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A STUDY OF THE PATHOGENIC INFLUENCES OF THREE STRAINS OF *PASTEURELLA MULTOCIDA* ON THE RABBIT - AN EXPERIMENT TO CONTROL THE EFFECTS BY SPIRAMYCIN

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INTRODUCTION

Statistics from different laboratories show that more than 50 % of adult rabbits either die or are eliminated due to respiratory diseases, or due to an associated disease (i.e. abcesses, mammitis, metritis, abortions stillbirths, otitis). Respiratory disease is the main cause of financial loss for the French rabbit farmer.

Pasteurella multocida (serotype A or D) (Pm) is often involved in these disorders. It is a gram-negative bacteria, non-specific to the rabbit and is quite harmless.

During our preliminary experiments, when we inoculated young rabbits with different strains of Pm, we noticed varying pathogenic results. This why we sought to test the pathogenic capacity of the three strains of Pm when administEred to rabbits.

Moreover, at the time of previous trials on rabbits as to the tolerance of SPIRAMYCIN, (an antibiotic from the macrolide family) we observed, in addition to the abscence of toxicity, a certain improvement in respiratory disorders when the product was administered in normal doses. We also know that Spiramycin is especially active on gram-positive bacteria and on mycoplasms.

It diffuses particularly well in the bronchial secretions and it concentrates in the macrophages. This considerably increases its activity in vivo.

Bearing in mind that this antibiotic is widely used in breeding and that, although Spiramycin and either Oxytetracyclin or Trimethoprim-sulphamid are usually used in association with each other, we sought to test Spiramycin alone in our experiment to inoculate with Pm.

METHODS AND MATERIALS

For the experiment our set-up consisted of 12 groups of 10 New Zealand rabbits (INRA strain 77) from the Magneraud rabbit farm. Each group was numbered from 1 to 12.

Treatment	Spira. "A" 0 ppm	Spira. "B" 300 ppm	Spira "C" 600 ppm
0 (control)	1	2	3
Pm. ref A5	4	5	6
Pm. A9	7	8	9
Pm. A5 Mag.	10	11	12

In other words there were 120 rabbits. These were divided up after weaning at 30 days, into even groups according to weight, and each was identified individually. Their mothers showed no signs of respiratory illness.

The rabbits were housed in individual Californian cages and placed in a building with static ventilation which was isolated from the rest of the farm. They were kept in cages in order to avoid any outside environmental effects. The food pellets, which were distributed haphazardly, were only supplemented with 0 (A), 300 (B) or 600 ppm of Spiramycin RP (C). These feeds were given from D0 (weaning) to D14. Then, all the rabits were given the same control feed A until slaughter. For each treatment the animals were inoculated on D7 with the strain of *Pasteurella multocida* (Pm) ref. A5 (groups 4, 5 and 6), A9 (groups 7, 8 and 9) and A5 Mag. (groups 10, 11 and 12). The rabbits were inoculated through the nose by injecting 0.1 ml of culture containing 10^4 germs per rabbit.

Control measures

Animals were weighed individually on D0, D7, D14, D21 and D42. Throughout the whole of the experiment their individual liveweight was recorded, along with the ADG per period for each treatment.

Feed distributed in the cages was first weighed in order to calculate the average feed conversion ratio (FCR) per group for each treatment. This was carried out for the periods D0-D7, D7-D14, D14-D21 and D0-D21.

Deaths were recorded along with the age, weight and any clinical signs present. An autopsy was carried out on all dead animals.

Feed analysis produced the following results :

	A	B	C
Dry Matter (%).....	89.6	87.6	87.4
Total Crude Protein (%) ..	15.6	15.3	15.2
Raw fiber (%)	15.75	15.9	15.7
Mineral Matter (%)	8.85	8.6	8.2

These feeds were very similar, and on a nutrional scale correspond to a typical feed for fattening rabbits.

The amount of Spiramycin in the different samples was :

Diet A (control) ..... :	absence of Spiramycin
Diet B (300 ppm) ..... :	290 ppm
Diet C (600 ppm) ..... :	562 ppm

This corresponds well with the expected results for the different treatments.

