

# PATHOLOGY

01 - BOUCRAUT-BARALON C., PETIT F., PY R., BERTAGNOLI S., CHANTAL J.

## **Molecular epidemiology of Myxoma virus by pulsed field gel electrophoresis.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 1-8.

Laboratoire associé INRA-ENVT de Microbiologie moléculaire - Ecole Vétérinaire 23, chemin des Capelles, 31076 Toulouse cedex (France).

The pulsed field gel electrophoresis technique was used to determine the genomic sizes of seven Myxoma virus strains with distinctive pathogenic characteristics. The genome of some attenuated viral strains, in particular european vaccinal ones, showed deletions of 5 to 13 kb. This technique is suitable to study viral genomes in infected cells, without any further purification. Restriction pattern of DNA from different strains after *in situ* digestion of lysed infected cells in agarose plugs revealed a strain polymorphism able to distinguish genomes with similar sizes. Polymerase chain reaction amplification of some genomic locations, including virulence genes permits the characterization of deleted regions and possibly localization of new pathogenesis genes. Implications of these molecular studies for construction of new vaccinal vectors are discussed.

02 - CASTELLINI C., CENCI T.\*, SCUOTA S.\*, LATTAIOLI P., BATTAGLINI M.

## **Myxomatosis : possible implication in the use of artificial insemination.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 9-15.

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The Authors investigated the possible elimination of Myxomatosis virus in semen of bucks experimentally infected with a wild virus, and the possible manifestation of disease in does inseminated with infected semen. Semen samples were taken by artificial vagina twice a week. The virus was sometimes found in the semen and it was reisolated by experimental infection of rabbits, cell culture infection and electronic microscopy techniques. Afterwards we have studied possible transmission of disease by genital way, using the current practices of artificial insemination. Thus, some does have been infected by a semen preparation artificially contaminated with the wild virus. The does inseminated with infected semen died at different times during the gestation. Seminal elimination of virus, which was found in some animals even in the presymptomatic period, could represent a risk in the use of artificial insemination.

03 - CHALARENG C., PILLIEN F., BOURY M., TASCA C., DE RYCKE J., MILON A.

## **Adhesion and cytopathic effect mutants: towards new oral vaccines against rabbit O103 colibacillosis.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI,

La Rochelle 6-7 déc. 1994, 17-24.

Laboratoire associé INRA/ENVT de Microbiologie moléculaire, Ecole Nationale Vétérinaire, 23 chemin des Capelles, 31076 Toulouse cedex (France).

Increasing knowledge on the mechanisms of pathogenicity of the EPEC-like O103 *E. coli* strains that are responsible of enterocolitis in weaned rabbits is leading to new hopes in the field of oral vaccination using live non virulent strains. In this respect, we produced mutants that do not express either the adhesin AF/R2 (Adhesive Factor/Rabbit 2) or a new cytopathic effect (CPE) resulting of the *in vitro* interaction of virulent strains and HeLa cells. The pathogenicity of these mutants was tested in weaned rabbits. The AF/R2<sup>-</sup> mutant showed highly significant loss of pathogenicity, but still induced diarrheas and mortality in some animals. On the contrary, the CEP<sup>-</sup> mutant was completely devoid of residual pathogenicity and may be a good candidate to vaccination.

04 - CHOW T.T., PEETERS J.E., GEEROMS R.

## **Pathogenicity and electrophoretic soluble protein profiles (SDS-PAGE) of 50 enteropathogenic and saprophytic strains of *Escherichia coli* obtained from commercial rabbit farms.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 25-33.

Institut National de Recherches Vétérinaires - Groeselenberg, 99 - 1180 Bruxelles (Belgium)

The pathogenic effect and the soluble protein profile (SDS-PAGE) of 50 field strains of *Escherichia coli* has been tested: 36 strains have been isolated from diarrhoeic rabbits showing histologic lesions of colibacillosis (attachment and effacement of microvilli), 14 strains were issued from healthy rabbits. The strains belonged to six different pathotypes (bio/serotypes 1+/O109, 2+/O128, 2+/O132, 3-/O15, 4+/O26 and 8+/O103) or to non pathogenic bio/serotypes. The pathogenicity has been evaluated on base of zootechnic performance (weight gain and feed consumption), diarrhoea, mortality and histologic lesions. The results confirmed the high pathogenicity of strains 3-/O15, 4+/O26 and 8+/O103 and the weak pathogenicity of neonatal strains (1+/O109) for weaned rabbits. The pathogenicity of strains 2+/O128 and 2+/O132 was variable; some of these strains exerted the same pathogenic effect as strains 3-/O15 and 8+/O103. None of the saprophytic strains induced intestinal lesions. The results of SDS-PAGE showed that the protein profiles of enteropathogenic strains were more homogeneous than those of non pathogenic strains. Among the strains belonging to the respective bio/serotypes only discrete differences in electrophoretic profiles have been found.

