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DOMESTIC RABBIT ENTEROPATHIES. LICOIS D. *INRA, BioAgresseurs, Santé, Environnement, Nouzilly, France.*

Digestive disorders are the main cause of morbidity and mortality, in fattening rabbit and are responsible for important economic losses in industrial rabbit farms. Among the specific causes of the intestinal pathology, parasites (coccidia) and some bacteria, mainly enteropathogenic *E. coli*, are predominant. Since 1997, a new gastrointestinal syndrome called epizootic rabbit enteropathy (ERE), close to another digestive pathology, the mucoid enteropathy, has appeared in Europe with a high incidence on mortality and morbidity. The etiological agent of this emergent disease was still not identified for the moment. During the last ten years, thanks to the rise of biotechnologies and molecular biology, considerable progress were obtained with regard to the identification of the virulence factors and to the knowledge of the mechanisms of pathogenicity of the rabbit *E. coli*. In parallel, beside coccidiostats available for the treatment of coccidiosis, new prophylactic prospects based on vaccination were developed against the coccidia. In spite of difficulties, an experimental model to study ERE has been established and efforts are concentrated by some teams to identify and isolate the pathogenic agent(s) of this disease. Associated with the understanding of the physiopathology of the gut, at least in part under the influence of

nutritional factors (both not developed here), the data obtained in these different areas would lead to new perspectives of control of the rabbit enteropathies.

MOLECULAR PROFILING OF THE MAJOR BACTERIAL SPECIES IN THE RABBIT CAECUM AS AFFECTED BY THERAPEUTICAL DOSES OF ANTIBIOTICS. ABECIA L.^{*,†}, MCEWAN N.R.[†], NEWBOLD C.J.[‡], FONDEVILA M.^{*}, BALCELLS J.^{*} **Dpt. de Prod. Animal y Ciencia de los Alimentos, Univ. de Zaragoza, Spain; †Rowett Res. Inst., Aberdeen, Scotland; ‡Inst. of Rural Studies, Univ. of Wales, Aberystwyth, Wales*

Intensive rabbit production is hampered by pathologies that restrict animal growth and often cause important death rates. The use of therapeutical doses of antibiotics added to feed is a common practise in such situation. However, to what extent these treatments may affect bacterial population and consequently nutrient utilisation by growing rabbits is as yet poorly understood. This paper makes use of Denaturing Gradient Gel Electrophoresis (DGGE), a molecular profiling technique, to study population shifts within the major bacterial species in the caecum of the rabbit following dietary supplementation with three of these antibiotics (bacitracin, chlortetracycline and tiamulin). The resulting profiles were interpreted by carrying out pair wise comparisons and measuring Hamming Distances between profiles. The dendogram resulting from this analysis demonstrates that there is a relatively large

variation in the major bacterial species present in control animals (as assessed by the long branches in the dendrogram). Branches of a similar length were also observed for samples collected from animals that had been fed bacitracin. However, less variation was observed in the samples from animals fed either chlortetracycline or tiamulin. These observations suggest that the biodiversity levels of the major bacterial species present in the caecum of the rabbits is less following feeding with either chlortetracycline or tiamulin, and that the effects of these antibiotics is more profound than that seen in the bacitracin.

SELECTIVE CULTURE MEDIUM TO ISOLATE *CLOSTRIDIUM SPIROFORME* FROM RABBIT GUT.

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Enteritis due to *Clostridium spiroforme* is an emerging problem of intensive rabbit breeding. To control the infection a laboratory support is needed both to diagnose the clostridiosis and to target the therapy. The aetiology can be suspected on the basis of the typical lesions and the bacterioscopic examination of the large intestine content. The isolation of *Clostridium spiroforme* is necessary to confirm the diagnosis and to evaluate the drug susceptibility. Isolation of *Clostridium spiroforme* is quite difficult because it requires a rich culture media and needs strictly anaerobic conditions; the microorganism has a low surviving time and produces small colonies not easy to identify. Moreover the isolation can fail because of overgrowing of other anaerobic or facultative anaerobic species (i.e. *Proteus* spp.). Thus the isolation procedure is too related to the subjective ability of the technician. To avoid the contamination problems a selective culture media (SM) to isolate *Clostridium spiroforme* was standardized. The commercial PAB medium (Perfringens Agar base, Oxoid) was supplemented with SFP (Shahidi-Ferguson-Perfringens Selective Supplement,

Oxoid) and added with a 5% of sheep red blood cells, 500 µg/ml of tylosin (Tylosin tartrate, Sigma) and 12,5 µg/ml of rifampicin (Rifampicin powder, Sigma). The SM was tested with 105 samples of different clinical significance and compared with the control medium. Results evidenced the ability of the SM to isolate *Clostridium spiroforme* in 79% of samples. Best results were obtained with samples without chemical and physical pre-treatment, so that it was enhanced the sensitivity of the medium while reducing both time and tools consuming. Moreover the capacity of the medium to isolate pure colonies from samples of high clinical relevance (47.5% of isolation against 13.3% of control media) reduces the necessity of specialized personal and makes the procedure easier to standardize.

REPORT OF ENTEROPATHOGENIC *E. COLI* (EPEC) ISOLATED FROM ENTERIC OUTBREAKS IN ITALIAN INTENSIVE RABBIT HERDS.

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Enteropathogenic *Escherichia coli* (EPEC) can hurt the rabbit intestinal wall with the *eae* gene products characteristic of human EPEC strains; they can be classified into sero/biotypes. The present paper updates the Italian EPEC distribution and examines the isolation frequency of EPEC strains during the 1999-2004 period. The biotype classification of 6274 *Escherichia coli* field strains, the *eae* gene detection in 2106 isolates, as well as the O typing of 498 strains, allowed us to consider the B12, B14, B20, B30 *eae*⁺ and B28 *eae*⁺ biotypes as the most frequent panel of the EPEC strains and the B19, B31, B30 *eae*⁻ and B28 *eae*⁻ biotypes as the most frequent panel of the not EPEC strains. Compared to biotyping, O-typing did not give additional information seeing that most of O103 strains resulted EPEC, while not all EPEC belonged to O103 serogroup and several resulted not typeable. At the present

colibacillosis represents one of the most important causes of enteric disease in Italian rabbit breeding units. During the last 5 years of diagnostic experience a low reduction of EPEC strains was observed from 1999 to 2002, then an increase was registered and, at present, approximately 45% of *E. coli* isolates possess the *eae* gene.

MEASUREMENT OF RABBIT'S INTESTINAL VILLUS: PRELIMINARY COMPARISON OF TWO METHODS. ALVES A., PINHEIRO V., MOURAO J.L., PIRES I., OLIVEIRA J., GAMA A. *CECAV – Univ. de Trás-os-Montes e Alto Douro, Vila Real, Portugal.* aalves@utad.pt

This work applies the microdissection technique, described by Clarke (1977), and the conventionally prepared sections stained with haematoxylin and eosin to samples from rabbit small intestine. Twenty-four rabbits, previously brooded under identical conditions with a basal diet were slaughtered at 24 (n=8), 32 (n=8) and 42 (n=8) days of age to collect samples for laboratory analysis. Two adjacent sections of ileum were collected, one was fixed in 10% neutral formalin for paraffin processing and the other immersed in Clark's fixative for microdissection. Intestinal architecture was evaluated by measuring villi height and width and crypt depth in both methods. The results show that measurements of villi and crypts in histological sections were consistently shorter than those obtained in microdissection technique. Microdissection method gives accurate information and insight into the relative sizes and shapes of villi and crypts, does not cause retraction artefacts related with dehydration, but as a limited time of execution and observation, while HE preparations are almost eternal. Besides that, HE preparations are also suitable for pathologic diagnosis. In conclusion we think that the microdissection method complements the conventionally haematoxylin and eosin method.

THE 16S r-DNA RFLP PROFILE OF TOTAL DNA OF INTESTINAL BACTERIA UNDER PATHOLOGICAL CONDITIONS. BADIOLA I.^{*}, PÉREZ DE ROZAS A.M.^{*}, ROCA M.^{*}, CARABAÑO R.[†], GARCÍA J.[†], ROSELL J.M.[‡] **CRESA (UAB - IRTA). Barcelona. ignacio.badiola@irta.es* [†]Dpt. de Prod. Animal. E.T.S.I. Agrónomos. Madrid. [‡]NANTA, Madrid, Spain.

The study of complex microbial communities by culture-independent techniques has improved the global knowledge of the microbial ecology of complex ecosystems. Differences in the positions of cleavage sites for restriction enzymes in a universal gene, as 16S r-DNA, are a powerful tool to analyse the microbial ecology of complex microbiota, as gut systems are, and a good method to analyse bacteria that are difficult to culture. The aim of the present study was to compare the intestinal bacteria communities present in the gut of healthy or sick rabbits, by restriction fragment length polymorphism (RFLP). Total DNA was extracted from the intestinal contents, collected in ethanol, by the QIAamp DNA Stool Mini Kit (QIAGEN), with some modifications. A fragment of the bacterial 16S r-DNA gene was amplified by PCR with CTACGGGAGGCAGCAGT and CCGTCWATTC MTTTGAGTTT primers and digested with five different restriction enzymes (*Alu* I, *Rsa* I, *Hpa* II, *Sau* 3A I or *Cfo* I). Some intestinal disorders are characterised by a dysbiosis, with an increment in the number of the bacterium that cause the characteristic symptomatology of the disease. Divergences in 16S r-DNA sequences between bacterial species can produce specific RFLP profiles because of differences in the cleavage sites of the restriction enzymes. With this method we have observed some RFLP profiles characteristic of different digestive bacterial disorders as Epizootic Rabbit Enteropathy, colibacillosis, clostridiosis or antimicrobial dysbacteriosis. The construction of a data base with the RFLP profiles associated with different diseases will be a useful instrument to assist the laboratorial diagnostic in rabbit pathology.

