Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 1389-1400

A COMPARATIVE STUDY OF THE VIRULENCE OF *Pasteurella multocida* FROM RABBITS (*O. cuniculus*)

P. RIDEAUD*, P. COUDERT**, P. MERCIER* and P. HERVOUET***

* INRA - Domaine du Magneraud - 17700 SURGERES
** INRA - Unité de pathologie du lapin - 37380 MONNAIE
*** BIOVAC - Lisses CE4517 91045 EVRY CEDEX

INTRODUCTION

Respiratory disease is presently the most important, specific disease in commercial rabbitry. The economic importance is linked with the frequency and the severity of this disease (1, 2, 3), and with the fact that it affects mainly maternities and particularly during the last week of pregnancy. period (4). The almost systematic occurence of chronic infections in the middle ear (5, 6, 7) worsens the situation and broadly explains the inefficacy of antibiotic treatments reported by most authors (8, 9, 10, 11). *Pasteurella multocida (P. multocida)* is the major aetiological agent.
All rabbit farms are contaminated by *P. multocida* without there being any apparent pathology. In order to make a diagnosis, a prognosis and a reasoned epidemiological study, it was necessary to better define the pathogenesis of *P. multocida*. Several works concern the relative frequency of the different capsular and somatic serotypes and certain authors have tried to establish a connexion between the serotype and the severity of the disease on rabbit farms. Their findings are contradictory and therefore no conclusion can be drawn (12,13,14,15). On the other hand, many of these investigations were carried out in laboratory rabbits flocks, where the epidemiological conditions and the frequency of *Pasteurella* infections are quite different from those to be found on commercial flocks (16,17,18,19).
Little experimental work has been published on the pathogenic influence of *P.multocida*, and few if any concern the comparison of strains. In the present study, we compared the pathogenic effects of 17 strains of *P. multocida* and looked for a simple experimental method to predict the pathogenic effects.

MATERIALS AND METHODS

* Animals and Buildings - Conditions

Three hundred and ninety-five 28 day old New-Zealand rabbits were used (INRA strain 1077). These young rabbits came from our experimental farm (Le Magneraud) and the breeding conditions have already been described (26). These rabbits were born from clinically healthy does (coryza free) and were housed in 1 m2 cages. The volume of the building was 120 m3 and a temperature of between 15°C and 20°C was maintained. The animals were fed *ad libitum* (see Table 1). After each trial the building was washed and fumigated with formaldehyde and all of the water circuits were chlorinated.

* Experimental design

In order to test the 17 strains of *P. multocida*, ten consecutive trials were carried out (figure 1).

FIGURE 1 : Experimental design for one trial

Inoculum	Strain of *P. multocida***						Control
	S1	S2	S3	S4	S4	Ref5***	uninoculated
dose 1 : 10^6CFU	5*	5	5	5	5	-	-
dose 2 : $\neq 10^6$CFU	5	5	5	5	5	5	-
No inoculum	-	-	-	-	-	-	5

CFU = colony forming units. * Number of animals. ** The number of strains varied from 3 to 5 per trial. The same strain could be tested several times in succession at differing doses. *** Our reference strain was strain 005 (21). An inoculum of 10^3 CFU was the minimum inoculum which systematically brought about 100 % mortality within 16 hours.

*** Strains of *P. multocida***

The species *multocida* of the *Pasteurella* genus was identified according to its physiological and biochemical characteristics (22, 23). The anatomical and geographical origins of the samples are to be found in Table 2. The isolates from rabbits are capsular type A. (24, 25, 26). Amongst the 32 strains kept in our laboratory, (the bacteria were stored at -70°C in 1 ml of brain heart infusion containing 15 % glycerol) 17 were selected after being cloned according to two criteria :

- they were representative of the somatic types found in France (27,28)
- the range of the diameter of the colonies after 24 hours incubation was as wide as possible.This last criterion was observed by the following method :
  - numeration of the bacteria by several dilution and plating of aliquots onto serum agar plates (19 ml tryptose Difco and 1 ml horse serum).
  - twenty-four hours incubation at 37°C. When approximately 10 colonies were detectable that were measured with a steroscopic microscope (magnification 9 x accuracy $\pm$ 0,125 mm). These measurements were taken on two occasions for 20 colonies (40 measurements per strain).
  - similar measurements were also performed after 13, 15, 17, 29, 21 and 23 hours of incubation to measure the rate of growth of the colonies for each strain.