05 - CHOW T.T., PEETERS J.E., GEEROMS R.

## **Inhibitory effect of polymyxin B on faecal enteropathogenic *Escherichia coli* (serotype O109 / biotype 1+) output after experimental infection of weaned rabbits.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 35-38.

Institut National de Recherches Vétérinaires - Groeselenberg 99 - 1180 Bruxelles (Belgium)

The inhibitory effect of polymyxin B, on the reduction of faecal

*Escherichia coli* output was evaluated after oral experimental infection. Rabbits were inoculated with  $10^7$  bacteria of an enteropathogenic strain of *E. coli* belonging to biotype 1+/serotype O109 seven days after starting medication. Twenty-four rabbits were divided in 3 equal groups of eight animals: one unmedicated infected group and two medicated infected groups. Medicated groups received respectively:  $5 \times 10^5$  and  $1 \times 10^6$  units (U) of polymyxin B per litre of water during 24 days. Faecal *E. coli* output was monitored by rectal sampling and semi-quantitative evaluation. Only  $1 \times 10^6$  U/l was able to reduce faecal output distinctly.

**06 - DROUET-VIARD F., COUDERT P., LICOIS D., BOIVIN M.**  
**Vaccination trial against *Eimeria magna* coccidiosis using a precocious line.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 39-43.  
INRA, Station de Pathologie Aviaire et Parasitologie, 37380 Nouzilly (France)

The rabbits of 8 suckling litters were vaccinated at 25 days (25 d group) with one of 2 doses ( $3.5 \times 10^3$  or  $3.5 \times 10^4$  oocyst) of a precocious line of *Eimeria magna*, and weaned at 28 days. The rabbits of eight at 28 days weaned litters were vaccinated in the same conditions at 29 days (29 d group). Nine rabbits from the same coccidia free colony served as control. At 34 days, all the rabbits were submitted to a challenge inoculation with a standard line of *E. magna*. A post-vaccination reduction of the growth rate is observed in the 29 d group only, probably in relation with the increase of the sensibility to coccidiosis with age. No post-vaccination diarrhea or mortality was observed in any group. After the challenge inoculation, no growth rate reduction was observed in the 29 d group. In the 25 d group, a growth reduction was observed, wider with the smallest dose. Four to ten days after the challenge, the oocyst daily out put in the vaccination groups was reduced by 10 to 1 000 times when compared to the control ( $3.4 \times 10^5$  to  $2.4 \times 10^7$  vs  $1.7 \times 10^8$ ).

**07 - DROUET-VIARD F., COUDERT P., ROUX C., LICOIS D., BOIVIN M.**

**Study of the immunity transmitted to young rabbits by does immunized against *Eimeria magna*.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 45-52.  
INRA, Station de Pathologie Aviaire et Parasitologie, 37380 Nouzilly (France)

Coccidia free rabbit does were immunized by repeated inoculations of  $10^4$  oocysts of *Eimeria magna* precocious line, before and during gestation. The oocyst output was completely controlled after the third inoculation and no sign of illness was ever observed. The serum antibodies (Ab) were titrated by an ELISA method. In the controls, the Ab titers were constantly below 0.25 DO. In immunized does, the Ab level began to increase 4 weeks after the first inoculation. Booster inoculations given 7 and 4 days before parturition produced a high increase of Ab level. One half of the females received an injection of sporozoites extracts 2 and 17 days after parturition; these injections produced a dramatic increase of the Ab level compared with that of the non treated females. The serum Ab of the weanlings were titrated at 29 and 39 days of age. In all groups, the Ab titers were very low but a slightly higher level was observed in the groups

corresponding to the immunized does. The dramatic increase of the Ab titers of the does injected with sporozoites had no effect on the Ab titers of their litters. To test the protection transmitted by the mothers, the weanlings were inoculated with  $10^5$  oocysts of the same *Eimeria magna* precocious line and the oocyst output was measured. The whole excretion of oocysts was identical ( $6 \times 10^7$  oocysts) in all groups, and the decrease in weight gain was identical to that of inoculated controls. We can conclude that the does' immunity did not protect the litter against an infection with *E. magna*.