NEW CHARACTERISTICS, PREVENTION AND CURE TECHNIQUE OF COCCIDIOSIS IN RABBIT. BAO J.C., ZI L.G., Q.H.K., C.Z., W.S.R., YU T.H. *Mountain Area Res. Inst. of Agricultural Univ. of Hebei. Baoding. China. hebaugz1@sohu.com*

In this paper, the happening of coccidiosis in rabbit were investigated and its new characteristics and trends were concluded in late ten years. Coccidiosis was enlargement of happening time, universality of anti-drug, seriousness of drug poisoning, complexity of compound infect non-typicality of clinical symptoms and increase of mortality. *Prevent experiment:* the young rabbits (1~3 months old) were selected and divided into 3 groups according to baby weight and weaning time new anti-coccidiosis drug "Kill-coccidiosis" (1884 animals) were made and got the better results compared with the traditional drugs (such as Robenidine (1586 animals) and Coyden (1462 animals). They were observed for 6 weeks. The results showed that the mortality of Kill-coccidiosis, Robenidine and Coyden groups were respectively 2.9%, 22.9%, 18.4%. The Kill-coccidiosis group was significant different with the other two groups ($P < 0.01$). The dead rabbit were autopsied to observe the symptom and the mortality was calculated. *Cure experiment:* The selected sick baby rabbits were divided into 3 groups and fed with different drugs (Kill-coccidiosis (482 animals), Robenidine (266 animals) and Coyden 258 animals). The observation lasted 3 weeks and the cured percentage calculated. The experiment result showed the dead amounts of Kill-coccidiosis, Robenidine and Coyden group was 4.77%, 22.18%, 21.32% respectively. So the Kill-coccidiosis was a kind of effective drug for coccidiosis. The methods which was effective preventing coccidiosis were pointed out.

USE OF A 3-/O15 Deae ENTEROPATHOGENIC E. COLI VACCINE IN A RABBITRY WITH MIXED ENTEROPATHY PROBLEMS: SPREADING CHARACTERISTICS AND PROTECTIVE EFFECT. BOHEZ L.*, MAERTENS L.†, LAEVENS H.*, STAKENBORG T.*, PEETERS J.*, VANDEKERCHOVE D.* **Vet. & Agrochem. Res. C., Brussel, †C. for Agronomical Res., Melle, Belgium. dovan@var.fgov.be*

In a rabbitry affected by colibacillosis, complicated by epizootic enteropathy, preliminary screenings detected the presence of enteropathogenic *E. coli* (EPEC) pathotypes 1+/O109 and 2+/O128. A trial was performed involving 108 newly weaned rabbits, in order to evaluate the capacity of an attenuated EPEC strain of pathotype 3-/O15, deleted in the *ea* gene, to spread between cages, and to assess its protective effect. Litters of six were distributed homogeneously over a vaccination and a control group, housed in a separate building but under comparable environmental conditions. Rabbits were caged per three. In the vaccination group, the rabbits of three cages were vaccinated *per os* with 109 CFU of the vaccine strain. The other cages had side, corner or no direct contact with the vaccinated rabbits. The experiment lasted for five weeks. Rabbits were assessed for clinical symptoms, *E. coli* excretion, serum antibodies, feed intake and body weight. Rabbits that died during the experiment were necropsied. The vaccine strain did not spread on a detectable level between the cages, indicating that all rabbits in a rabbitry need to be vaccinated. The wildtype *E. coli* strains isolated belonged to pathotypes 1+/O109 and 2+/O132. No significant differences were found between the control and the vaccination group for diarrhoea, mortality, necropsy lesions, coprological or bacteriological results. Nevertheless, the serological results of an EspB-based ELISA yielded a significant difference between the vaccinated and control rabbits. This might indicate that the vaccine strain effectively limits wildtype strains in their possibilities to colonise and thus immunostimulate the rabbits. The production parameters of the vaccinated rabbits and the other rabbits were similar, indicating that the vaccine strain has no adverse effect in this respect.

AN ATTENUATED 2+/O132 Dtir ENTEROPATHOGENIC E. COLI OFFERS CROSS PROTECTION AGAINST A 3-/O15 CHALLENGE AND PARTIAL PROTECTION AGAINST AN 8+/O103 CHALLENGE BOHEZ L., STAKENBORG T., LAEVENS H., PEETERS J., VANDEKERCHOVE D. *Vet. and Agrochem. Res. C., Brussel. dovan@var.fgov.be*

Enteropathogenic *E. coli* (EPEC) induce severe diarrhoea in rabbits and cause important losses after weaning. In Belgium and the Netherlands the bio/serotype 3-/O15 is the most prevalent, while in France, Spain and Italy EPEC of the 8+/O103 and less frequently 4+/O26 types are detected. An effective vaccine would protect rabbits against EPEC-associated diarrhoea, and limit antibiotic use. One of the virulence characteristics of EPEC is the mechanism of attachment and effacement (A/E). The adhesion of the bacterial outer membrane protein intimin to the translocated receptor of bacterial origin in the enterocyte's cell membrane (Tir), results in intimate attachment and is followed by effacement of the enterocyte's microvilli. We have created an attenuated mutant, as shown by experimental infection, by deleting the *tir* gene of EPEC strain 82/90 (2+/O132 ?*tir*). The rabbits vaccinated *per os* with this live attenuated strain were protected against a heterologous challenge with a 3-/O15 strain and partially protected against a heterologous challenge with an 8+/O103 strain. Previous experiments with a 3-/O15 EPEC strain deleted in the *eae* gene, thus unable to produce intimin, showed that this ? *eae* mutant induced insufficient cross protection against other pathotypes. This led to the conclusion that none of the LEE gene products, other than intimin, offered a sufficient cross protection in vaccination-challenge trials with heterologous EPEC strains. In the experiments presented here, it was shown that an attenuated strain still producing the highly immunogenic intimin was not capable either of offering sufficient cross protection. A study identifying immunoprotective antigens of EPEC should be performed. Its results might allow the construction of one or more attenuated mutants that could be used as vaccine strains, and offer perspectives towards the control of colibacillosis in meat rabbits.

DIFFERENT ADMINISTRATION METHODS FOR THE 3-/O15 Deae EPEC VACCINE STRAIN PROTECTING MEAT RABBITS AGAINST A 3-/O15 CHALLENGE: PRELIMINARY RESULTS. BOHEZ L., STAKENBORG T., LAEVENS H., PEETERS J., VANDEKERCHOVE D. *Vet. and Agrochem. Res. Centre, Brussel, Belgium.* dovan@var.fgov.be

Six groups of six individually housed SPF rabbits, five weeks of age, were analysed for presence of enteropathogenic *E. coli* (EPEC), *Clostridium spiroforme*, rotavirus and coccidiosis. All animals were negative for all pathogens tested, except one, which was positive for *C. spiroforme*. Four groups were vaccinated with an attenuated EPEC strain of the 3-/O15 pathotype (*Deae*) at 1.5×10^9 CFU/ml. The first group received the vaccine directly in the oral cavity by use of a syringe. The second group received the vaccine via the drinking water in individual water bottles. In the third group, the vaccine was sprayed on the rabbits' fur, and in the fourth group it was sprayed on the feed. The fifth (unvaccinated challenged) and sixth group (negative control) were not vaccinated. Three weeks later, groups one to five were challenged *per os* with 10^7 CFU of a 3-/O15 wildtype (WT) strain. The negative control group did not receive a challenge. All vaccinated rabbits excreted the vaccine strain on a detectable level, except three animals from group 2. After challenge, all rabbits from the unvaccinated challenged group excreted the WT strain in high numbers. Excretion in the vaccinated groups was much more limited. No significant differences were observed for body weight after challenge, due to some loss of virulence of the challenge strain, in combination with the rather small groups and the individual differences, causing large standard deviations. However, for feed intake a significant difference was observed after challenge between the unvaccinated challenged group and the negative control group ($P=0.0053$), whereas no significant difference was found between the negative control group and the vaccinated groups. Symptoms of enteritis were seen in only one rabbit, of the unvaccinated challenged group. We conclude that all administration methods tested resulted in colonisation of the rabbits'

gut by the vaccine strain. There are indications that all methods might yield an effective protection against a challenge infection with a 3-/O15 WT strain. However, the experiment must be repeated with more rabbits per group and a more virulent challenge strain.