*** Inoculation**

These strains were cultivated for 6 hours at 37°C in tryptose broth Difco supplemented with 5% hors serum. Inocula of 107 to 102 CFU were prepared from dilutions in physiological saline.
The animals were infected by intramuscular injection of 0,1 ml of bacterial suspension in the back.

*** Autopsy and bacteriological procedures**

The animals were observed each day in order to note any clinical signs. The survivors were killed on the 6th day, and an autopsy was carried out on all animals. We looked for, and characterised, *P. multocida* in any organs with lesions. The characteristics (cultural, biochemical and antigenic) were compared with those of the inoculated strain.

*** Determination of lethal dose 50 (LD50) for mice**

Mice were infected by an intraperitoneal injection (0.2 ml) of different tenfold dilutions. Five mice per dilution were used and 7 dilutions per strain were carried out. LD50 was calculated (29). The mortality was followed for a period of 10 days.

RESULTS

The results recorded (except for the mortality rate) were obtained with an inoculum fo 10^6 CFU. This dose was used systematically for all the strains.

*** Mortality and Clinical Signs**

For all of the 10 consecutive trials, none of the uninoculated control animals either died or developed lesions. For the inoculated animals, three groups were observed (Table 3). These were classified according to the pathogenic characteristics of the *Pasteurella* strains inoculated. This order has been retained for all the other tables.

1st group : four strains killed all the animals within 16 hours with an inoculum of 10^2 or 10^3 CFU. Only the animals infected with strains 005 and 2610 developed clinical signs of prostration half an hour to an hour prior to death.

3rd group : Six days after inoculation, six strains caused no deaths, even with 10^6 or 10^7 CFU. Previous trials showed no modification of these results after the 6th day. No clinical signs were observed in this group.

2rd group : this was defined by its differences with the two previous groups. The mortality varied from 20 % to 90 % for an inoculum of 10^6 CFU. The last deaths were observed between 3 and 5 days after inoculation. Although the strains A3B and A5B killed 90 % and 70 % of the rabbits respectively, no deaths were observed on the day following inoculation, by contrast to the strains of the 1st group. In general, the rabbits showed signs of prostration, followed by signs of nervous disorder, several hours before death.

The search for a exotoxin of the 17 strains of *P. multocida* showed that they are non toxogenic. The Elisa kit, commercialized by DAKO, whose technique is described by Kobisch, was used to test these strains (30).

*** Lesions**

The nature and the importance of the lesions could be quite different according to the strain (table 4).
In 1st group, certain strains (005 and 2610) caused pleuropneumonia, whereas others caused mainly hepatic and/or splenic lesions. In the latter case the liver was congested and friable, the spleen was diffluent, hypertrophied and its surface was irregular. All the animals showed signs of septicaemia. No abcesses were observed.
In the 2nd group, the dominant lesions were the outcome of a purulent infection, which was more pronounced in animals dying late. However, more specific lesions were noted for certain strains :

- hematoma observed at the point of inoculation (2971)
- enteritis (A5B)
- peritonitis (A3B, A5B, 5123, A9B, TG2, 0100)
- sub-cutaneous abcesses spreading from the point of inoculation towards the abdomen (5123).

In the 3rd group, only strain 5129 caused significant lesions, Moreover, the inoculum of 10^7 caused 2 deaths in 5 animals.
From these criteria for mortality and lesions we produced an index of pathogenic characteristics enabling to divide group 2 into 2 sub-groups (Table 4). For the last 3 strains, either mortality ceased later and was lower, or the survivors had no lesions (as in the case of strain A9B).

The results obtained with weaker or stronger inocula (not reported here) confirm and precise those which have already been described (10^6 CFU).

*** Comparison between the pathogenic influence on rabbits and the LD50 test on mice.**

Although the test on mice was carried out with fewer strains, it is relatively representative of the pathogenic influence observed on rabbits. The strain 5123 appears to be poorly classified by this test, but it can be observed that if LD50 is weak, mortality began on day 5, whereas for the other 2 strains, to which LD50 is inferior at 3.2.101 (ref. 005 and 2610), mortality began on day 1.