RESULTS AND DISCUSSION

MORTALITY (see Table n° 1 and 2)

Table n° 1 : Number of dead rabbits

Treatment	A				B				C			
Group	1	4	7	10	2	5	8	11	3	6	9	12
Number of dead rabbits/group	0	9 *90%*	0	0	0	9 *90%*	1 *10%*	0	2 *20%*	9 *90%*	1 *10%*	0
Number of dead rabbit/treatment	9 *(22.5%)*				10 *(25%)*				12 *(30%)*			

Table n°2 : Characteristics of dead rabbits

Group	N° rabbit	Age (D)	Weight dead rabbits (g)	Lésions	AWG 0-7D (g)	Feed intake 0-7D (g)	FCR 0-7 D
3	163	40	940	Pleuropneumonia	44.71	107.14	2.40
	016	37	455	Enteritis	24.28	21.43	-
1+2+3	2 rabbits	-	-	---	-	-	-
4	167	39	1035	Pleurisy	50.00	115.71	2.31
	075	40	1010	Pleuropneumonia	39.86	110.00	2.76
	153	40	920	Pleurisy	41.43	122.86	2.97
	004	38	1130	Pleurisy	56.29	125.71	2.23
	095	39	930	Pleurisy + enteritis	40.71	87.14	2.14
	005	39	965	Pleurisy + enteritis	48.00	113.57	2.37
	112	39	925	Pleurisy	51.14	112.14	2.19
	081	39	800	Pleurisy	30.86	79.29	2.57
	085	38	840	Pleurisy + enteritis	45.57	110.71	2.43
5	114	39	1010	Pleurisy	55.43	108.57	1.96
	077	39	885	Pleurisy	39.14	107.14	2.74
	166	39	1015	Pleurisy	46.00	130.71	2.84
	031	39	880	Pleurisy	43.57	100.00	2.30
	007	39	900	Pleurisy	46.00	119.29	2.59
	032	39	890	Pleurisy	43.86	94.29	2.15
	141	39	855	Pneumonia	49.57	121.43	2.45
	098	39	985	Pleurisy	54.43	127.14	2.34
	162	39	1015	Pleurisy	50.00	110.71	2.21
6	111	39	1010	Pleuropneumonia	46.86	119.29	2.55
	146	35	-	Diarrhoea	-	-	-
	056	35	-	Diarrhoea	-	-	-
	030	39	1035	Pleurisy + enteritis	42.29	100.00	2.36
	057	39	965	Pleurisy	48.00	65.71	1.37
	001	35	-	Diarrhoea	-	-	-
	179	39	1005	-	45.57	115.71	2.54
	115	39	960	Pleurisy	45.29	102.14	2.26
	024	35	-	Diarrhoea	-	-	-
4+5+6	27rabbits	38.4	960	---	-	-	-
8	065	59	-	Pleuropneumonia	42.43	105.71	2.49
9	025	46	-	Pleuropneumonia	46.00	99.29	2.16
7+8+9	2 rabbits	-	-	---	-	-	-
10+11+12	0	-	-	---	-	-	

The effects of Spiramycin

Both tables show that there is no significant difference in the overall mortality rate for treatments A, B and C (22.5%, 25% and 30% respectively).

The Spiramycin supplement in treatments B and C gave no protection, against respiratory problems following an infection. This was due to a very pathogenic strain of Pm.

This confirms our observations during previous experiments : Spiramycin is harmless at a dose of 300ppm, but as soon as the dose reaches 600ppm the intestinal flora is slightly disturbed. In fact 4 rabbits from group 6 and 1 rabbit from group 3 died from diarrhoea at 35 days (D5) and at 37 days (7) respectively i.e. before being inoculated with pasteurella.

The effects of Pasteurella

We can confirm that our strain of *Pasteurella multocida* is extremely virulent : it killed 90% of the rabbits within 48 hours of being inoculated. As is to be expected with this strain, the autopsy revealed lesions essentially due to pleurisy. Unlike previous experiments, this time we had created typical breeding conditions. Moreover, the surprising fact is that 1 out of 10 rabbits in each treatment proved resistant to inoculation.