EVALUATION OF THE EFFECTIVENESS OF SOLUBLE BACITRACIN (BACIVET S[®]) IN DRINKING WATER COMPARED TO BACITRACIN IN THE FEED (ALBAC[®]), DURING AN EXPERIMENTAL REPRODUCTION OF EPIZOOTIC RABBIT ENTEROPATHY SYNDROME. BOISOT P.^{*}, DUPERRAY J.^{*}, GUTONVARCH A.^{*}, RICHARD A.[†], LICOIS D.[‡], COUDERT P.[‡] ^{*}*Evalis, Vannes.* [†]*Alpharma, Verrières le Buisson.* [‡]*INRA, Unité Bio-Agresseur, Santé, Environnement, Nouzilly, France*

The effectiveness of soluble bacitracin in drinking water (Bacivet S[®]) compared to bacitracin in the feed (Albac[®]) was tested, during an experimental infection of ERE syndrome. Treatments were used before or after the experimental contamination. Weanlings (168) were divided at 32 days of age into 4 groups: contaminated but not medicated control group, group with bacitracin 100ppm in the feed (Albac[®]), 2 groups medicated with Bacivet S[®] (0.675 g/l of drinking water) before (preventive use) or after (curative use) contamination. Results of this study confirm the efficiency of bacitracin in ERE syndrome conditions with a significant reduction in mortality and morbidity compared to the non treated group. A preventive use of Bacivet S[®] is as effective as bacitracin 100ppm in the feed (Albac[®]) during the acute period of the disease. The curative use of Bacivet S[®] during 14 days, after the observations of the first symptoms, also reduces rabbits mortality and morbidity compared to the control but is less effective than a preventive use.

GENETICALLY ENGINEERED ENTEROPATHOGENIC *E. COLI* STRAIN PROTECTS RABBITS AGAINST COLIBACILLOSIS. BOULLIER S., NOUGAYRÈDE J-P., MARCHES O., TASCA C., MILON A. *Ecole Vétérinaire, Toulouse, France.*
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Enteropathogenic *E. coli* (EPEC) is a well-established cause of serious diarrhoea in young children and in different animal species like rabbits (REPEC). EPEC strains induced a specific «attaching-effacing» lesion, characterized by a destruction of the enterocyte brush border and an intimate bacterial attachment. Bacterial effectors are coded in the locus of enterocyte effacement (LEE). Inactivation of every gene of the LEE leads to a decrease of virulence, although bacteria could still colonize the intestinal tract. The aim of this study was to generate a vaccinal REPEC strain to protect rabbits in breeding units. We inactivated by allelic exchanges two genes of the LEE, coding proteins injected into the host cell by a type III secretion system: the gene coding EspB, a protein forming a pore in the host cell membrane and the gene coding Tir, a protein injected in the host cell surface and representing the receptor of another bacterial protein, Intimin. Tir-intimin interaction allows intimate attachment of bacteria to cells. In the first part of our study we showed that the vaccinal strain, E22DTir/EspB, was completely safe: it did not induce diarrhoea nor any histological intestinal lesions but it was still able to colonize the intestinal tract. We then showed that E22DTir/EspB was able to protect rabbits against an early (7 days post vaccination) and a late (28 days post vaccination) virulent challenges with the parental strain E22. In addition, the vaccinal strain blocked the shedding of the virulent strain, decreasing the risk of bacterial transmission between rabbits. Antibodies against LPS O103, the bacterial adhesin AF/R2 and the Intimin were detected as soon as 7 days post E22DTir/EspB inoculation. Anti-AF/R2 Abs could blocked bacterial adhesion *in vitro*. These results indicated that E22DTir/EspB is a good vaccine candidate to protect weaned rabbits against REPEC infections in fattening breedings, efficient with a single inoculation dose.

VIRULENCE GENES AND ANTIMICROBIAL RESISTANCE PATTERNS OF ENTEROPATHOGENIC *E. COLI* FROM RABBITS IN SOUTHERN ITALY. CAMARDA A., PENNELLI D., BATTISTA P., MARTELLA V., GRECO L., ALLOGGIO I., MAZZOLINI E.* *Dpt. di Sanità e Benessere degli Animali. Univ. degli Studi di Bari. Italy.* †*Ist. Zooprof. Sp. delle Venezie Sez. di Udine. Italy.* a.camarda@veterinaria.uniba.it

A total of 56 *E. coli* strains isolated from enteritis outbreaks in 28 rabbitries were biotyped and checked for the presence of the *eae*, AF/R1 and AF/R2 genes. Antimicrobial resistance to gentamicin (GM10), amikacin (AN30), tetracycline (TE30), erythromycin (E15), spiramycin (SP100), enrofloxacin (ENR5), trimethoprim/sulphamethoxazole (SXT), flumequine (AR30), amoxicillin (AMX 25), apramycin (APR30), difloxacin (DFX10), marbofloxacin (MAR5), nalidixic acid (NA30), neomycin (N30), colistin (CL50), streptomycin (S10). on 55 *E. coli* isolates has been carried out. Either the virulence genes *eae* or AF/R2 were detected in 20 of the 56 isolates, belonging to 10 distinct farms (35,71% of the farms). The AF/R1 gene, encoding for the fimbrial adhesin, was not detected in any of the isolates. Ten biotypes were distinguished: 5 rhamnose (Rh) + (B16, B17, B24, B25, B28) and 5 rhamnose (Rh) - (B0, B1, B8, B9, B12) The *eae* gene was detected in 39,13% of the Rh- strains and 77,77% of such strains was also AF/R2+. The *eae* gene was not so significantly common among the Rh+ strains (21,21%). Nevertheless, the AF/R2 gene was detected in 42,85% of the Rh+ and *eae*+ isolates. The biotypes 8 and 24 were highly common in the investigated area. The *eae* and AF/R2 genes were mostly identified in such *E. coli* biotypes. Evaluation of drug resistance showed that all the isolates (100%) were E15-resistant. High percentages of resistance were also found to SP100 (98,2%), SXT (92,8%), TE30 (87,5%), S10 (73,2%), GM10 (71,4%) and N30 (69,6%). A variety of multiple resistance patterns was observed in all the *E. coli* strains tested.

RESULTS OF SEROEPIDEMIOLOGICAL SURVEYS FOR THE DETECTION OF NATURAL ANTI-RHD ANTIBODIES INDUCED BY THE NONPATHOGENIC RABBIT CALICIVIRUS (RCV) IN MEAT RABBITS. CAPUCCI L.* †, CERRONE A.* †, BOTTI G.* †, MARIANI F.* †, BARTOLI M.* †, LAVAZZA A.* † †*Ist. Zooprof. Sp. della Lombardia e dell'Emilia Romagna.* †*OIE Ref. Centre for Haemorrhagic Disease of Lagomorphs, Brescia.* capucci@bs.izs.it. †*Ist. Zooprof. Sp. del Mezzogiorno, Portici, Italy.*

Rabbit haemorrhagic disease virus (RHDV) is a non-cultivable calicivirus that infects rabbits (*Oryctolagus cuniculus*) and causes outbreaks of an acute fatal hepatitis, firstly described in China in 1984. Another virus, named rabbit calicivirus (RCV), related to the RHDV was identified in healthy rabbits in Italy in 1996. This virus is avirulent, replicates in the intestine at a low titre and presents a 92% genomic identity with RHDV. In addition, seroepidemiological data have shown the presence of 'naturally acquired' antibodies in Europe either from 1975 to 1987 i.e. before the first evidence of the disease and in colonies where RHD had never been recorded or vaccination performed, thus suggesting the existence of one or more non-pathogenic viral strain antigenically related to RHDV. In order to check the diffusion of RCV in Italian rabbitries we conducted a survey respectively in 39 farms in North Italy (Lombardia and Triveneto) and 21 farms in South Italy (Lazio, Campania and Basilicata) by testing non vaccinated 80 dd old meat rabbits at slaughterhouse. The results indicate the presence of "natural antibodies" presumably induced by RCV i.e. over 80% of animals showing titres $3 \text{ } 1/20$ in almost 30% of farms controlled.

INTESTINAL EMPHYSEMA IN A RABBIT DOE (*Oryctolagus cuniculus*). CAYO V.* †, PERIS B.* †, GARCÍA V.* †, SEGURA P.* †, MARTÍNEZ J.* †, ORTEGA J.* †, CORPA J.M.* †*Dpt. At. Sanitaria, Salud Pública y Sanidad Animal. Univ. Cardenal Herrera-CEU. Moncada, jmcorma@uch.ceu.es* . †*Consell. de Agr., Pesca y Alimentación. Estación exp. agr. de Carcaixent. Spain.*

Intestinal emphysema is a rare condition characterized by numerous thin-walled, gasfilled cystic structures, a few millimetres to several

centimetres in diameter, in the gut wall and on the serosal surface. These are located mainly in the small intestine, although the large intestine, mesentery, and mesenteric lymph nodes may be involved. Microscopically, the gas bubbles are present within lymphatic vessels. A pleocellular inflammatory reaction may be evident in the walls of the cysts. This report describes a case of intestinal emphysema in a rabbit doe from an ecological farm and different etiologic causes are discussed. It is proposed that the intestinal emphysema may have been caused by a combination of bacterial, nutritional, and perhaps host factors. An unbalance diet may have been the leading factor.