*** Comparison of the virulence of the strains of *P. multocida* according to the type and diameter of the colonies**

In this trial no relationship was established between the somatic type and virulence (Table 4). However, the second criterion studied showed a positive correlation between the dimension on the colonies after 13 hours of incubation, and virulence (Table 5). The strains with a diameter 1.4 mm were the most pathogenic and of a septicaemic nature. All the other strains had a diameter twice as small and had a smaller and delayed pathogenic effect.
The average diameter for the three groups of *P. multocida* was significantly different ($P < 1$ %) for at least 24 hours of incubation (Table 6).

DISCUSSION

The main data of the literature concerning the experimental infection of rabbits with *P. multocida* are summarized in Table 7. Generally speaking, the doses indicated by the authors are much higher than those we used but it did not seem necessary to use doses higher than 10^6 CFU. Striking differences were observed in the pathogenic effects of the strains (Table 7). The extremes varied from 0 % mortality for a period of 26 days (34) to 100 % mortality within 24 hours (32,33). OKERMAN's strain produced identical results in our own trials (Table 3, strain P197), even with very low doses (10^2 CFU). Three other of our isolates showed the same degree of pathogenesis, associated with a septicaemic nature, which is not often pointed out (31,32,33,8,).
A second group of *P. multocida* (Table 4) was characterized by a variable mortality, but this was always later than in the previous group. The lesions in the latter group were the most important and the most diverse. This diversity has already been pointed out by several authors, rhinitis, pneumonia and pleurisy being the most frequent lesions (34, 35, 36, 37). However it became quite clear during our work, some strains caused specific lesions (The A5B strain induced important lesions on numerous organs, hematoma were frequently observed with strain 2971, and spreading abcesses were usual with strain 5123). This second group was not very homogeneous and, on the basis of the importance of mortality and/or lesions, it could be divided into two sub-groups (indexes 2 and 3 of Table 4).
A third group seemed to be weakly or not at all, pathogenic under our experimental conditions.
Although the means of inoculation can influence the lethality (40,45), or the nature of the lesions (31,39,38), the pathogenic characteristics of strains 005 (ref. strain) and 3729 used in this trial, were identical to those obtained by intranasal inoculation with the same strains (21). No correlation can be demonstrated between the somatic A serotype of the strains and their pathogenic properties. The serotypes A5 et A7, for example, are to be found in group 1 as well as in group 3.

The origin of the strain allows to suspect its septicaemic nature. Nevertheless, it is necessary to ascertain the acuteness of the disease. In the case chronic infections we were able to find the slightly pathogenic strains in the liver (results not published), the middle ear and the brain (7), those being linked to the intraneural migration of the *Pasteurella multocida* (5).
The LD50 test on mice (Table 5) was correlated with the pathogenic influence observed on the rabbit, but mice respond differently to certain strains. OKERMAN recorded the same findings (40).
Other author (41) has obtained the same, apparently contradictory results, but this could probably be due to the fact that the appearance of pasteurellosis often depends more on the environmental conditions than on the pathogenic properties of the strain.
The relationship which we have shown between the diameter of the colonies after 13 and 24 hours of incubation and the virulence of the strains (Tables 5 and 6), appears as providing a means of prediction the virulence of a strain. After 13 hours, we can identify the most virulent strains : they have a diameter twice the size of less virulent strains. Analysis of the works of OKERMAN follows the same lines (40). Other authors (42,43,44) suspected the existence of a relation between the type of the colonies (mucoid, smooth, rough) and the virulence. Work is underway to determine the origin of the difference in diameter of the colonies and also to better define the mechanisms of the pathogenicity of the non toxigenic strains (exotoxin).

ACKNOWLEDGEMENTS

We should like to thank Mr OKERMAN (Laboratory for Microbiology and infectious Diseases, Faculty of Veterinary Medecine, Gent, Belgium) and D. RABOTEAU, J. BELLEREAUD and her team (Rabbit Farm of the Magneraud) for their technical assistance, and J.L. WIDIEZ for statistical analysis.

ABSTRACT

Several strains of *Pasteurella multocida*, isolated from New Zealand rabbits, were tested for their pathogenic effect on rabbits after intramuscular injection. The pathogenic characteristics of the strains were recorded over a period of 6 days by analysing mortality, along with the nature, the rapidity and the gravity ot the lesions observed. Lesions specific to certain strains were also noted.
The lethal dose 50 (LD50) on mice, correlates quite well with the results obtained in rabbits. No relation sent was observed between the somatic serotype of the strains and their pathogenic characteristics. On the other hand, there were significant differences between the average diameter of the colonies after 13, 17 and 24 hours of incubation and the virulence of the bacteria. The septicaemic strains of *P. multocida* could be distinguished from the other strains by the size of their colonies after 13 hours. After 17 and 24 hours, the strains which formed the smallest colonies were only slightly, or not at all, pathogenic.

Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 1389-1400

TABLE 1 : COMPOSITION OF THE RABBIT FEED

Humidity	14 %	Vitamin A	11 000 UI/kg
Crude protein	15.5 %	Vitamin D3	1 000 UI/kg
Unrefined fats	2 %	Vitamin E	40 mg/kg
Raw fiber	15 %	Copper	25 mg/kg
Crude ash	3 %		

TABLE 2 : ORIGINS AND CHARACTERISTICS OF THE STRAINS OF P. multocida

Strains	Geographical origin	Anatomical origin	Serotype *	Diameter of the colonies (24 h) mean ± standard deviation	
005**	Ile de France	bone marrow	A5	3.47	0.22
2610	Vienne	liver	A7	3.28	0.20
P197	Belgium	bone marrow	A7	3.23	0.20
23055	Magneraud	middle ear	A5	3.09	0.17
A3B	Ile de France	sinus	A3	2.83	0.27
A5B	Ile de France	sinus	A5	1.79	0.18
2971	Vienne	liver	A5	2.31	0.21
5123	Vienne	sinus	A9	2.50	0.28
A9B	ILe de France	sinus	A9	2.79	0.20
TG2	Tours	middle ear	A3	2.71	0.15
0100	Vienne	lung	A7	2.02	0.27
5129	Vienne	sinus	A9	1.97	0.26
4590	Creuse	sinus	A9	1.95	0.21
A7B	Ile de France	sinus	A7	1.98	0.19
31048	Magneraud	sinus	A5	1.93	0.18
4384	Vienne	cutan.abcess	A3	1.48	0.18
3729	Haute Vienne	sinus	A3	1.83	0.16

* According to the classification of CARTER-NAMIOKA ** Reference strain

TABLE 3 : VIRULENCE OF 17 STRAINS OF P. multocida AT DIFFERENT DOSES : STUDY OF THE MORTALITY

STRAINS	DOSES 10^2	10^3	10^4	10^5	10^6	10^7
005	11/11*	23/23	10/10			
2610	5/5	5/5		5/5	10/10	
P197	5/5	13/13			10/10	
23055		5/5			13/13	
A3B	0/5	1/10			9/10	
A5B		1/5			7/10	5/5
2971		0/5		2/5	6/10	5/5
5123		0/5	0/5	2/10	4/10	1/5
A9B		0/5			5/10	5/5
TG2		0/5			2/10	4/5
0100			0/5	0/5	2/10	
5129				0/10	0/10	2/5
4590				0/5	0/10	0/5
A7B		0/5			0/5	
31048				0/5	0/10	1/5
4384	0/5				0/10	0/5
3729					0/10	0/10

* Number of deaths/number of animals inoculated

TABLE 4 : COMPARISON OF THE PATHOGENIC EFFECT OF THE 17 STRAINS OF *P. multocida* WITH 10^6 CFU

STRAINS	Number of rabbits	MORTALITY No	MORTALITY Day*	LESIONS Deaths Observations	LESIONS Deaths No	LESIONS Killed day 6 Observations	LESIONS Killed day 6 No	INDEX of Pathogenic effects
A5: 005	23	23	1	Pleuropneumo.	23			1
A7: 2610	10	10	1	Splenomegal.	10			1
				Pleurosy q	7			
				Lung hepatitis	3			
A7: P197	10	10	1	Splenomegal.	10			1
				Friable liver	10			
				Lung hepatitis	1			
A5:23055	13	13	1	Friable liver	10			1
				Splenomegal.	10			
				Lung hepatitis	1			
A3: A3 B	10	9	3	Peritonitis	7	no lesion	1	2
				Pleuropneumo.	6			
				Abcess p.i***	4			
A5: A5 B	10	7	3	Enteritis	6	Peritonitis	1	2
				Peritonitis	6	Abcess p.i**	2	
				Pleurosy	6			
				Nephritis	4			
				Abcess p.i	2			
A5: 2971	10	6	3	Pleurosy	5	no lesion	4	2
				Hematoma p.i	5			
A9: 5123	10	5	3	Diffus. abcess	5	no lesion	5	2
				Peritonitis	2	Peritonitis	1	
A9: A9 B	10	5	4	Peritonitis	5	no lesion	5	3
				Pleuropneumo.	3			
				Enteritis	2			
A3: TG2	10	2	4	Pleurosy	1	Pleurosy	1	3
				Peritonitis	2	Abcess p.i	6	
				Abcess p.i	1	Pneumonia	1	
A7: 0100	10	2	5	Pleurosy	1	no lesion	3	3-4
				Peritonitis	1	Abcess p.i	5	
				Friable liver	1			
A9: 5129	10	0	6			Pleuropneum.	2	4-3
						Necrosis liver	3	
						Nephritis	2	
						Abcess p.i	1	
						no lesion	2	
A9: 4590	10	0	6			Abcess p.i	2	4
						no lesion	8	
A7: A7 B	5	0	6			Abcess p.i	4	4
						Peritonitis	1	
A5: 1048	10	0	6			Abcess p.i	2	4
						no lesion	8	
A3: 4385	10	0	6			Abcess p.i	7	4
						no lesion	3	
A3: 3729	10	0	6			Abcess p.i.	9	4
						no lesion	1	