The Pm A9 strain led to few clinical problems. However, two rabbits (one each in groups 8 and 9) died at 59 days (D9) and at 46 days (D16) respectively. They showed pleuropneumonic lesions.

This strain may be equally pathogenic, but distinctly less virulent than ref. A5.

The Pm strain A5 Magneraud did not cause any problems and would appear to be only slightly, or not at all pathogenic.

Performance (Refer to tables 3A, 3b, 3c and 3 d)

The effects of Spiramycin

The evolution of liveweight and daily growth during this trial show that treatment B (300ppm Spira) gives excellent results : on D42 the final liveweight was up 64g (+ 2.7%) and the ADG was at 1.15g (+ 2.9%) compared with control A.

On the other hand, the results of treatment C (600ppm Spira) show a disturbing effect on the digestive system. This is confirmed by the death rate. The liveweight and the ADG for the first 3 weeks are low : this is particularly evident from D0 to D14, the period when the supplemented feed was given. However, it should be noted that the compensatory growth in the second half of the fattening period gives a final result better than the control : + 0.90g of ADG (+ 2.25%).

A study of feed intake shows that this is higher for treatment B for each period of the first 3 weeks, when compared with treatment A : at D21 the daily feed intake was up 5.4g (+ 4.4%). This proves that Spiramycin does not suppress the appetite at this dosage. A distinctly lower feed intake can be observed for treatment C, especially from D0 to D14, the period when the supplemented feed was given.

There is no overall difference with regard to the FCR during the period D0-D21. However, this was disturbed in most of the groups of treatment C when the rabbits were fed with the supplemented diet.

The effects of Pasteurella

The control groups (N° 1, 2 and 3) did very well, since a liveweight of 2.374g and an ADG of 41.03g in 42 days of fattening was recorded. Their average FCR for the first 3 weeks was 2.93.

It is impossible to interpret the results of the groups inoculated with Pm ref. A5 (N0 4, 5 and 6) since there were only three survivors (1 per treatment).

Compared with other groups, the results were slightly down for the groups inoculated with Pm A9 (N° 7, 8 and 9).

Thus the liveweight on D42 was 2.331g compared with 2.374g for the control (- 1.8%) and the ADG was 39.95g against 41.03g (- 2.6%).

Moreover, a slightly lower feed intake was observed following inoculation. (D7 to D21).

Finally, the groups inoculated with Pm A5 Mag. (N° 10, 11 and 12) showed strictly no difference compared with the control as far as growth and feed intake were concerned. This tends to confirm that this *pasteurella* strain is apathogenic.

CONCLUSION

The added Spiramycin in the feed gave no protection against consecutive respiratory infections due to a very pathogenic strain of Pm.

However, we were able to confirm the specific effects of Spiramycin, according to the incorporated dose, on rabbits bred and housed in traditional conditions, and affected by a respiratory disease.

The addition of 300ppm of Spiramycin to the feed during 14 days (a therapeutic dose) is entirely tolerated. It does not inhibit the feed intake and, in general, gives better results than the control.

A dose of 600ppm during 14 days (double the therapeutic dose) entailed the risk of disturbing the intestinal flora. This caused diarrhoea and a drop in the growth rate, along with a reduced feed intake, during the period when the supplemented feed was given.

In other respects, the results of this trial help to deepen our knowledge of the pasteurella strains tested under traditional breeding conditions.

The Pm strain ref. A5 proved to be extremely virulent, since 90% of the inoculated rabbits died within 48 hours, displaying pleurisy lesions or pleuropneumonia.

The Pm A9 strain did not engender any spectacular clinical problems, but did bring about 2 later deaths, both with pleuro-pneumonic lesions, accompanied by a slight drop in growth rate and a low feed intake, following inoculation.

This strain could be considered as averagely pathogenic.

Finally, the Pm A5 Mag. did not cause any disorders and performed identically to the control. This strain therefore seems to be apathogenic.