A SURVEY OF ZONOTIC AGENTS IN ITALIAN RABBIT SLAUGHTERHOUSES. CERRONE A.*¹, MARIANI F.*², CIABRELLI M.*³, GALIERO G.[†], DE CARLO E.[†], FIORETTI A.[‡], BAIANO A.[‡], BARTOLI M.*⁴ *Ist. Zooprof. Sp. del Mezzogiorno, Portici. annacerr@tin.it*, [†]*Ist. Zooprof. Sp. del Mezzogiorno. Sz. di Salerno, Fuorni.* [‡]*Dpt. di Patologia e Sanità Anim. Univ. degli Studi di Napoli Federico II. Italy. fioretti@unina.it*

From June 2002 to February 2003 five rabbit slaughterhouses were selected in Campania region (Italy) on the basis of their geographic location and their slaughtering capacity. Samples from muscular tissue, carcass swabs, organs and carcass washing water were collected and examined for *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter spp.*, *Yersinia enterocolitica*; surface air system was used for *Dermatophytes* and hygienic indicators of the slaughtering process were evaluated with the enumeration of coagulase - positive *Staphylococci*, of *E. coli* and of total aerobic mesophilic count. Three different serotypes were respectively detected each one in three of the five monitored slaughterhouses: *S. Indiana* (from carcass washing water), *S. blockley* and a non-typifiable strain (in muscular tissue). Three different strains of *Listeria monocytogenes* were detected in two of the five processing plants; in particular two strains were isolated from muscular tissue and one strain from organs. All the rabbit carcasses were negative for *Campylobacter spp.* and *Yersinia*

enterocolitica. Environmental monitoring and hygienic control have shown the presence of dermatophytes above all during stunning (<42 CFU/m³), skinning (<32 CFU/m³), evisceration (<30 CFU/m³) and packing phase (<28 CFU/m³) that, together with other hygienic indicators and, particularly, the enumeration of *E. coli* signal the necessity of an increasing attention to the implementation of codes of good manufacturing practice (GMP). Greater care during evisceration and accurate meat inspection procedures should be encouraged to prevent secondary contamination from environment of the slaughtering plant.

PARASITOLOGICAL ZONOSIS IN RABBIT MEAT: RESULTS OF SEROEPIDEMIOLOGICAL SURVEY FOR THE INVESTIGATION OF ENCEPHALITOOZON CUNICULI, TOXOPLASMA GONDII AND CHLAMYDIA PSITTACI IN ITALIAN RABBITRIES. CERRONE A.*¹, MARIANI F.*², CIABRELLI M.*³, GALIERO G.[†], DE CARLO E.[‡], FIORETTI A.[‡], BAIANO A.[‡], BARTOLI M.*⁴ *Ist. Zooprof. Sp. del Mezzogiorno, Portici, annacerr@tin.it*. [†]*Ist. Zooprof. Sp. del Mezzogiorno. Fuorni.* [‡]*Dpt. di Patologia e Sanità Animale. Univ. degli Studi di Napoli Federico II. Italy.*

During June 2002 and February 2003 1,800 blood samples were collected from 5 slaughtering plants located in Campania region (Italy) which were supplied by 21 rabbitries situated in central and southern Italian regions, such as Campania, Lazio, Basilicata, Molise, Calabria. The aims of this study were to use the Carbon Immunoassay (India ink) test (CIA) and FC test to determine respectively the prevalence of specific zoonotic agents such as *E. cuniculi*, *T. gondii* and *Chlamydia psittaci* in fattening rabbits. For *E. cuniculi* of the total number of 1,800 sera examined, 490 were positive and they represented 27.2%. All supplying breeding farms were positive with a percentage from 10% (4/40) to 57.5% (23/40) of total sera collected from each slaughtered batch. Sera resulted less positive for *T. gondii*: of 1,800 sera only 50 (2.7%) were positive; these dates were relative to 6/21 breeding farms. Serological Chlamydia screening resulted totally negative.

ABDOMINAL PREGNANCIES IN FARM RABBITS.

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Abdominal pregnancy is defined as the implantation and development of one or several segmented ova or embryos in the peritoneal cavity. Although this has been reported in several species, it is considered as a low incidence process. It is classified as a primary abdominal pregnancy, if there is no evidence of uterine rupture, with presumed regurgitation of early embryos from the uterine tube and as a secondary abdominal pregnancy, when there is evidence of uterine rupture. During a necropsy study of 550 adult fertile female New Zealand white rabbits (*Oryctolagus cuniculus*) from two rabbit farms in Valencia (Spain), the main causes of elimination were studied. Twenty-eight abdominal pregnancies were diagnosed. Seven animals showed no lesions in their reproductive tract. The remaining twenty-one animals showed acute or chronic lesions in the reproductive tract. The classification as a primary or secondary condition is discussed. It may be concluded therefore that extrauterine pregnancies would not be such an unusual finding in rabbits (7.8% in one of the studied), and that this premise should be considered in the diagnostic approach when assessing rabbit doe pathology. New husbandry systems in rabbits such as artificial insemination are factors to be considered.

EVALUATION OF THE EFFICACY OF CYCOSTAT® 66G AGAINST COCCIDIOSIS IN FATTENING RABBITS UNDER CONTROLLED FIELD CONDITIONS.

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This study investigated the efficacy of Cycostat® 66G against rabbit coccidiosis on farm level in fattening rabbits. Three hundred thirty five of four weeks old rabbits from two different breeding farms were divided into three different experimental groups: uninoculated untreated (UIUT), inoculated treated (IT) and inoculated untreated (IUT). The experimental inoculation

was low (13200 oocysts per rabbit with different pathogenic or very pathogenic *Eimeria* species) in order to mimic a natural contamination. Body weight gain and feed conversion were significantly better for the inoculated animals receiving feed supplemented with Cycostat® 66G compared to inoculated untreated animals. The oocyst excretion was strongly reduced (more than 85%) for *E.piriformis* and *E.flavescens* + *E.intestinalis* in treated groups vs. untreated groups whilst the excretion of less pathogenic species (*E.magna*, *E.media* and *E.perforans*) was less reduced (30 to 40%). At the end of the trial, none of the most pathogenic species were seen. Mortality caused by coccidiosis was low in all groups. At the end of the fattening period there was a clear positive effect of the treatment on the carcass quality and on the ratio carcass weight/live weight. The efficacy of anticoccidial drug is lesser with the batch of rabbits having the most precarious health status.

STUDY OF EARLY PHENOMENA DURING EXPERIMENTAL EPIZOOTIC RABBIT ENTEROPATHY: PRELIMINARY RESULTS.

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This study uses the effectiveness of the bacitracin for better knowledge of the various phases of the ERE in particular in early hours which follow the inoculation. Six groups of animals were used including 3 treatments with Bacivet S at different time: A group treated before inoculation. A group treated before inoculation but whose treatment stopped 20h after inoculation. A group only treated 20h after inoculation. An inoculated reference group and never treated. Two Uninoculated but treated reference groups. Three parameter were studied: growth, mortality and rumbling noises (borborygmus). The whole of the studied parameters is very coherent and lead to the same interpretation of the results. A significant fall of growth is observed before the 20th h following the inoculation in all the inoculated groups. The treatment with the bacitracin allow to remove mortality and the rumbles but not this initial fall of growth. The treatment started as soon as 20h

after inoculation is much less effective. Even with a preventive treatment stopped only 20h after inoculation one observes a delay of several days for the apparition of the disease (fall of growth, appearance of the rumbles) and the total mortality is reduced. Very few pathogens can explain this early fall of the growth. The bacitracin is antibiotic which make it possible to control the disease very well thus probably the pathogen but not the physiopathological disturbances of the first hours. The intervention of an exogenic toxin as soon as the moment of the contamination seems likely.

CHARACTERIZATION OF *E. COLI* STRAINS ISOLATED FROM RABBITS WITH ENTERITIS IN LOMBARDIA AND EMILIA ROMAGNA (NORTH ITALY) DURING THE PERIOD 2000-2003. D'INCAU M., PENNELLI D., PACCARINI M., MACCABIANI G., LAVAZZA A., TAGLIABUE S. *Ist. Zooprof. Sp. della Lombardia e dell'Emilia Romagna, Brescia, Italy.* mdincau@bs.izs.it

Colibacillosis are the most important cause of enteritis in rabbit breedings (Milon *et al.*, 1999). In this paper we report the results of the serotyping of 711 *E. coli* strains, isolated during the period 2000-2003 in Lombardia and Emilia Romagna from European rabbits (*Oryctolagus cuniculus*) with enteritis. The aims of the study were first to investigate which selected O-antigens were mostly associated with rabbits enteric disease and then to detect other pathogenic factors like toxins (VT, LT, CNF) and *eae* genes. The results show that O2 and O103 are the most frequently identified serogroups, being respectively 25.9% and 25.2%, i.e. 79 and 77 out of 305 O-antigens recognized. The *eae* gene was identified in 185 of the 711 strains investigated (26%); toxins were found in 5 strains only (2 VT1, 2 VT2, 1 LT). The identification of O103 serogroup was associated with the presence of the *eae* gene in 60 out of the 77 (77.9%) strains isolated. Such result confirms the importance of the contemporaneous presence of both these pathogenic factors in *E. coli* strains responsible for outbreaks of enteritis in rabbits. Comparing these results with those previously obtained in the same geographic area

during the triennium 1997-1999 (Finazzi *et al.*, 2000), it is evident that we observe both a significant increase of the O2 and O157 serogroups' detection and an important decrease of the O103 detection. Results concerning the serotyping of other O-antigens are substantially superimposing.

