

PASTEURELLA EPIDEMIOLOGY :  
EFFECT OF THE AGE OF WEANLING RABBITS

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INTRODUCTION

Systematic necroscopic research carried out on female breeders has shown that more than 50 % of them are affected by Pasteurella otitis even when there are no obvious symptoms (1 and 2), in practice the frequency would appear to be even higher (2). Since the does were often infected, we sought to find out at what age young rabbits become Pasteurella carriers. The aim was to precise when and how the rabbits spread infection, with a view to establishing prophylactic methods of eradication.

MATERIALS AND METHODS

A series of 4 experiments are recorded here. They were all carried out in the same building, equipped with adjoining cages. The diet was free from any antibiotic.

The strain of Pasteurella used was a Pasteurella multocida A5 strain. This was the reference strain (005 Ref) of our laboratory and was identified by its cultural and biochemical characteristics.

Experiment N°1

Search for spontaneous contamination before weaning

On a conventional rabbit farm, but one that was free from respiratory infections, (3 to 5 % of females were killed each year because of coryza suppurated) a total of 102 young rabbits, the offspring of 30 females, were selected at the age of 15, 21 and 28 days (2 to 4 rabbits per litter). The doses were checked for Pasteurella, either by nasal swabs or by other various samples, after being killed. Pasteurella was looked for the young rabbits by swabbing the oropharynx and the sinuses once the animals had been killed.

Experiment N°2

Search for spontaneous contamination before weaning

The post-mortem swabs were much more reliable than the swabs taken in vivo, but the results were unquantifiable. We therefore repeated the previous experiment and looked for Pasteurella after removing and grinding the oropharynx. These trials were carried out on fifty-four 28 day old rabbits, the offspring of 18 females (2 to 3 rabbits per litter). The bacteriological technique enabled us to detect 100 germs (Pasteurella multocida) per gram per organ.

Experiment N°3

Experimental contamination in the oropharynx

Weanlings from SPF females were inoculated by a culture broth onto the oropharynx. This culture contained 10 to 1000 Pasteurella, according to the groups (0.1 ml per animal) ; 18 groups of 4 or 5, 28 to 42 day old rabbits

were used (63 were inoculated and 15 served as controls). On the day of inoculation, they were placed in a building where the atmosphere contained 30 to 50 ppm ammonia. They were killed 1 to 9 days later, according to the group. Swabs were taken from the nasal cavities and the oropharynx after aseptic dissection of 9 animals on days 1, 2, 3, 6 and 9.

#### Experiment N°4

##### Experimental contamination in the nasal cavities

The same procedure as previously used was repeated on 40, 28 day old animals, the inoculum ( $10^3$  or  $10^4$  of Pasteurella according to the groups) was injected into to each nostril by means of a microsyringe. The search for Pasteurella was carried out by removing and grinding the entire nasal cavity, and by classical techniques on the other organs. Three animals were killed each day for necroscopic and bacteriological examination.

#### RESULTS

Experiments N° 1 and 2 : Table 1

TABLE 1 : SEARCH FOR PASTEURELLA

Origin of the rabbits	In the does		In the young rabbits after autopsy		
	Nasal swabs living does	Nasal swabs post-mortem	Nasal cavities	Oropharynx	Total
20 coryza free does	14 +	ND	0/40	0/40	0/80
	6 -	6 +	0/40	0/40	
18 coryza free does	8 +	ND	ND	0/22*	0/54
	10 -	ND	ND	0/32*	
10 does with coryza	10 +	ND	2+/22	3+/22	3/22

ND : not done

\* Quantitative analysis of ground oropharynx (experiment N° 2)

All the young rabbits born to coryza free females were uninfected with regard to the organs sampled, even when the does were Pasteurella carriers. Only 3 out of 22 rabbits from does with coryza (coryza which was, however, never suppurated) proved positive (two came from the same doe).

#### Experiment N°3

After oral contamination, none of the animals were found to be Pasteurella carriers. It can be presumed that the inoculum was swallowed before implantation took place. Nevertheless, the strength of ammonia caused a catarrh coryza in some young rabbits from the 2<sup>rd</sup> day.

#### Experiment N°4

On the other hand, the nasal inoculation provoked a 100 % death rate within 3 days. The importance of this phenomenon was only modified by 24 hours with the strongest inoculum. Lesions were seen to appear quickly in the respiratory system, and also in the middle ear. The controls did not display any macroscopic lesions, which proves that ammonia did not play a major role with regard to lesions. No animal showed any signs of coryza.