* The day of death taken into account is the day the last animal of the group died ** Abcess at point of inoculation

TABLE 5 : *P. multocida* : COMPARISON OF PATHOGENIC EFFECT ON RABBITS AND MICE AND CLASSIFICATION OF THE STAINS ACCORDING TO THE DIAMETER OF COLONIES

Test of PE* on the rabbit (intra-muscular inoc)		Test of PE on the mouse LD 50	Classification of the strains according to the decreasing diameter of the colonies after:								
			13 H.			17 H.			24 H.		
Strain	Index	CFU at LD 50	Strain	Index	Diameter	Strain	Index	Diameter	Strain	Index	Diameter
005	1	3.2×10^1	2610	1	1.72	2610	1	2.28	2610	1	3.47
2610	1	$<1.4 \times 10^1$	005	1	1.45	005	1	2.07	005	1	3.29
P197	1	ND**	P197	1	1.43	P197	1	1.95	P197	1	3.23
23055	1	ND	23055	1	ND	25055	1	Na	23055	1	3.09
A3B	2	1×10^2	5129	3	0.90	5123	2	1.68	A3B	2	2.83
A5B	2	1.4×10^4	2971	2	0.75	TG2	3	1.64	A9B	3	2.79
2971	2	1.8×10^3	TG2	3	0.69	5129	4-3	1.42	TG2	3	2.71
A9B	3	1.2×10^5	A3B	2	0.65	2971	2	1.24	2971	2	2.31
TG2	3	ND	31048	4	0.65	A9B	3	1.21	0100	3-4	2.02
0100	3-4	ND	4384	4	0 62	31048	4	1.11	A7B	4	1.98
5129	4-3	ND	5123	2	0.59	4590	4	1.01	5129	4-3	1.97
4590	4	ND	A5B	2	0.54	4384	4	0.99	4590	4	1.95
A7B	4	1.3×10^6	0100	3-4	0.50	A5B	2	0.93	31048	4	1.93
31048	4	ND	A7	4	0.46	0100	3-4	0.87	3729	4	1.83
4384	4	ND	4590	4	0.39	A7B	4	0.83	A5B	2	1.79
3729	4	3.5×10^7	3729	4	0.29	3729	4	0.75	4384	4	1.48

* PE = Pathogenic effect

** ND = Not Done

TABLE 6 : THE AVERAGE DIAMETER OF THE COLONIES OF THE 3 GROUPS OF *P. multocida* AFTER DIFFERENT PERIODS OF INCUBATION

GROUP	INCUBATION PERIOD 13 H			17 H			24 H		
	Mean ±	SD*	VC**	Mean ±	SD	VC	Mean ±	SD	VC
1	1.52a	0.16	10.82	2.09a	0.17	8.39	3.27a	0.22	6.78
2	0.63b	0.17	26.68	1.28b	0.34	26.87	2.42b	0.42	17.56
3	0.55c	0.29	43.15	1.02c	0.25	24.78	1.86c	0.25	13.76

* Standard deviation ** Variation coefficient The mean of the groups followed by a, b or c significantly different at P < 1%