GASTRO-INTESTINAL AND EXTERNAL PARASITOSE OF RABBIT IN BENIN. FAROUGOU S.*, KOUTINHOUI B.*, KPODEKON M.*, DUOGNON P.*, DJAGO Y.†, ADEHAN R.*, AHLINCOU F.† *Unité de Rech. Cunicole et Cavicole (URCC), Ecole Polytech. d'Abomey, Cotonou. kpodekon@bj.refer.org. †Centre Cunicole de Rech. et d'Information, Cotonou, Bénin

An inventory of rabbit gastro-intestinal and external parasitoses in south-Benin was carried out from February to April 2003 in eight rabbit farms. For the external parasitoses, only the mange was highlighted. On 480 examined rabbits, 76 were carrying lesions of mange. As well ear mange as that of the head and the body were observed with a more important frequency of the first form. Concerning the internal parasitoses, the helminthosis were relatively rare: on 480 samples, only 22 were positive in 4 farms out of the 8 prospected. Two species of nematodes were revealed: *Graphidium strigosum* (more frequent) and *Trichostrongylus retortaeformis*. All the farms were parasitized by the coccidia with a higher OPG in the fattening rabbits. The eleven *Eimeria* species generally found in rabbit were identified whose more frequent is *E. magna* followed by *E. media*.

RECURRENT OUTBREAKS OF AMYXOMATOUS MYXOMATOSIS IN A COMMERCIAL RABBITRY ATTRIBUTED TO CONTACT TRANSMISSION. FARSANGA.*, MAKRANSZKI L.*, DOBOS-KOVÁCS M.†, VIRÁG G.‡, FÁBIÁN K.*, BARNÁ T.*, KULCSÁR G.*, KUCSERA L.*, SOÓS T.*, VETÉSI F.† *Inst. for Vet. Medicinal Products, Budapest. makra@oai.hu †Dpt. of Pathology and Forensic Vet. Medicine, Fac. of Veterinary Sci., Budapest, Hungary. ‡Inst. for Small Animal Research, Gödöllo, Hungary. virag@katki.hu

An outbreak of atypical form of myxomatosis has struck a rabbit farm in Hungary. The disease appeared in winter when presence of mosquitoes

and fleas is not common. The virus was isolated from eyelid specimen of a naturally infected rabbit. Genetic analysis of the isolated virus was carried out by polymerase chain reaction (PCR) and direct sequencing. The primers were designed on the basis of the major envelope gene (Env) of the Lausanne reference strain in the GenBank. The viral proteins were examined by SDS-PAGE. The isolated virus (ref. No: BP04/2001) was able to infect the susceptible animals directly, by contact way. The disease was characterised by respiratory clinical signs of the upper respiratory tract, conjunctivitis and high mortality by the 11-14 day after infection. Aerogenic infection with strain BP04/2001 resulted in 100% morbidity among the susceptible animals. Sequencing the amplified 400 bp-long DNA revealed 97% homology with the Env gene of the Lausanne strain, which proves that strain BP04/2001 is a variant of the Lausanne strain having been enzootic throughout Europe. The live vaccine strain used in Hungary against myxomatosis, which is also a Lausannerivated strain, protected the animals. According to the protein examinations a protein in size 200 kDa is not expressed in the strain BP04/2001. This is the first report on atypical myxomatosis in Central-Europe. The virus spreads in aerogen route and may cause severe losses in rabbit population.

STUDY ON CONTROL TECHNOLOGY OF INFECTIOUS RHINITIS OF RABBIT. GU Z.L., CHEN B.J., KAN Q.H., REN W.S., HUANG Y.T., HUANG R.L. *Mt. Area Res. Inst. of Hebei Agriculture Univ., Baoding, China. hebaugzl@sohu.com*

The relationship between rabbit infectious rhinitis and environmental factors (density, level, cage locations, feeding system, variety, age and season) were observed to study the breaking rules. The optimal drug "Bi Gang Jing" were elected among seven kinds of therapeutic schedules. The effective level and reoccurrence rate were 100% and 87.47% respectively in a case of treatment and reached significant level ($P < 0.05$ or $P < 0.01$). The control of environmental factors was also pointed out.

RESULTS OF THE TECHNICAL MANAGEMENT OF FOUR RABBIT FARMS IN BENIN. KPODEKON MR.*[†], DJAGO Y.[†], FAROUGOU S.[†], COUDERT P.[‡], LEBAS F.[§] **Unité de Rech. Cunicole et Cavicole; Cotonou, Bénin; kpodekon@bj.refer.org. †Centre Cunicole de Rech. et d'Information, Cotonou Bénin. ‡Lab. de Pathologie du Lapin, INRA, Centre de Tours-Nouzilly, France. §Station de Rech. Cunicoles, INRA, Centre de Toulouse, France*

A study was carried out in 2000 in four rabbit units to evaluate the level of the technical management of the rabbit farms of south - Benin. The data collected by the farmers themselves made it possible to note a mean level of the zootechnical performances. The average size of the whole litter is 6. An average of 5,6 young rabbits are born alive of which 4,8 are weaned. The interval between littering is evaluated to 73 days, that is to say an average of 6 littering per annum. From one farm to another considerable variations are observed among some zootechnical parameters such that the number of born alive per litter. It shows that there is an exploitable genetic characteristic on the level of the rabbit breeding in Benin.

AN UPDATE ON THE PRESENCE AND SPREADING IN ITALY OF RABBIT HAEMORRHAGIC DISEASE VIRUS AND OF ITS ANTIGENIC VARIANT RHDVa. LAVAZZA A.*[†], CERRONE A.[†], AGNOLETTI F.[‡], PERUGINI G.[§], FIORETTI A.[#], BOTTI G.*[†], BOZZONI G.*[†], CERIOLI M.*[†], CAPUCCI L.*[†] **Ist. Zooprof. Sp. della Lombardia e dell'Emilia Romagna and OIE Ref. C. for Haemorrhagic Disease of Lagomorphs, Brescia. alavazza@bs.izs.it. †Ist. Zooprof. Sp. del Mezzogiorno, Portici. ‡Ist. Zooprof. delle Venezie, Treviso. §Ist. Zooprof. dell'Umbria e delle Marche, Macerata. #Fac. Medicina Vet., Univ. "Federico II", Napoli, Italy.*

Rabbit haemorrhagic disease virus (RHDV) is a non-cultivable calicivirus that infects rabbits (*Oryctolagus cuniculus*) and causes an acute fatal hepatitis, firstly described in China in 1984. The first consistent antigenic variant originated from the classical strain, called RHDVa, was identified almost contemporaneously in Italy and Germany in 1997. We report here the results of diagnostic studies in order to compare the rate of diffusion of RHDV and RHDVa in Italy since its first detection. From 2000 to 2003 RHDVa has

been identified in 53,7% of the cases and, in particular during the last two years, a total of 201 cases of RHD were diagnosed, 128 (63,7%) of which resulted RHDVa. The higher percentages (70-100%) were found in those regions where is concentrated most of intensive rabbit production (Lombardia, Emilia Romagna, Piemonte, Veneto and Campania). This survey shows that RHDVa is present in most part of Italy and that it is rapidly replacing the RHDV "classical" strain. The importance of such variant is discussed with reference to vaccine preparation and application.

SEROLOGICAL EVALUATION OF THE IMMUNITY INDUCED IN COMMERCIAL RABBITS BY VACCINATION FOR MYXOMATOSIS AND RHD. LAVAZZA A.*[‡], GRAZIANI M.[‡], TRANQUILLO V.M.*[‡], BOTTI G.*[‡], PALOTTA C.*[‡], CERIOLI M.1, CAPUCCI L.*[‡] **Inst. Zooprof. Sp. de lla Lombardia e dell'Emilia Romagna and †OIE Ref. Centre for Haemorrhagic Disease of Lagomorphs, Brescia, alavazza@bs.izs.it, ‡Meril Italia spa, Milano.*

For both Rabbit Haemorrhagic Disease and Myxomatosis viruses, vaccination is made in order to increase the specific humoral and/or cellular immune response of the animals and protect against disease. The aim of our work is to detect the humoral immune response, using competition ELISAs (cELISA) as diagnostic tests, in breeders and fattening rabbits vaccinated with different types of vaccines and by variable route of administrations against myxomatosis and RHDV. Difference in serological response were analysed by mixed model on a logarithmic transformation of titres. In all the vaccinated groups a specific seroconversion was observed. As expected the highest average values were detected among multiparous breeders, which had been previously vaccinated several times. For breeders, the linear mixed models for serological responses show a very important interaction effects between "Day of Sampling" and "Kind of Vaccination" group. The most relevant difference in fattening rabbits vaccinated for the first time against myxomatosis was, as expected, the absolute lower levels of antibodies detected. The linear mixed model for Myxomatosis

serological response in fattening rabbits show a significant interaction between "Day of Sampling" and "Kind of Vaccination". Moreover, by using RHDV serological methods (cELISA and anti-isotype IgG-IgA-IgM ELISAs) all the rabbits tested at 28 days of age showed passive antibodies of maternal origin against RHD. Therefore we cannot exclude that the presence of antibodies at different titres at the moment of first vaccination could have conditioned the effect of immunization. The results obtained in this study demonstrate that serological methods could be a valid aid in monitoring the efficacy of vaccination for both Myxomatosis and RHD. Using methods having a high level of specificity, it was possible to detect seroconversion in groups of rabbits vaccinated with different type of vaccines. Indeed, serology and particularly the combination of RHDV anti-isotypes ELISAs could help to improve the quality of vaccination by determining the more convenient moment to administrate the first vaccination in relation to the disappearance of maternal antibodies.