TABLE 2 : MORTALITY AND SYMPTOMS AFTER NASAL INOCULATION OF  $10^3$  OR  $10^4$  PASTEURRELLA

Day	MORTALITY		AUTOPSY				Animals killed (No = 9)			Controls
	Inoculum		Dead animals (No = 22)							
	$10^3$	$10^4$	Tracheitis	Pneumonia	Otitis	Coryza	Tracheitis	Pneumonia	Coryza	no lesion
1	1/20 (+3)*	4/15	5/5	5/5	0/5	0	0/3	0/3	0	5/5
2	2/16 (+3)*	6/11	5/5	5/5	2/5	0	3/3	3/3	0	5/5
3	8/11 (+3)*	5/5	12/12	12/12	7/12	0	3/3	1/3	0	5/5

(+3)\* three animals were killed each day for autopsie

From a bacteriological point of view, all the organs sampled were positive : nasal cavities (25/25), oropharynx (25/25), lungs (14/14), brain (12/13). Only 2 out of the 9 rabbits killed were negative ; all the control animals were negative. The amount of germs contained in the nasal cavities (3.5 g) was from  $7.10^2$  to  $5.10^5$  in the animals killed, and from 2 to  $35.10^6$  in the animals which died. The day of examination had no bearing on the results.

#### DISCUSSION

Experiments 1 and 2 show that it is not easy to infect young rabbits with Pasteurella and that contamination is slow ; these experiments confirm our previous results (3).

This epidemiological survey, alongside other research of the same type not reported here, leads us to conclude that, when the general hygiene conditions are satisfying, it is exceptional for young rabbits to be contaminated before the third week, and at least not by the Pasteurella strains which are spontaneously rampant in the rabbit farms from which the young rabbits were taken.

Even the experimental contamination (experiment N°3) with a very virulent strain administered orally, did not give the results that could be expected according to various authors (4, 5, 6, 7). In fact, they found that young rabbits could be contaminated at a very early age when the dam produced a nasal secretion, and that the initial point of infection was the oropharynx. They considered that the drinking pipette was the privileged agent of contamination. Moreover, the fact that the control animals remained unaffected confirms that transmission through the atmosphere is not easy (8). When the animals were inoculated intranasally (experiment N°4), the rapidity of mortality and the lesions observed are in contrast with the previous results. Septicaemia is the only explanation, and the examinations carried out on the brain would confirm this. The atmosphere rich in ammonia certainly favoured the implantation of Pasteurella. MORISSE has already described this technique (9), although he did not produce mortality with an inoculum nevertheless 100 times higher, but with a different strain. We therefore need to verify up to what doses these results can be reproduced with other strains and also to what extent ammonia is necessary with the more virulent strains. With regard to the epidemiological research undertaken, this trial shows that it is possible to detect contaminations of at least  $10^3$  germs in the young rabbit. Complementary experiments are underway to estimate the minimum threshold of detection.

REFERENCES

- 1 M. BALENCON, P. RIDEAUD, P. COUDERT, 1982. Etude bactériologique des otites et torticolis chez les reproductrices réformées. 3ème Journées de la Recherche Cunicole. Paris comm. N° 29.
- 2 P. COUDERT, P. RIDEAUD, M. BALENCON, 1986. Pasteurellose non respiratoire en élevage intensif : l'otite moyenne des lapines reproductrices. Cuni-Science, Vol 3, Fasc 2, 1-6.
- 3 P. COUDERT, D. LICOIS, J. BESNARD, 1988. Establishment of a specified pathogen free rabbit breeding colony without hysterectomy and hand-rearing procedures. 4th Congress of the World Rabbit Science Association, Budapest, october 1988, vol. 2, 137-148.
- 4 H.T. HOLMES, N.M. PATTON, P.R. CHEEKE, 1983. Pasteurella contaminated watering valves : its incidence and implications. J. Appl. Res. 6 : 123-124.
- 5 H.T. HOLMES, N.M. PATTON, P.R. CHEEKE, 1984. The occurrence of Pasteurella multocida in newborn and weanling rabbits. J. Appl. Res. 7 : 17-20.
- 6 H.T. HOLMES, 1989. Studies on selected topics of Pasteurella multocida infection in laboratory rabbits. Ph. D. Thesis Oregon State University.
- 7 N.M. PATTON, H.T. HOLMES, P.R. CHEEKE, 1984. Respiratory pasteurellosis : incidence in young rabbits and mechanisms of transmission. 3rd Congress of W.R.S.A. Rome.
- 8 R.F. DIGIACOMO, C.R.D. JONES, C.M. WATHES, 1987. Transmission of Pasteurella multocida in rabbits. Lab. Anim. Sci., 37 : 621-623.
- 9 J.P. MORISSE, 1978. Infection pulmonaire expérimentale Pasteurella multocida : influence d'un facteur irritant (NH<sub>3</sub>) sur la réceptivité du lapin. Rec. Med. Vet. 154 (10) 859-863.

ABSTRACT

In order to find out when young rabbits become infected by Pasteurella, two sets of experiments were carried out. In the first one, we tested 15 to 28 day old rabbits born of Pasteurella females showing no symptoms : all of them were free from Pasteurella. In the second set, naive young rabbits were infected with 10<sup>3</sup> or 10<sup>4</sup> Pasteurella multocida, either in the oropharynx or in the nasal cavities. Only the second route caused infection and a 100 % death rate within 3 days, without any signs of respiratory disease. These experiments show that it is not easy to infect weanling rabbits with Pasteurella, and that contamination is slow.