TABLE 7 : MAIN REFERENCES CONCERNING EXPERIMENTAL INFECTIONS WITH *P. multocida*

Authors	Anatomic origin of P. multocida	Inoculum CFU	Serotype P.m	Method of inoculation	Mortality	Period of observation (in days)
OKERMAN, 1981(32)	bone marrow	$2\text{-}6.10^6$	A1*	nebulization	4/4	J 1
" 1990(33)	" "	1.10^7	"	sub-cutaneous	10/10	J 1
SOKKAR, 1986(39)	lung	4.10^7	?	per bone	(3)14/15	(J3)J28
		2.10^7	?	intranasal	(3)15/15	(J3)J28
LU, 1987(46)	mucus	5.10^8	A3*	intranasal	10/16	J14?
MORISSE, 1979(31)	?	1.10^4	A3**	intravenous	8/10	J 6
		1.10^8	"	intranasal	4/10	J12
KPODEKON, 1983(5)	spleen	10^9 ?	?	trig. nerve	0/8	J29
	middle ear	"	?	intranasal	0/4	J29
				intravenous	2/2	(J1)J23
DILLEHAY, 1991(49)	lung	4.10^8	A3*	intranasal	5/10	J14
DIGIACONO,1987(37)	?	$1,75.10^8$	A12*	intranasal	0/ 4	J21
		$0,4.10^9$	A3*	"	2/ 4	J 7
PERCY, 1985(35)	lung	3.10^{10}	A3*	intranasal	1/ 6	J14
	otitis	"	A12*	"	0/ 6	J14
PERCY, 1986(38)	lung	1.10^{10}	A3*	nebulization	0/ 6	J14
		$10^7\text{-}10^9$	"	intravenous	0/ 6	J14
		$10^8\text{-}10^{10}$	"	intracheal	1/ 6	J 5
NAKAGAWA, 1986(47)	?	$10^2\text{-}10^5$	?	intranasal	0/25	J14
WATSON, 1975(48)	mucus	5.10^9	?	intran+stress	0/ 9	J21
MORISSE, 1978(34)	lung,mucus	4.10^5x4	A**	intranasal	0/ 9	J26
CHENGAPPA, 1980(36)	mucus	10^{10}	A?	sub-cutaneous	0/ 4	J15
		"	"	intranasal	0 /4	J15

Serotype according to the classification * of CARTER-HEDDLESTON ** of CARTER-NAMIOKA

REFERENCES

1 KOTSCHE W., GOTTSCHALK C. 1983. Krankheiten der Kaninchen und Hasen. 3., Uberarbeitete Auflage. Jena : VEB Gustav Fischer Verlag, 1983.- 335 p.

2 LEBAS F., COUDERT P., ROUVIER H., ROCHAMBEAU H.de 1986. The Rabbit. Husbandry, health and production. Rome : (FAO) Food and Agriculture Organization, 1986. 235 p. (FAO Animal Production and Health Series N°21) ISBN:92-5-101253-9.

3 VETESI F.. 1990. Hygiene of the domestic Rabbit. Anatomy, physiology, reproduction, management, nutrition and pathology. (Hong.) Budapest (Hongrie) : Mezogazdasagi Kiado, 1990.- 248 p. ISBN : 963-234-197

4 COUDERT P., BRUN J.M. 1989. Production et morbidité des lapines reproductrices : étude comparative de quatre genotypes. Genet.Sel.Evol., 1989, 21, 49-65.

5 KPODEKON M., 1983. Pathologie et pathogenie des complications auriculaires et encephaliques de la pasteurellose du lapin d'elevage. Ann.Rech.Vet., 1983, 14, 225-232.

6 COUDERT P., RIDEAUD P., BALENCON M., 1986. Pasteurellose non respiratoire en elevage intensif : l'otite moyenne des lapines reproductrices. Cuni-Sciences, 1986, 3(2),1-6.

7 BALENCON P., RIDEAUD P., COUDERT P.,1982. Etude bactériologique des otites et torticolis chez les lapines reproductrices réformées. 3ème Journées de la recherche Cunicole. ITAVI-INRA , Paris comm. N°29.

8 FLAT R.E., Bacterial diseases. In Weisbroth SH, Flat RE, Kraus AL, eds. The biology of the laboratory Rabbit. Acad. Press . New York. 1974, 194-236.

9 HOLMES H.T., 1988. Studies on selected topics of Pasterella multocida infection in laboratory Rabbits. Diss. Ph.D.:Microbiology: Oregon State Univ. 1988. 244p

10 SPANOGHE L., OKERMAN L.. 1989. Prevention of rabbit pasteurellosis by an inactivated vaccine - Field experiments. Cuniculture, N°87, 16(3):169-173.