MICROBIOLOGICAL CONTROL OF THE ENVIRONMENT IN AN INTENSIVE RABBIT REARING. MARTINO P.A.*[‡], LUZI F.[‡], VERGA M.[‡]

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The microbiological conditions of farm environment are very important to obtain good performance (e.g., daily live weight gains, feed conversion rate, meat quality, etc.), even if this parameter is often disregarded. Rabbits are particularly sensitive to environmental conditions and to stress due to changes in some parameters (e.g., temperature, relative humidity, etc.). Aim of this work is the evaluation of the number and kind of bacteria and fungi, using exposed plates with microbiological media, to which rabbits, bred in reproductive shed with forced ventilation, have been exposed. This work was performed in a meat rabbit farm located in the province of Bergamo (Lombardia region), in the North-West of Italy. Rabbits (commercial hybrids) are housed in two separate sheds with forced ventilation; one of them is dedicated to

does and nests (reproduction sector) and the other one is for fattening rabbits. Metal cages are situated, for the 1st shed, on one level; on the contrary, for the 2nd on two levels (California systems). The environmental samples were collected using “opened plates”, during springtime (March-June) in doe’s shed and nests. *Micrococcus luteus* and *Staphylococcus* spp. were the most frequently isolated bacteria and among yeasts *Rhodotorula rubra*, with a total charge always more than 50 UFC/plate. Among environmental fungi there is an important differentiation; beside *Aspergillus* spp., *Penicillium* spp. and *Alternaria*, there are *Fusarium*, *Saksenaea* (a *Mucorales* species) and *Curvularia*. For dermatophytes, high prevalence of *Trichophyton mentagrophytes* has been observed (from 50% to 70%); this microorganism is typical of rabbit farms and, like *Microsporium canis*, it’s a zoonotic agent. The data reported in this paper demonstrate that a good environmental control, particularly a microbiological control, can be very useful for maintaining good health status in farm animals.

EXPLORING THE EPIDEMIOLOGY OF LAPINE ROTAVIRUSES: EVIDENCE FOR SPREADING OF ROTAVIRUSES DISPLAYING THE NEWLY-RECOGNISED P[22] VP4 GENE ALLELE IN ITALY.

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An epidemiological survey was carried out to investigate the distribution of the VP7 and VP4 antigenic specificities of lapine rotaviruses (LRV) in Italy. Rotaviruses were identified in rabbitries from different geographical regions of Northern and Southern Italy. The VP7 and VP4 specificities of the Italian LRV strains were determined by either PCR genotyping or sequence analysis. Almost all the strains (14 out of 16) were characterised as P[22],G3, confirming the presence of viruses resembling the newly recognised P[22] LRVs in Italian rabbitries. The P[22] VP4s detected were about 87.3 to 91.5% identical at the amino acid level to the prototype

P[22] LRV strains (160/01, 229/01 and 308/01), identified in 2001 in Italy. Only 1 strain was characterised as P[14],G3 and 1 sample was a mixed infection of P[14],G3 and P[22],G3 LRVs. These data are suggestive of an almost complete replacement of the P[14] allele by the P[22] in Italian rabbitries.

FOOD SAFETY IN RABBIT MEAT PRODUCTION.

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The Food and Agriculture Organization of the United Nations (FAO) in collaboration with the World Health Organization (WHO) has worried about an integrative focus regarding innocuous food quality and management in all the productive chain in the countries belonging to it. Meat is exposed to different pollutants all along the production process until it reaches the final consumer. That is why it is necessary to establish a risk reduction system in its productive process to assure and certify the products. The problems regarding innocuous products originate from the fact that they should be capable of being traced or prevented from the very beginning of the productive chain by using Good Agriculture Practices (GAP), Good Manufacturing Practices (GMP), Standard Sanitization Operation Procedures (POES) and the Hazard Analysis of Critical Control Points (HACCP). All these elements are intimately related with the gradual implementation of the ISO 9000:2000 system. The successful application of the system requires management decisions and a multidisciplinary team highly committed to food safety. The Mexican Government within its regulation functions has to make up a series of rules regarding the regulation of the food production chain that guarantees it with a certified quality label. Rabbit meat production cannot escape this reality.

TOLERANCE OF DECOQUINATE IN THE RABBIT.

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Decoquinatate is one of the molecules that is effective against coccidia in many animal species. Many trials conducted on rabbit farms, at incorporation rates of 60 ppm in the feed, have made it possible to confirm the interest of using it. The study presented here aims to assess the safety margin for the use of decoquinatate as incorporated in a feed for fattening rabbits. Five lots of 36 rabbits received respectively from weaning: a control feed without decoquinatate, a 72 ppm decoquinatate-supplemented feed, a 120 ppm decoquinatate -supplemented feed, a 200 ppm decoquinatate-supplemented feed and a 340 ppm decoquinatate-supplemented feed. The same mortality, morbidity, feed intake and growth were observed in all 5 lots, whatever the rates of decoquinatate incorporated in the feed. As the 200 ppm decoquinatate dose corresponds to 3 times the necessary and sufficient dose to an efficient treatment of coccidia in the rabbit, it may then be considered that the safety margin for the use of decoquinatate incorporated in the feed is wide.

EFFICACY OF INTRADERMAL RHD VACCINATION USING VARIOUS ADJUVANTS ON FATTENING RABBITS. PAGÈS-MANTÉ A., ALFONSO M. *Lab. Hipra, S.A. Girona. Spain* apm@hipra.com

There is a clear necessity to protect fattening animals to prevent the losses that an outbreak of Rabbit Hemorrhagic Disease (RHD) can cause in industrial rabbit farms. Inactivated vaccines against RHD with different adjuvants are available in the market as well as several vaccination devices. The purpose of this study was to assess the efficacy of various RHD vaccines with different adjuvants when administered by intradermal route by measuring the serological immune response and the resistance to an intramuscular challenge with RHD virus in fattening rabbits. Five batches of animals were vaccinated: Batch 1 or control rabbits were inoculated phosphate buffer solution (PBS) by intradermal route. Batches 2 and 3 were vaccinated with half-dose of oil-based Cunipravac-RHD® (Laboratorios Hipra) by subcutaneous and intradermal route respectively. Batch 4 received an experimental

aluminum hydroxide-based vaccine with the same antigenic composition as Cunipravac-RHD®. Aluminum hydroxide-based Dercunimix® (Merial) was administered to Batch 5 according to manufacturer's indications. Vaccinations did not affect health status of rabbits but produced transient local reactions at the inoculation site. Serological response at 29 days post-vaccination was not complete and varied widely between groups. In contrast, total protection after challenge was reached in Batches 2, 3 and 5. When using the intradermal route half-dose of the oil-based vaccine (Batch 3) conferred a protection comparable to a complete dose of the aluminium hydroxide-based vaccine (Batch 5) after challenge with RHD virus. Our results suggested that oil-based vaccines were more effective controlling a RHD viral infection regardless of the inoculation route used compared to the aluminium hydroxide-based vaccines based on the identification of RHD virus from vaccinated challenged animals.

BIOTYPE AND SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS OF RABBIT *E. coli*. PISONI A.M.* , PICCIRILLO A.†, GALLAZZI D.* , AGNOLETTI F.‡, GRILLI G.* *Dpt. of Vet. Pathology, Hygiene and Public Health. Univ. of Milan. guido.grilli@unimi.it. †Dpt. of Public Health, Comparative Pathology and Veterinary Hygiene. Univ. of Padua. ‡Ist. Zooprof. Sp. delle Venezie. Italy.

Non-invasive Enteropathogenic *Escherichia coli* (EPEC) represents one of the most frequent pathogens involved in fatal enteropathy of commercial rabbits in Italy. We here report the characterisation of the biotype and the resistance to antimicrobial agents of 60 strains of *E. coli* isolated from 150 rabbits affected by diarrhoeic enteropathy. Parasitological, bacterioscopic, bacteriological and histological examinations were performed on caecal samples. Biotyping was made according to Camguilhem and Milon (1989), and the antibiotic resistance against 18 different antibiotics was evaluated using the Kirby-Bauer method. All the strains were isolated from *caeca* that showed catharral to haemorrhagic entero-tiflitis and, microscopically, attachment/effacement (A/E) lesions. Twelve different biotypes were

identified, among which B12 and B14 biotypes were constantly present. Antibiotic resistance patterns varied considerably among the biotypes and within the same biotype among the rabbitries. Only fluoroquinolones resulted highly efficacious against *E. coli*. The results suggest to adopt quarantine measures for restocking rabbits in order to identify potential reservoirs of EPEC as well as to determine the prevalent biotype/biotypes and its/their susceptibility to antimicrobial agents.

HEALTH STATUS OF DOMESTIC RABBITS IN THE IBERIAN PENINSULA. INFLUENCE OF THEIR ORIGIN. ROSELL J.M.*, DE LA FUENTE L. F† *NANTA SA; Madrid, Spain. j.rosell@nutreco.com. †Prod. Animal I, Fac. de Veterinaria, León, Spain.

A retrospective study was carried out on the health status of does on 115 intensive production rabbitries in the Iberian peninsula, to evaluate the effect of the “animal origin” variable used on these farms, on the percentage of females affected by coryza, mastitis and sore hocks. 277 samplings were carried out on a total of 13,941 suckling females, with a mean size of 50.3 does examined per sampling, during 3 years (January 2001-February 2004). On all the farms the same origin was used for the does as for the bucks (or semen), which belonged to one of the following five groups: S, O, M, I and U. The effect of other variables (whether all the does were given injectable antibiotics on the occasion of parturition or the use of antiseptic aerosols on the hocks, and of footrests) on the prevalence of the previously mentioned diseases was also evaluated by ANOVA. In the case of coryza the influence of the origin of the animal is evident. Rabbitries with the “S” origin had 49.31 percent affected does in comparison with 27.75% on the following group of rabbitries (“O” origin). In the case of mastitis, farms where antibiotics had been used were eliminated, so that 177 samplings were analysed. In this case, origin of the animals was a risk factor in mastitis. For sore hocks, rabbitries using footrests were not included, so 153 samplings in which 7,699 examined does did not have footrests were analysed; in this case the prevalence of sore hocks was significantly

higher in groups “S” (15.26% affected does) and “U” (10.73%), the percentage for both being above the overall mean of 9.19%. An evaluation of the possible causes of differences between origins would be useful in future studies.