**08 - ESSLINGER J., HERRMANN G., BLOBEL H.**

**Adhesion of *Pasteurella multocida* to rabbit macrophages.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 53-60.  
Bacteriologie et Immunologie, Faculté de Médecine Vétérinaire, Justus Liebig Universität, Frankfurterstr. 107, 35392 GIESSEN (Germany)

Of 27 *Pasteurella multocida* strains belonging to serotypes A, B, D and E, all 14 strains of serotype A adhered strongly to alveolar macrophages (AM) from rabbits, but not to peritoneal or blood macrophages. Strains of serotype B, D or E adhered only weakly or not at all. Furthermore, the A-strains adhered exclusively to AM in frozen lung sections of rabbits.

Adhesion to AM *in vitro* was markedly reduced by pretreatment of the bacteria with hyaluronidase or preincubation of the macrophages with hyaluronic acid. These findings may indicate a possible role of hyaluronic acid (contained in the capsule of serotype A *pasteurellae*) in adhesion and subsequent colonization.

**09 - FOUCAULD J. de, FOURNIER D.**

**Evaluation of the protection against myxomatosis and viral haemorrhagic disease of rabbit conferred by a bivalent vaccine administered intradermally.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 61-65.  
RHÔNE MÉRIEUX, 29, Avenue Tony Garnier, 69007 Lyon (France)

A group of rabbits was vaccinated intradermally at 4 weeks of age with a homologous SG33 strain myxomatosis vaccine and at 10 weeks of age with a bivalent vaccine containing the "myxomatosis" (SG33 strain) attenuated component and the "rabbit haemorrhagic disease" (VHD) component. Some of the vaccinated rabbits, along with unvaccinated control rabbits, were challenged with a virulent myxomatosis virus, approximately 4 months after vaccination with the bivalent vaccine. All the control rabbits developed myxomatosis within 4 days. None of the vaccinated animals were affected with the disease. One year after vaccination with the bivalent vaccine, all the remaining vaccinated rabbits along with control rabbits, were challenged with a virulent VHD virus. Eight out of ten control rabbits and one out of twelve vaccinated animals died.

As a result of these preliminary trials we can envisage a vaccination schedule for breeding animals which includes a bivalent vaccine against viral haemorrhagic disease of rabbits and myxomatosis, being given intradermally.

**10 - GACA-LAGODZINSKA K., PROVOT F.\*, P. COUDERT P.\***  
**Salinomycin tolerance in the rabbit. Efficiency against three rabbit coccidia species.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 67-72.

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\* INRA Station de Pathologie Aviaire et de Parasitologie 37380 Nouzilly (France)

The anticoccidial effect of Salinomycin against *E. flavescens*, *E. intestinalis*, and *E. stiedai* where tested in experimentally infected rabbits.

The drug was used at different level (12 to 80 ppm) in feed and its tolerance by the rabbits was evaluated.

It was demonstrated from two trials using 248 rabbits that there were a negative regression between weight gain or the feed consumption and the increase in concentration of Salinomycin. With 18-19 ppm in feed, all symptoms of intestinal or liver coccidiosis are eliminated and this dose is well tolerated.

**11 - HENDRICKX W., BOLS L., DE WITTE J.**

**Transfer from a conventional level to a "minimal disease level" in a rabbit farm. Evaluation of the performances five years later.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 73-80.

N.V. VERLABREED S.A., Kapellestraat 70, 9800 DEINZE, (Belgium)

In view of the failure of antibiotic treatments to improve the living conditions of a selected Cunistar breed on a long-term basis, it was decided to use a more radical method. A new closed production unit protected from external contaminants was built. It was populated by young rabbits originating from the best females of the selection unit born by aseptic Caesarean and artificially suckled. The implanted intestinal flora originated from a healthy rabbit was bacteriologically controlled. Very strict hygienic measures have been taken. The checks done during the next 5 years have demonstrated that these techniques as a whole allowed to create and maintain a breeding unit of rabbit free of the main specific pathogenic agents (Minimal Disease Level : MDL). This change in sanitary status is expressed by a spectacular and durable improvement in the rabbits' zootechnical performances and in particular in mortality after weaning stabilizing at approximately 2 %.