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TABLE n° 3 (a) : PERFORMANCE (Liveweight)

TREATMENT	A				B				C				TOTAL			
	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ
Weight at weaning D0 (g)	1	10	650	85	2	10	655	78	3	10	647	71	1-2-3	30	650	76
	4	10	640	81	5	10	655	79	6	10	652	80	4-5-6	30	649	78
	7	10	652	69	8	10	654	84	9	10	651	68	7-8-9	30	652	71
	10	10	651	76	11	10	661	83	12	10	657	74	10-11-12	30	656	75
Total		40	648	75		40	656	78		40	652	71		120	652	74
Weight at D7 (g)	1	10	983	111	2	10	941	101	3	10	907	179	1-2-3	30	943	134
	4	10	958	96	5	10	987	96	6	6	996	75	4-5-6	26	978	90
	7	10	1006	107	8	10	954	126	9	10	960	82	7-8-9	30	973	105
	10	10	993	34	11	10	989	108	12	10	964	123	10-11-12	30	982	103
Total		40	985	97		40	968	106		36	952	125		116	967	109
Weight at D14 (g)	1	10	1267	121	2	10	1237	113	3	8	1266	82	1-2-3	28	1256	105
	4	1	1195	-	5	1	1475	-	6	1	1180	-	4-5-6	3	1283	166
	7	10	1289	112	8	10	1240	139	9	10	1187	128	7-8-9	30	1239	129
	10	10	1290	87	11	10	1306	114	12	10	1223	130	10-11-12	30	1273	114
Total		31	1279	104		31	1268	127		29	1221	116		91	1257	117
Weight at D21 (g)	1	10	1531	113	2	10	1524	121	3	8	1579	74	1-2-3	28	1542	108
	4	1	1450	-	5	1	1830	-	6	1	1400	-	4-5-6	3	1560	235
	7	10	1562	176	8	10	1547	151	9	9	1499	123	7-8-9	29	1537	149
	10	10	1563	98	11	10	1593	126	12	10	1511	159	10-11-12	30	1556	130
Total		31	1549	129		31	1564	139		28	1523	129		90	1546	132
Weight at D42 (g)	1	10	2337	149	2	10	2369	116	3	8	2429	107	1-2-3	28	2374	127
	4	1	2185	-	5	1	2845	-	6	1	2140	-	4-5-6	3	2390	395
	7	10	2306	239	8	9	2379	194	9	9	2310	142	7-8-9	28	2331	193
	10	10	2358	184	11	10	2386	195	12	10	2390	197	10-11-12	30	2378	186
Total		31	2329	187		30	2393	183		28	2366	162		89	2362	178

TABLE n° 3 (b) : PERFORMANCE (AWG)