EXCRETION OF TILMICOSIN IN MILK OF RABBITS AFTER SUBCUTANEOUS ADMINISTRATION. SAGGIORATO M.*, PRADELLA G.*, FEDRIZZI G.†, MENOTTA S.†, SCANDURRA S.†, ANFOSSI P.‡, CASAROTTI M.§, GIOVANARDI D.# *Eli Lilly Italia Div. Elanco Animal Health. Sesto Fiorentino. saggiorato_marco@lilly.com †Ist. Zooprof. Sp. della Lombardia e dell'Emilia Romagna – Reparto di Merceologia degli Alimenti di Origine Animale. Bologna. ‡Dpt. di Sanità Pub. Vet. e Patologia Animale. Ozzano Emilia. §Agricola Alimentare Italiana. S. Martino B.A. #Lab. Diagnostico Tre Valli. S. Martino B.A.. Italy.

To check the pattern of tilmicosin excretion in milk and to determine the best therapeutic schedule for the treatment of mastitis in does, 3 groups of does were administered one single subcutaneous dose (20 mg/kg) before parturition, a single dose after parturition, or two doses, one before and one after parturition. Milk concentrations of tilmicosin were determined by a validated HPLC method and were compared to the minimal inhibitory concentrations (MIC) for *Staphylococcus aureus* strains isolated from clinically mastitic does. Treatments were well tolerated and no adverse reactions were observed. Tilmicosin peak concentrations in milk were attained on the first day in does treated once before parturition (1.51 ± 1.30 ig/g), and in those treated twice, before and after parturition (3.05 ± 1.88 ig/g). When tilmicosin was administered once the day after parturition, peak concentrations in milk were detected on the 2nd experimental day (group B: 2.33 ± 1.47 ig/g). The administration of two doses produced the highest concentrations of tilmicosin at all sampling times, with differences from the other therapeutic schedules being significant on the first 3 days. Close to 50% of the *S.aureus* isolates would be sensitive to the milk concentrations of tilmicosin obtained in does treated twice, before and after parturition. As the intensive use in rabbitries of antibiotics has been associated with

an overall increase of pathogen resistance, these results indicate that tilmicosin is a potential tool for the therapy of rabbit mastitis, and that it could represent an alternative to the usual approaches for the therapy of this disease.

CHARACTERIZATION OF GROWING RABBIT MORBIDITY AND MORTALITY IN A RABBITRY IN CHAPINGO, MEXICO. SALCEDO-BACA R. *, MARTÍNEZ-GARCÍA G.E.†, MONTESINOS-R.L.I.‡, GÓMEZ-LORENCE M.* *Dpt. de Preparatoria Agrícola, †Dpt. de Zootec., Univ. Autónoma Chapingo, Texcoco, México. rames@correo.chapingo.mx. ‡FMVZ, UNAM, Coyoacan, México D.F., México.*

The objectives of this study was to characterize morbidity and mortality in a rabbitry during summer 2003. Animals from this study were at Chapingo, Mexico, which is located at 19° 29' N latitude, 98° 53' W longitude, and 2250 m over sea level altitude. The climatic formula of the place is Cb (wo)(w)(i')g, with 571.5 mm of precipitation and average temperature of 15.2 °C. Animals were managed in 3 band or sets with 14 d period within bands. The cages were cleaned and disinfected before any movement of animals, ones the rabbits were weaned they received a preventive 10 d period antibiotic. Records from rabbits during the fattening period of the three bands red (R), n=523, green (G), n=432, and yellow (Y) n=449, were taken from July to September 2003. Morbidity (MOB) and mortality (MOT) were recorded daily in each cage. Sex and signs of the ill or dead animal were recorded. Autopsy was performed to 223 dead animals. Tissue samples were taken in approximately 20 % of animals of each of the kind of death (diarrhoea, pneumonia, and other). The tissue samples from 43 rabbits were analyzed in the Pathology laboratory from the FMVZ, Universidad Nacional Autónoma de México. The first group of animals (R band) was used to test two rabbit commercial formulas (A and B) with antibiotics or prebiotics and probiotics (Bio-Mos and Acid-Pak respectively, Alltech, T.M.). Previously to the analysis arcsin transformation was applied to the independent variables and proc GLM form SAS (V8) was utilized. Graphs were made in Excel (Microsoft T.M.). Morbidity

rate was 16, 19, and 34% for G, R and Y bands respectively. Mortality rate was 12, 13 and 21% for G, Y and R bands respectively. Difference between bands in morbidity and mortality patterns are shown graphically. Y band had a higher proportion of deaths due to pneumonia whereas in the other 2 bands the main mortality cause was diarrhoea. When the main cause of mortality was diarrhoea the sex proportion of mortality was 70-30 (R band) and 60-40 (G band) for males and females respectively. Pneumonia explained 70% of mortality in Y band, where the sex proportion was 50-50. This suggests that males are more sensible to diarrhoea than females are. The percentage of affected organs and the microorganism identified are shown.

COMPARISON OF INNER ORGANS WEIGHT AND SOME HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF TRANSGENIC AND NONTRANSGENIC RABBITS. SUVEGOVÁ K. *, JURCIK R. *, CHRENEK P. *, GAZOVICOVA Z. †, RAFAY J. *, HANUSOVA E. * *Res. Inst. for Animal Prod., Nitra. suvegova@vuzv.sk. †G-Lab, Nitra, Slovak Rep.*

Chosen anatomical and physiological parameters were compared in F2 generation transgenic rabbits produced by microinjection of WAP-hFVIII gene construct (10 animals) and rabbits of initial population in conformable age and average weight (20 animals). The animals were bred in equal conditions similar to intensive industrial ones. The transgenic rabbits of average weight 2.81 kg and control animals of average weight 2.83 kg were slaughtered. Before slaughtering the blood for haematological and biochemical analysis was taken from the central ear artery. Autopsy and pathological examination of vital organs were performed. The laboratory scales with the accuracy of 0.01 g were used to determine weight of chosen organs: heart, lungs, liver, kidney right, kidney left, spleen, both adrenal glands. Haematological values (white blood cells WBC, red blood cells RBC, blood haemoglobin HGB, haematocrit HGT, mean corpuscular volume MCV, mean corpuscular haemoglobin MCH, mean corpuscular haemoglobin concentration MCHC, platelets PLT) were determined by Sysmex KX-

21Nä Automated Haematology Analyzer. Biochemical parameters (total protein, glucose, urea, creatinine and aspartat-aminotransferase AST, alanine-aminotransferase ALT and gammaglutamyltransferase activities GMT) were assessed by laboratory analyzer COBAS INTEGRA 400 plus using Roche diagnostic tests. Pathological examination of vital organs showed relatively more pathological changes in transgenic rabbits. Our results showed no significant differences of chosen inner organs weight between transgenic and nontransgenic animals except of weight of lungs (transgenic rabbits 14.06 g, nontransgenic 16.82; $P < 0.01$). We observed statistically significant higher values of WBC (transgenic 12.37.10 9/l – nontransgenic 9.42.10 9/l; $P < 0.001$) and PLT (567.10.10 9/l – 481.64.10 9/l; $P < 0.05$), and lower values of MCH (19.85 pg – nontransgenic 21.01 pg; $P < 0.01$) and MCHC (29.49 g/dl – 30.30 g/dl; $P < 0.05$) in blood of transgenic rabbit. Significant differences were found in the concentration of urea depending on the group (transgenic 6.78 mmol.l-1 and nontransgenic 5.23, $P < 0.001$), creatinine (transgenic 73.20 μ mol.l-1 and nontransgenic 62.66, $P < 0.01$), and total protein (65.78 g.l-1 and 61.97 g.l-1, $P < 0.05$). Highly significant differences were obtained also in AST (transgenic 0.41 ikat.l-1 and nontransgenic 0.27, $P < 0.001$), and in GMT (transgenic 0.17 ikat.l-1 and nontransgenic 0.10, $P < 0.001$) activity.

ILEAL MUCOSAL EFFECTS OF VITAMIN E IN EXPERIMENTALLY INFECTED RABBITS WITH ENTEROPATHOGENIC *E. COLI* O103. TSALIE E.^{*}, KALDRYMIDOU E.^{*}, KOUZI K.[†], POUTAHIDIS TH.^{*}, XYLOURI E.[‡], ILIADIS N.[§], SARRIS K.[§], ABAS Z.[#] ^{*}Lab. of Pathology, Vet. Medicine, Aristotle Univ. of Thessaloniki. [†]Lab. of Histology-Embryology, Medicine school, Aristotle Univ. of Thessaloniki. [‡]Dpt. of Anatomy & Physiology of Farm Animals, Agr. Univ. of Athens. [§]Lab. of Microbiology, Vet. Medicine, Aristotle Univ. of Thessaloniki. [#]Dpt. of Agr. Development, Democritos Univ. of Thrace, Greece.