11 GAERTNER D.J.1991. Comparison of penicillin and gentamicin for treatment of pasteurellosis in Rabbits. Lab.Anim.Sci., 1991, 41(1), 78

12 CHENGAPPA M.M., MYERS R.C., CARTER G.R.. 1982. Capsular and somatic types of Pasteurella multocida from Rabbits. Can.J.Comp.Med., 1982, 46, 437-439.

13 LU Y.-S., PAKES S.P., STEFANU C., 1983. Capsular and somatic serotypes of Pasteurella multocida isolates recovered from healthy and diseased rabbits in Texas. J.Clin.Microbiol., 1983, 18, 292-295.

14 RIMLER R.B., BROGDEN K.A., 1986. Pasteurella multocida isolated from rabbits and swine : serologictypes and toxin production. Am.J.Vet.Res., 1986, 47(4), 730-737.

15 HERVOUET P., NOUAILLE L. 1985. Epidemiologie de la staphylococcie et de la pasteurellose du Lapin dans les pays de Loire. Cuniculture, 1985, (65), 275-276.

16 SCHARF R.A., MONTELEONE S.A., STARK D.M.. 1981. A modified barrier system of maintenance of Pasteurella-free rabbits. Lab.Anim.Sci., 1981, 31(5), 513-515.

17 DIGIACOMO R.F., GARLINGHOUSE L.E.Jr., VAN HOOSTER G.L.Jr., 1983. Natural history of infection with Pasteurella multocida in rabbits. J. Am. Vet. Med. Assoc., 1983, 183(11), 1172-1175.

18 HAGEN K.W. 1967. Effect of antibiotic-sulfonamide therapy on certain microorganisms in the nasal turbinates of domestic Rabbits. Lab.Anim.Care, 1967, 17(1), 77-80.

19 KAWAMOTO E., SAWADA T., MARUYAMA T., 1990. Prevalence and Caracterization of Pasteurella multocida in Rabbits and their environment in Japan. Jpn. J. Vet. Sci. 1990. 52(5): 915-921.

20 LU Y.S., RINGLER D.H., PARK J.S., 1978. Characterization of Pasteurella multocida isolates from the nares of healthy Rabbits and Rabbits with pneumonia. 1978. Lab. Anim. Sci. 28: 691-697

21 RIDEAUD P., COUDERT P., 1992. Pasteurella epidemiology: effect of the age of weanling Rabbits. 1992. Proceedings of the Vth Congress of the World Rabbit Scientific Association. Corvallis (USA)

22 NAMIOKA S.. 1978. Pasteurella multocida - Biochemical characteristics and serotypes. Methods Microbiol., 1978, 10, 273-292.

23 ESCANDE F., 1985. Taxonomie des genres Pasteurella et Actinobacillus. Paris: Université Paris-Sud, Centre d'Orsay, 1985.- 142p.

24 CARTER G.R., 1952. The type specific capsular antigen of Pasteurella multocida. Can.J.Comp.Med., 30:48-53.

25 CARTER G.R.1955. Studies on Pasteurella multocida. I. A hemagglutination test for the identification of serological types.Am.J.Vet.Res., 1955, 16, 481-484.

26 CARTER G.R.. 1963. Proposed modification of the serological classification of Pasteurella multocida. Vet.Rec., 1963, 75(47), 1264-1265.

27 NAMIOKA S., MURATA M.. 1961. Serological studies on Pasteurella multocida. II. Characteristics of somatic (O) antigen of the organism. Cornell Vet., 1961, 51, 507-521.

28 NAMIOKA S., BRUNER D.W.. 1963. Serological studies on Pasteurella multocida. IV. Type distribution of the organisms on the basis of their capsule and O groups. Cornell Vet., 1963, 53, 41-53.

29 REED L.J., MUENCH H., 1938. A simple method of estimating fifty per cent end point. Amer. J. of Hygien. 27:493-497.

30 KOBISCH M., BLANCHARD B., MORVAN P., LABBE A., TOQUIN D., LE MENEC M., MORVAN H.. 1991. L'Exotoxine de Pasteurella multocida d'origine porcine. Etude comparative de trois techniques de détection. Recl.Med.Vet., 1991, 167(5), 407-411.

31 MORISSE J.P. 1979. Prophylaxie medicale de la "Pasteurellose" du lapin : essai de trois vaccins. Recl.Med.Vet., 1979, 155(9), 693-702.