**12 - LAFARGUE-HAURET P., JARRIN D., RICCA V., ROUILLE R.**

**Amoxicillin : toxicity for the rabbits**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 81-84.

Sanders Aliments - 17 quai de l'Industrie - 91200 Athis-Mons (France)

Toxicity of amoxicillin incorporated in rabbit pelleted feed (0, 5, 10, 20 and 40 ppm) has been evaluated on 5 groups of 10 rabbits from 42 to 55 days of age. There was no mortality in the control group. For the other groups, mortality ranged from 10 to 60 %, while the feed intake and the weight gain decrease proportionally to the amoxicillin increase. The practical conclusion of this study is, even the lowest concentration of amoxicillin has a growth depressive effect.

**13 - LICOIS D., HUMBERT J.F., CERE N., AFANASSIEFF M., CHANTELOUP N.**

**Genetic characterization of the *Eimeria* from the rabbit and isolation of an *Eimeria media*-specific DNA probe : a tool for the diagnostic and the study of coccidia from the rabbit.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 85-90.

INRA, Station de Pathologie Aviaire et de Parasitologie, 37380 Nouzilly (France)

A study of the genetic polymorphism was performed in rabbit coccidia, using the Random Amplified Polymorphic DNA (RAPD). The comparative analysis of the amplified fingerprints showed that the DNA patterns obtained were very different according to the species. On the other hand, species-specific fingerprints could be observed. In *E. media*, the analysis of the RAPD products showed weak differences between strains or lines but however allowed to differentiate the lines deriving from the multiplication of a single oocyst. After identification of a 800 base pairs (bp) fragment specific of *E. media*, this fragment was isolated, cloned and DIG-labelled. After southern blot, we observed that this probe was specific of *E. media*. Sequencing of the 5' and 3' ends of this probe, allowed us to determine 2 primers that have been used in a Polymerase Chain Reaction. An amplified product of 750 bp has been proved to be specific of *E. media* showing that this technique could be a useful tool for different purposes (diagnosis, epidemiological studies...).

**14 - ORSENIGO R., GIUSTI A M.\*, PEETERS J.E.\*\*,**  
**SCANZIANI E.\*, GALLAZZI D.\*, ENICE F.\***

**Histological and immunohistochemical investigations in rabbits experimentally infected with enteropathogenic *Escherichia coli* and *Clostridium spiroforme* and fed with different levels of starch and fat.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 91-98

Istituto di Zootecnica Generale,

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Eighty rabbits, divided in 8 groups of 10 animals each have been examined. Six groups were infected orally with *Escherichia coli* (EPEC) strain, belonging to sero/biotype O132/2+, and/or with *Clostridium spiroforme* strain NCTC 11493, while two groups were kept as negative controls. All animals were fed with one of two iso-energetic and iso-proteic feeds but with a different level of fat and starch. Our results showed the influence of diet on enteropathogenic organisms proliferation. Histological lesions were more pronounced in the caecum of rabbits feeded with a hyperlipidic diet, sacrificed 8 days p.i. and infected with both organisms or only with *E. coli* O132/2+. *C. spiroforme* has never been observed. Location and distribution of *E. coli* O132/2+ were also detected with an immunohistochemical procedure, using a primary antibody against *E. coli* capsular antigen, when these organisms were not found in routine histological preparations.

15 - PEETERS J.E., GEEROMS R., CHOW T.T.

**Pathogenicity of 6 strains of *Eimeria magna*, *media* and *perforans* in weanling rabbits and anticoccidial effect of 1 ppm of diclazuril**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 99-104.

Institut National de Recherches Vétérinaires, Groeselenberg 99 - 1180 Bruxelles (Belgium)

The influence of 3 *Eimeria* species on zootechnical performance was evaluated. Simultaneously, the anticoccidial effect of diclazuril was tested. Rabbits infected with  $10^5$  oocysts of *E. perforans* or *E. media* or with  $5 \times 10^4$  oocysts of *E. magna* showed a weight gain reduction of 15%, 41% or 38 %, whereas FCR was altered by 8 %, 33 % or 27 % three weeks afterwards ( $p < 0.05$ ). There was no significant difference in pathology between different isolates of the same species. A mixed infection with the 3 species exerted a stronger effect ( $p < 0.05$ ). Low mortality was detected after infection with *E. media* or *E. magna* or after mixed infection. Oocyst output reached 62, 163, 139 and 160 x  $10^6$  oocysts after infection with *E. perforans*, *E. media*, *E. magna* or after a mixed infection. Incorporation of 1 ppm of diclazuril in the feed prevented mortality and reduced oocyst output of *E. magna*, *E. media* and *E. perforans* by 94 %, 71 % or 56 % ( $P < 0.05$ ). Weight loss was only partially prevented. After a mixed infection including the 3 species, weight gain was significantly improved by 35 % in comparison with infected unmedicated controls, whereas no noticeable improvement of average daily gain reduction was noticed in comparison with non infected controls. Oocyst output was reduced by 71 %. In case of *E. magna*, a distinct difference in anticoccidial effect was established according to the isolates tested : oocyst output of strain U93/298 was reduced by 88.5 % against 99.95 % in case of infection with strain U93/407. In case of the former strain the drug had no influence on zootechnical performance, whereas treatment after infection with the latter strain resulted in a significant improvement of weight gain and FCR. Performance was comparable to that of uninfected unmedicated controls.