TREATMENT	A				B				C				TOTAL			
	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ
AWG D0-D7 (g)	1	10	47.57	5.13	2	10	40.81	5.68	3	10	42.06	9.83	1-2-3	30	43.48	7.56
	4	10	45.34	7.28	5	10	47.39	5.02	6	6	45.55	1.92	4-5-6	26	46.18	5.46
	7	10	50.52	6.83	8	10	42.87	7.45	9	10	44.07	5.77	7-8-9	30	45.82	7.33
	10	10	48.73	4.89	11	10	46.93	5.59	12	10	43.84	10.81	10-11-12	30	46.50	7.59
Total		40	48.04	6.18		40	44.50	6.41		36	43.69	8.09		116	45.47	7.10
AWG D7-D14 (g)	1	10	40.64	4.48	2	10	42.36	3.74	3	8	44.20	7.31	1-2-3	28	42.27	5.22
	4	1	42.14	-	5	1	51.43	-	6	1	41.43	-	4-5-6	3	45.00	5.58
	7	10	40.43	5.46	8	10	40.86	5.57	9	10	33.64	11.60	7-8-9	30	38.31	8.48
	10	10	42.43	4.86	11	10	45.21	5.17	12	10	38.29	14.45	10-11-12	30	41.98	9.42
Total		31	41.20	4.79		31	43.09	5.21		29	38.42	11.90		91	40.96	8.03
AWG D14-D21 (g)	1	10	37.71	4.60	2	10	41.00	2.36	3	8	44.82	7.50	1-2-3	28	40.92	5.64
	4	1	36.43	-	5	1	50.71	-	6	1	31.43	-	4-5-6	3	39.52	10.01
	7	10	39.00	11.23	8	10	43.86	6.58	9	9	45.16	8.47	7-8-9	29	42.59	9.07
	10	10	39.07	5.51	11	10	41.07	2.98	12	10	42.00	19.62	10-11-12	30	40.71	11.54
Total		31	38.53	7.34		31	42.26	4.64		28	43.45	13.11		90	41.34	9.05
AWG D0-D21 (g)	1	10	41.98	2.34	2	10	41.39	3.49	3	8	44.32	2.63	1-2-3	28	42.44	3.03
	4	1	42.71	-	5	1	39.38	-	6	1	49.33	-	4-5-6	3	43.81	5.06
	7	10	43.32	5.93	8	10	42.53	3.97	9	9	40.70	4.67	7-8-9	29	42.23	4.88
	10	10	43.41	2.08	11	10	44.40	3.80	12	10	40.66	5.89	10-11-12	30	42.83	4.38
Total		31	42.89	3.73		31	42.99	3.95		28	41.68	4.77		90	42.55	4.15
AWG D21-D42 (g)	1	10	38.38	5.53	2	10	40.21	3.05	3	8	40.45	3.43	1-2-3	28	39.63	4.15
	4	1	35.00	-	5	1	48.33	-	6	1	35.24	-	4-5-6	3	39.52	7.63
	7	10	35.45	7.18	8	9	38.99	3.91	9	9	38.62	3.96	7-8-9	28	37.61	5.39
	10	10	37.86	4.99	11	10	37.76	4.35	12	10	41.86	5.64	10-11-12	30	39.16	5.22
Total		31	37.16	5.82		30	39.30	4.11		28	40.18	4.59		89	38.83	5.02
AWG D0-D42 (g)	1	10	40.18	3.20	2	10	40.80	2.04	3	8	42.38	2.60	1-2-3	28	41.03	2.72
	4	1	38.86	-	5	1	48.83	-	6	1	37.31	-	4-5-6	3	41.67	6.25
	7	10	39.38	4.89	8	9	40.86	3.39	9	9	39.66	2.85	7-8-9	28	39.95	3.77
	10	10	40.63	3.31	11	10	41.08	3.86	12	10	41.26	3.85	10-11-12	30	40.99	3.56
Total		31	40.03	3.72		30	41.18	3.35		28	40.93	3.28		89	40.70	3.46

TABLE n° 3 (c) : PERFORMANCE (Daily feed intake)

TREATMENT	A				B				C				TOTAL			
	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ
Feed intake D0-D7 (g)	1	10	107.29	10.16	2	10	106.14	15.56	3	10	92.14	26.56	1-2-3	30	101.86	19.37
	4	10	107.64	14.86	5	10	114.21	11.95	6	6	103.45	20.23	4-5-6	26	109.20	15.22
	7	10	113.50	19.84	8	10	106.43	8.80	9	10	103.79	10.43	7-8-9	30	107.90	14.05
	10	10	108.57	9.71	11	10	111.07	11.39	12	10	100.71	16.74	10-11-12	30	106.79	13.29
Total		40	109.25	13,92		40	109.46	12.18		36	99.64	19.07		116	106.34	15.72
Feed intake D7-D14 (g)	1	10	122.86	12.02	2	10	132.71	18.08	3	8	122.05	16.51	1-2-3	28	126.15	15.90
	4	1	112.14	-	5	1	165.00	-	6	1	120.71	-	4-5-6	3	132.62	28.37
	7	10	125.14	14.92	8	10	132.72	12.75	9	10	108.21	15.41	7-8-9	30	122.02	17.37
	10	10	125.64	6.70	11	10	143.22	13.29	12	10	117.86	25.44	10-11-12	30	128.91	19.65
Total		31	124.15	11.40		31	137.14	15.85		29	115.79	19.66		91	125.91	18.02
Feed intake D14-D21 (g)	1	10	137.43	6.27	2	10	142.00	5.81	3	8	142.77	15.52	1-2-3	28	140.59	9.63
	4	1	134.28	-	5	1	167.14	-	6	1	134.28	-	4-5-6	3	145.24	18.97
	7	10	137.07	21.34	8	10	140.43	11.85	9	9	136.51	11.94	7-8-9	29	138.05	15.34
	10	10	141.00	10.42	11	10	143.14	8.12	12	10	138.21	24.54	10-11-12	30	140.79	15.66
Total		31	138.36	13.59		31	142.67	9.69		28	138.83	17.68		90	139.99	13.88
Feed intake D0-D21 (g)	1	10	122.52	8.21	2	10	126.95	11.09	3	8	121.31	9.41	1-2-3	28	123.76	9.62
	4	1	115.24	-	5	1	151.67	-	6	1	124.29	-	4-5-6	3	130.40	18.97
	7	10	125.24	16.82	8	10	126.52	9.12	9	9	116.11	8.40	7-8-9	29	122.85	12.62
	10	10	125.07	5.88	11	10	132.48	8.36	12	10	118.93	13.84	10-11-12	30	125.49	11.12
Total		31	123.99	10.94		31	129.39	10.36		28	118.89	10.63		90	124.26	11.36

