity varies between 56.38% and 1.15% (Table 2); "early" inoculated rabbits are significantly more morbid compared to "middle" and "old" inoculated rabbits, whatever the strain used. At D46, morbidity varies from 47.25 to 0% according to the group. Morbidity in "early" and "middle" inoculated rabbits is significantly higher than in "old" inoculated rabbits. After D49, there is no significant difference between groups.

Table 2: Morbidity in % according to the strain inoculated and inoculation date

Strain / Inoculation date	A "early"	A "middle"	A "old"	B "early"	B "middle"	B "old"	p value
D43	56.4 ^{c}	34.4 ^{b}	2.1 ^{a}	24.0 ^{b}	1.2 ^{a}	1.2 ^{a}	<0.001
D46	47.3 ^{c}	29.8 ^{c}	10.5 ^{b}	38.5 ^{c}	30.2 ^{c}	0.0 ^{a}	<0.001
D49	29.1	19.3	21.3	16.5	17.5	11.5	NS
D56	6.8	18.6	15.1	10.4	16.7	15.2	NS
D63	0.0	0.0	7.1	6.3	1.6	4.1	NS
D70	3.6	3.1	6.3	1.7	1.6	3.0	NS

Means with different letters on the same rows differ significantly.

Growth performances

There is no significant interaction between age and *E. coli* strain on body weight (Table 3). At D43, the age at inoculation had an effect on the body weight of rabbits. The body weight of "early" or "middle" inoculated rabbits is significantly lower than for "old" inoculated rabbits. The strain had an effect on live body weight at D43, D49 and D56; rabbits inoculated with the strain A are significantly lighter than rabbits inoculated with the strain B. At D63 and D70, there is no difference between groups.

Table 3: Live weight and Average Daily Gain according to the strain inoculated and inoculation date

	Strain / Inoculation date	A "early"	A "middle"	A "old"	B "early"	B "middle"	B "old"	p value Strain * Date	p value Date	p value Strain
Live Weight (g)										
D35	Means	942.77	943.64	949.59	947.70	948.59	944.92	NS	NS	NS
	SD	79.79	81.61	73.47	72.72	56.90	74.30			
D43	Means	1215.78 ^{x}	1312.06 ^{x}	1415.73 ^{y}	1349.35 ^{x}	1377.65 ^{x}	1414.49 ^{y}	NS	<0.001	0.005
	SD	83.64	94.61	102.91	99.19	69.24	93.04			
D49	Means	1467.36	1486.91	1553.74	1647.24	1621.55	1721.63	NS	NS	<0.001
	SD	142.03	126.64	128.10	123.67	127.51	105.55			
D56	Means	1824.85	1768.80	1807.56	1995.13	1928.44	1990.74	NS	NS	<0.001
	SD	188.03	163.25	136.12	145.04	154.61	143.26			
D63	Means	2224.63	2137.48	2121.90	2295.33	2273.89	2229.53	NS	NS	NS
	SD	202.96	153.32	147.80	181.61	149.46	191.73			
D70	Means	2555.81	2434.18	2398.08	2588.83	2538.65	2503.97	NS	NS	NS
	SD	192.03	162.17	186.04	158.01	126.25	213.97			
Average Daily Gain (g/d)										
35-43d	Means	34.13 ^{a}	46.05 ^{b}	58.26 ^{d}	50.20 ^{bc}	53.64 ^{cd}	58.70 ^{d}	<0.001		
	SD	8.87	5.27	4.22	7.36	3.40	3.12			
43-49d	Means	41.93 ^{bc}	29.14 ^{ab}	23.01 ^{a}	49.65 ^{c}	40.65 ^{bc}	51.20 ^{c}	0.007		
	SD	14.16	12.98	7.06	10.71	13.42	5.53			
49-56d	Means	51.07 ^{x}	40.27 ^{y}	36.28 ^{y}	49.71 ^{x}	43.85 ^{y}	38.44 ^{y}	NS	<0.001	NS
	SD	9.47	10.09	10.48	10.07	7.34	9.67			
56-63d	Means	57.11 ^{x}	52.67 ^{x}	44.91 ^{y}	42.90 ^{x}	49.35 ^{x}	34.10 ^{y}	NS	<0.001	<0.001
	SD	5.52	6.92	9.91	10.48	3.87	10.96			
63-70d	Means	47.32	42.39	39.47	41.94	37.83	39.20	NS	NS	NS
	SD	8.50	8.95	7.92	10.56	9.59	9.73			
35-70d	Means	46.08	42.58	41.39	46.89	45.45	44.55	NS	NS	NS
	SD	4.86	3.52	4.44	4.05	2.75	4.59			

Means with different letters on the same rows differ significantly; a, b, c and d are for the interaction age*strain; and x and y for the effect of age at inoculation.

Regarding the ADG between 35 and 43 days, and between 43 and 49 days, there is an interaction between the date of inoculation and the strain (Table 3). ADG35-43d is significantly lower in the

group Strain A "early" compared to the 2 "old" groups. ADG of the group Strain A "middle" is significantly lower than ADG of group "old" and Strain B "middle" but higher than rabbits from group Strain A "early". ADG43-49d is significantly lower in the group Strain A "old" compared to groups "early" and strain B "middle" and "old". After these periods, there is no interaction between date of inoculation and strain. From 49 to 56 days, an effect of the date of inoculation is observed; ADG of "early" inoculated rabbits is significantly higher than that of "middle" and "old" inoculated rabbits. From 56 to 63 days, ADG is significantly improved for "early" and "middle" inoculated rabbits compared to "old" inoculated rabbits. For this period, an effect of the strain appears; ADG is higher for rabbits inoculated with the strain A. There is no significant difference between groups for ADG from 63 to 70 and from 36 to 70 days of age.