Vitamin E effect on intestinal mucosal lesions caused by *E. coli* infection was examined. Sixty rabbits were challenged with the highly pathogenic strain E22 and additionally thirty of them daily administered 60 mg/kg b.w. of Vitamin

E. The lesions were evaluated histologically and computer-aided morphometry was used for the following measurements: Total mucosal thickness, Villous height, Crypt depth, Villous height/crypt depth ratio, Mononuclear and Polymorphonuclear cells at the submucosa and mucosa (tip of the villous and base of the crypt). The morphometric analysis showed significant differences, indicating that vitamin E may have some beneficial effects against REPEC E22 intestinal infection.

SCREENING OF HIGH AND LOW VIRULENCE *STAPHYLOCOCCUS AUREUS* ISOLATES FROM RABBITS FOR MSCRAMM GENES. VAN DRAEYNST D., HERMANS K., HAESBROUCK F. *Dpt. of Pathology, Bacteriology and Poultry Diseases, Ghent Univ., Belgium. dieter.vancraeynest@UGent.be*

At rabbit flock level, two types of *S. aureus* infections can be distinguished. In the first type, caused by low virulence strains, the infection remains limited to a small number of animals. The second type of infection is caused by high virulence strains, which spread throughout the rabbitry. The pathogenetic capacity of a particular *S. aureus* strain is attributed to a combination of extracellular factors and properties such as adherence. Twentyeight high virulence and 34 low virulence *S. aureus* isolates recovered between 1998 and 2003 were used to study the prevalence of genes encoding for microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). PCR assays were performed to detect *bbp* (encoding bone sialoprotein binding protein), *clfA* and *clfB* (encoding clumping factors A and B), *cna* (encoding collagen binding protein), *ebpS* (encoding elastin binding protein), *eno* (encoding laminin binding protein), *fnbA* (encoding fibronectin binding protein A), *fnbB* (encoding fibronectin binding protein B) and *fib* (encoding fibrinogen binding protein). All rabbit *S. aureus* strains harboured *clfA* and *clfB*. The prevalences of *ebpS*, *eno*, *fnbA* and *fib* did not reveal striking differences between high and low virulence strains. *FnbB* prevalence in high virulence isolates was significantly lower than in low virulence isolates and *can* was absent in

high virulence strains. It was remarkable that only high virulence strains were positive for *bbp*. This could lead to the development of a diagnostic PCR test to screen asymptomatic rabbits for the presence of these strains, in order to prevent the entry of these rabbits in unaffected rabbitries.

ANTIMICROBIAL RESISTANCE AND RESISTANCE GENES IN *STAPHYLOCOCCUS AUREUS* STRAINS FROM RABBITS. VANCRAEYNST D.*¹, HERMANS K.*², MARTEL A.*², VANECHOUTTE M.†¹, DEVRIESE L.A.*¹, HAESBROUCK F.*¹ ¹Dpt. of Pathology, Bacteriology and Poultry Diseases. ²Dpt. of Clinical Chemistry, Microbiology and Immunology, Ghent Univ., Belgium. dieter.vancraeynest@UGent.be

Fifty-six *S. aureus* isolates recovered between 1998 and 2003 from 31 rabbit farms with and without problems of chronic staphylococcosis, were screened for resistance to enrofloxacin, erythromycin, gentamicin, lincomycin, neomycin, penicillin and tetracyclines using the agar dilution test. For penicillin, a disk diffusion test was also performed. The detection of *tetP(B)*, *tet(K)*, *tet(L)*, *tet(M)*, *tet(O)*, *tet(T)*, *tet(W)*, *erm(A)*, *erm(B)* and *erm(C)* genes was done via a PCR assay. Four isolates showed resistance to erythromycin and lincomycin. These isolates were positive in the PCR assay for the *erm(C)* gene which encodes resistance to lincosamides, macrolides and streptogramin B antibiotics (Leclercq and Courvalin, 1991). Eleven strains were resistant to tetracyclines and all were positive for the *tet(K)* gene encoding active efflux of tetracyclines (Hirata *et al.*, 1998). In the agar dilution test, five isolates showed resistance to penicillin, whereas in the disk diffusion test 12 isolates showed resistance. Only one strain showed resistance to gentamicin, and all strains were susceptible to enrofloxacin and neomycin. This study demonstrates that resistance to antimicrobial agents in *S. aureus* isolates originating from rabbits is relatively rare compared to resistance in *S. aureus* isolates originating from other animals and from humans.

EFFICACY OF CIPROFLOXACIN AND ENROFLOXACIN IN THE TREATMENT OF A RESPIRATORY PASTEURELLOSIS OUTBREAK IN NEW ZEALAND RABBITS. VELÁZQUEZ O.V., ALONSO F.M.U., MENDOZA B.J., TALAVERA R.M., LAGUNAS B.S., MONTES DE OCA J.R. *Centro de Investigación y Estudios Avanzados en Salud Animal, FMVZ, UAEM. Toluca, México. vvo@uaemex.mx*

Pasteurellosis is an important economic disease in rabbit production. The objective of this study was to determine the individual therapeutic efficacy of fluoroquinolones such as ciprofloxacin and enrofloxacin on *Pasteurella multocida* in one outbreak of respiratory pasteurellosis in a New Zealand rabbit farm in central Mexico. The drugs were administered subcutaneously during three days in animal groups: A ciprofloxacin 20/mg/kg b. wt.; B enrofloxacin 10/mg/k b. wt.; C receiving a placebo 1 mL/k b. wt.; D untreated animals. The efficacy was evaluated as disease recovery and mortality reduction after treatment. *Pasteurella multocida* A3 was isolated from clinical cases and postmortem examination from acute or chronic lesions. The rabbit survival in groups A and C was higher with low mortality compared with untreated animals in which the clinical signs were persistent. Pathological findings related to the infection involved abscedative pneumonia and pleuritis and extrapulmonary lesions such as otitis, meningoencephalitis and subcutaneous abscesses. Our results indicate that ciprofloxacin and enrofloxacin are efficient as medical treatment of acute respiratory pasteurellosis in young rabbits, reducing the clinical cases and lesion severity and increasing survival in animals.

DIARRHEAL CASE IN SEMIINTENSIVE PRODUCTION OF NEW ZEALAND WHITE (NZW) RABBIT IN MEXICO CITY. "CHARACTERIZATION OF MACROSCOPIC AND MICROSCOPIC LESIONS". VENTURA E*¹, JUÁREZ M†¹, CANDANOSA E‡². ¹UNAM, FMVZ, CEIEPA México. D.F. ²UNAM, FMVZ, Dpt. de patología, México D.F. sk88@mexskate.zzn.com

The results were analyzed from 25 cases with clinical signs of diarrhea. The results reported, signs at the moment of necropsy, macroscopic

lesions and microscopic morphological diagnosis, the results from each case were contained in agreement of these three characteristics and the frequency was taken as percentage of presentation from different signs and lesions.

EFFECT OF A LIMITED ACCESS TO WATER ON MORTALITY OF FATTENING RABBITS.

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The aim of this trial was to study the effect of water restriction on mortality of fattening rabbits. Rabbits were housed in cages containing 8 rabbits. In the control group, the rabbits were given water *ad libitum*, but pelleted feed was restricted. In the other group, the rabbits were given water during 2.5 h per day, but feed was given *ad libitum*. Average mortality was of 15,1%, mainly caused by digestive problem. Water restriction led to a significant decrease of the mortality since in the control group, mortality was of 19,3%, whereas in the water restricted group, mortality was of 9.3%.

CHARACTERIZATION OF *PASTEURELLA MULTOCIDA* RECOVERED FROM LIVE RABBITS AT A SMALL-SCALE FARM PREVIOUSLY MANIFESTING DEATHS BY PYOTHORAX AND PYOMETRA.

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Long distance transport and relocation of adult rabbits into another small-scale farm was shortly followed by mating to the bucks of the same group. Within a 6 weeks period an outbreak of the respiratory syndrome occurred amongst these animals. Mortality of pregnant and nursing females, and their pups was 50% and 70% respectively. Main pathomorphological lesions

included pyothorax, pleural empyema, and pyometra or necrotised foetuses. The aboriginal rabbits of the farm remained healthy at the same period. Nasal and vaginal colonization with *P. multocida* was monitored on live rabbits of the relocated group and of the local animals soon after the outbreak. Proportion of the nasal carriers was 70% in the relocated group and that was around three times higher compared to the value obtained at the group of local rabbits. Vaginal colonization was detected at 33% of the females in the relocated group and each of vaginal positive females was nasal positive either. *P. multocida* isolates were genotyped with REP-PCR and relatedness of the products obtained with each isolates as template were calculated. The result showed that the strains originated from the recently introduced animals were clearly different from that of the local population. Strains isolated from the nasal and vaginal mucosa were different within a rabbit, and clustered together by other strains according the anatomical region of the isolaton. REP-PCR was proven to be highly discriminative beside its velocity and simplicity in characterisation of *P. multocida* isolates. Based on the results of the genetic analysis the most probable cause of the outbreak is a *P. multocida* strain that colonized the nasal mucosa of the mounting buck, and was carried from its original stock. Furthermore the severity of the outbreak could be influenced by the relocation as stress factor.

RABBIT HAEMORRHAGIC DISEASE IN CHINA.

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Some advanced application research and techniques into RHD in China are reviewed in this paper, including clinical and postmortem manifestations, anatomical changes, vaccination and antibody, and other control and prevention.