TABLE n° 3 (d) : PERFORMANCE (FCR)

TREATMENT	A				B				C				TOTAL			
	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ	Group	N	x	σ
FCR D0-D7	1	10	2.26	0.19	2	10	2.63	0.48	3	10	2.19	0.61	1-2-3	30	2.36	0.49
	4	10	2.40	0.30	5	10	2.43	0.28	6	6	2.28	0.46	4-5-6	26	2.38	0.33
	7	10	2.24	0.26	8	10	2.55	0.47	9	10	2.40	0.46	7-8-9	30	2.40	0.41
	10	10	2.24	0.23	11	10	2.39	0.36	12	10	2.38	0.49	10-11-12	30	2.34	0.37
Total		40	2.29	0.25		40	2.50	0.40		36	2.32	0.50		116	2.37	0.40
FCR D7-D14	1	10	3.05	0.37	2	10	3.15	0.45	3	8	2.81	0.47	1-2-3	28	3.02	0.43
	4	1	3.76	-	5	1	3.12	-	6	1	3.43	-	4-5-6	3	2.93	0.28
	7	10	3.12	0.41	8	10	3.30	0.51	9	10	4.03	2.85	7-8-9	30	3.48	1.68
	10	10	3.00	0.43	11	10	3.20	0.41	12	10	3.85	2.68	10-11-12	30	3.35	1.57
Total		31	3.04	0.39		31	3.21	0.44		29	3.60	2.29		91	3.28	1.34
FCR D14-D21	1	10	3.69	0.45	2	10	3.47	0.21	3	8	3.24	0.47	1-2-3	28	3.48	0.42
	4	1	2.71	-	5	1	3.03	-	6	1	2.34	-	4-5-6	3	3.75	0.49
	7	10	3.81	1.43	8	10	3.25	0.45	9	9	3.08	0.39	7-8-9	29	3.39	0.93
	10	10	3.66	0.47	11	10	3.50	0.27	12	10	6.76	11.68	10-11-12	30	4.64	6.69
Total		31	3.72	0.86		31	3.40	0.33		28	4.48	6.97		90	3.85	3.90
FCR D0-D21	1	10	2.92	0.17	2	10	3.08	0.31	3	8	2.75	0.27	1-2-3	28	2.93	0.28
	4	1	3.71	-	5	1	2.60	-	6	1	3.97	-	4-5-6	3	2.98	0.24
	7	10	2.91	0.31	8	10	2.99	0.20	9	9	2.87	0.27	7-8-9	29	2.92	0.26
	10	10	2.89	0.18	11	10	3.00	0.27	12	10	2.97	0.50	10-11-12	30	2.95	0.34
Total		31	2.90	0.22		31	3.02	0.25		28	2.88	0.37		90	2.94	0.29