32 OKERMAN L., SPANOGHE L.. 1981. Protective effects of inactivated Pasteurella vaccines in specific pathogen free Rabbits. Comp. Immunol. Microbiol. Infect. Dis., 1981, 2, 223-228.

33 OKERMAN L., DEVRIESE L.A., GEVAERT D., UYTTEBROEK E.,HAESEBROUCK F. 1990. In vivo activity of orally administered antibiotics and chemotherapeutics against acute septicaemic pasteurellosis in Rabbits. Lab.Anim., 1990, 24(4), 341-344.

34 MORISSE J.P. 1978. Infection pulmonaire experimentale a Pasteurella multocida. Influence d'un facteur irritant (NH3) sur la receptivite du Lapin. Recl.Med.Vet., 1978, 154(10), 859-863.

35 PERCY D.H., PRESCOTT J.F., BHASIN J.L., 1985. Pasteurella multocida infection in the domestic rabbit - Immunization with a streptomycin-dependent mutant. Can.J.Comp.Med., 1985, 49(2), 227-230.

36 CHENGAPPA M.M., MYERS R.C., CARTER G.R.. 1980. A streptomycin dependent live Pasteurella multocida vaccine for the prevention of Rabbit pasteurellosis. Lab.Anim.Sci., 1980, 30(3), 515-518.

37 DIGIACOMO R.F., DEEB B.J., BERNARD B.L., KLAASSEN J.M., et al.,1987. Safety and efficacy of a streptomycin dependent live Pasteurella multocida vaccine in rabbits. Lab.Anim.Sci., 1987, 37(2), 187-190.

38 PERCY D.H., BHASIN J.L., ROSENDAL S., 1986. Experimental pneumonia in rabbits inoculated with strains of Pasteurella multocida. J.Vet.Res., 1986, 50(1), 36-41.

39 SOKKAR S.M., MOHAMED M.A., FETAIH H., 1987. Pathogenesis of Pasteurella multocida in experimentally infected rabbits. Arch.Exp.Veterinaermed., 1987, 41(4), 516-521.

40 OKERMAN L., SPANOGHE L., DE BRUYCKER R.M.. 1979. Experimental infections of mice with Pasteurella multocida strains isolated from Rabbits. J.Comp.Pathol., 1979, 89, 51-55.
41 MUSHIN R., SCHOENBAUM M.. 1980. A strain of Pasteurella multocida associated with infections in rabbit colonies. Lab.Anim., 1980, 14, 353-356.
42 CARTER G.R., BIGHAN J.,1953. Dissociation and virulence in strains of Pasteurella multocida isolated from a variety of lesion. Can. J. Comp. Med., 1953, 17, 473-479.
43 CARTER G.R.1957. Studies on Pasteurella multocida. II. Identification of antigenic characteristics and colonial variants. Am.J.Vet.Res., 1957, 18(66), 210-213.
44 BROGDEN K.A.. 1980. Physiological and serological characteristics of 48 Pasteurella multocida cultures from Rabbits. J.Clin.Microbiol., 1980, 11(6), 646-649.
45 KPODEKON M., 1983. Etude experimentale de la pathogenie des meningites et encephalites lors de la pasteurellose du lapin. Ann.Rech.Vet., 1983, 14, 217-224.
46 LU Y.-S., PAKES S.P., MASSEY L., STAFENU C., 1987. A potassium thiocyanate extract vaccine prepared from Pasteurella multocida : a protects rabbits against homologous challenge. Infect.Immun., 1987, 55(12), 2967-2976.
47 NAKAGAWA M., NAKAYAMA K., SAITO M., TAKAYAMA S., WATARAI S., 1986. Bacteriological and serological studies on Pasteurella multocida infection in rabbits. Exp.Anim., 1986, 35(4), 463-470.
48 WATSON W.T., GOLDSBORO J.A., WILLIAMS F.P., SUEUR R.. 1975. Experimental respiratory infection with Pasteurella multocida and Bordetella bronchiseptica in Rabbits. Lab.Anim.Sci., 1975, 25(4), 459-464.
49 DILLEHAY D.L., PAUL K.S., DIGIACOMO R.F., CHENGAPPA M.M. 1990. Pathogenicity of Pasteurella multocida A3 in Flemish Giant and New Zealand White Rabbits. Lab.Anim., 1990, 25(4), 337-341.