Feed intake

Table 4: Feed intake (g/d/rabbit) according to the strain inoculated and inoculation date

Strain		A	A	A	B	B	B	**p value**	**p value**	**p value**
Inoculation date		"early"	"middle"	"old"	"early"	"middle"	"old"	**Strain * Date**	**Date**	**Strain**
Feed intake (g/d/rabbit)										
35-43d	Means	73.75 ^{a}	96.29 ^{bc}	108.89 ^{d}	91.03 ^{b}	102.53 ^{cd}	107.27 ^{cd}	0.003		
	SD	9.40	8.20	7.00	11.70	6.70	7.70			
43-49d	Means	75.44 ^{x}	74.03 ^{x}	100.05 ^{y}	96.81 ^{x}	101.80 ^{x}	133.29 ^{y}	NS	<0.001	<0.001
	SD	25.94	18.55	11.53	30.22	22.25	10.22			
49-56d	Means	122.69	109.34	107.92	126.92	115.35	122.91	NS	NS	NS
	SD	21.26	17.67	16.17	29.07	21.07	16.10			
56-63d	Means	170.77 ^{x}	157.00 ^{x}	138.39 ^{y}	152.90 ^{x}	157.64 ^{x}	127.68 ^{y}	NS	<0.001	0.041
	SD	18.42	14.15	18.91	27.22	9.176	18.58			
63-70d	Means	179.45 ^{x}	165.65 ^{x}	152.60 ^{y}	165.73 ^{x}	167.27 ^{x}	151.01 ^{y}	NS	0.001	NS
	SD	14.99	13.92	22.5	15.41	17.51	24.07			
35-70d	Means	124.37	121.10	121.82	126.51	128.94	127.69	NS	NS	NS
	SD	13.04	8.74	11.36	15.82	8.37	11.21			

Means with different letters on the same rows differ significantly; a, b, c and d are for the interaction; x and y for the effect of age at inoculation.

Regarding feed intake from 35 to 43 days of age, there is an interaction between the date of inoculation and the Strain used (Table 4). Animals in groups "old" eat significantly more than animals in groups "early". From 43 to 49 days, an effect of the date of inoculation is observed; "old" inoculated rabbits eat more than 'early" or "middle" inoculated rabbits; the same observation is made for 56-63d and 63-70d. The effect of the strain is observed for periods 43-49d and 56-63d; the strain B allows a higher feed consumption. On the whole period, there is no effect on feed intake. Feed conversion ratio is not calculated, because in these conditions it is too variable to interpret it.

CONCLUSION

In conclusion, this trial demonstrates the effect of the date of inoculation on mortality and growth performance, as previously described by Licois et al, 1992. Rabbits that are inoculated with *E.coli* near to weaning show lower survival but a greater growth of the survivors. Strain B seems to be less pathogen on rabbits inoculated "early" or "middle". In this trial, the "middle" and "old" inoculation of rabbits enables to achieve a mortality level which can permits to run trials that aim at reducing mortality due to colibacillosis. Thereby, the "middle" or "old" inoculation of rabbits with the strain A seemed to be a good compromise to meet our aim. The effect of the weaning weight could also have an effect on mortality, it could be interesting to study this.

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REFERENCES

Boisot, P., Duperray, J., Guyonvarch, A., Licois, D., Coudert, P. (2005). . Méthodologie de reproduction de l'Entéropathie Epizootique du Lapin (EEL) : contamination par contact direct ou indirect avec un ou plusieurs lapins inoculés avec l'inoculum TEC 2. In: *Journées de la recherche cunicole* (p. 257-260). Presented at 11. Journées de la Recherche Cunicole, Paris, FRA (2005-11-29 - 2005-11-30). Paris, FRA : ITAVI - Institut Technique de l'Aviculture.

Combes, S., Gidenne, T., Bouchet, S., Fortun-Lamothe, L., Bolet, G., Coureaud, G., Coureaud, G. (2013). . Lapereaux de la naissance au sevrage : Quels outils pour des lapereaux plus robustes ?. In: 15èmes Journées de la Recherche Cunicole (p. 63-77). Presented at 15. Journées de la Recherche Cunicole, Le Mans, FRA (2013-11-19 - 2013-11-20).

Licois D., Guillot JF., Mouline C., Reynaud A. Susceptibility of the rabbit to an enteropathogenic strain of *Escherichia coli* 0103: effect of animals' age. *Annales de Recherches Vétérinaires, 1992, 23 (3), pp.225-232*

Licois, D., Marlier, D. (2008). Pathologies infectieuses du lapin en élevage rationnel. *Prod Animales, 21* (3), 257-268.

Marlier, D., Dewrée, R., Delleur, V., Licois, D., Lassence, C., Poulipoulis, A., Vindevogel, H. (2003). . Description des principales étiologies des maladies digestives chez le lapin européen (Oryctolagus cuniculus). *Annales de Médecine Vétérinaire, 147, 385-392.*

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