16 - PEETERS J.E., ORSENIGO R., MAERTENS L.\*, COLIN M.\*\*  
**Promoting effect of dietary beet pulp on iota-enterotoxaemia (*C. spiroforme*) in rabbits at weaning**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 105-112.

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A total of 60 rabbits, weaned at 28 days of age, were fed one of two iso-energetic (10.2 MJ ADE/kg) and iso-proteic (175 g/kg) diets *ad libitum*. The diets differed in the main fiber source; 240 g/kg beet pulp were incorporated in diet P and 54 g/kg flax chaf and 300 g/kg alfalfa meal in diet F. As a result, diets P and F contained 142 and 163 g/kg crude fiber, 102 and 138 g/kg indigestible fiber, 343 and 311 g/kg NDF, 170 and 181 g/kg ADF and 46 and 66 g/kg ADL, respectively. Rabbits were inoculated orally with *Clostridium spiroforme* strain NCTC 11493. Utilization of diet P was followed by a significant 30 % increase of caecal weight and a 22 % increase in total volatile fatty acid (VFA) production, whereas caecal pH decreased by 0.27 units in comparison with diet F.  $\text{NH}_3$ -levels were unaffected. None of the diets significantly favoured proliferation of saprophytic

faecal *E. coli*. Diet P promoted iota-enterotoxaemia with reduced weight gain (-29 %) and impaired feed conversion (+40 %). Faecal *C. spiroforme* scores remained elevated during 24 days post-infection ( $P < 0.05$ ), two out of 10 rabbits died of enterotoxaemia and two more rabbits showed liquid diarrhoea. Experimental infection of rabbits fed diet F on the contrary was not associated with clinical signs.

17 - RIDEAUD P., COUDERT P.\*

**Identification of *Pasteurella multocida* pathogenic strains for rabbits.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 113-120.

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Previous results, formerly obtained, indicated a positive correlation between *in vitro* culture diameter of colony of different strains of *P. multocida* and their pathogenicity for the rabbit. This work, using 52 strains isolated from a "complexe cunicole", confirm the previous results. Furthermore it appears clearly that the strains forming small colonies are also deprived of ornithine decarboxylase (ODC-). The strains without ODC have a weak pathogenicity if any and it does not depend of the diameter of the colony. This criteria ODC+ or - is proposed in addition of the diameter of the colony to presume the pathogenicity of the strains of *P. multocida*. The classification of the species *multocida* among the genus *Pasteurella* is also discussed.

## REPRODUCTION

18 - P. ARVEUX P., TROISLOUCHES G.

**Effect of a lighting programme 8 h/4 h (L/D) on rabbit does reproduction.**

6èmes Journées de la Recherche Cunicole en France, INRA-ITAVI, La Rochelle 6-7 déc. 1994, 121-126.

UNCAA DPA Ets UCAAB - BP 19 - 02402 Chierry (France)

Nulliparous does from a strain selected for litter size, were divided in 2 equivalent groups of 18 each : Control group was submitted to lighting during 16 hours consecutively per 24 hours, experimental group was submitted to lighting during 2 periods of 8 hours each, separated with 4 hours darkness.

The trial stopped at the end of the 6<sup>th</sup> litter of the does (dead or culled does were not replaced during the trial).

Experimental group does performance was :

\* Lower mortality and culling rate (42.9 versus 71.4 %)

\* Better response to mating (interval between kindling and successful mating of 19.2 days versus 24.2) and increased fertility (82.6 % versus 67.6 %).

\* Does weight gain between kindling and weaning was higher ; feed intake was also higher (479 versus 446 g per day for litter plus multiparous doe from kindling to weaning).

\* Increased productivity (58.9 weaned rabbits per doe and per year versus 52.8)